EAU Guidelines

Investigation, Treatment and Monitoring of Late-Onset Hypogonadism in Males
ISA, ISSAM, and EAU Recommendations


Institute of Reproductive Medicine, University of Münster, Germany
Division of Endocrinology, Harbor-UCLA Medical Center, and Los Angeles BioMedical Research Institute, Torrance, CA, USA
Andrology Unit, Department of Urology, Martin-Luther-University, Halle, Germany
Department of Endocrinology/Andrology, Free University Hospital, Amsterdam, The Netherlands
Department of Endocrinology, Academisch Ziekenhuis, Gent, Belgium
Department of Endocrinology, Centre Hospitalier Universitaire, Sart-Tilman, Liège, Belgium
Faculty Life Science, Bar-Ilan University, Israel
Division of Geriatric Medicine, St. Louis University, St. Louis, MO, USA
Department of Urology, University Clinic of Brussels, Erasme Hospital, Belgium
General Clinical Research Center, Harbor-UCLA Medical Center, and Los Angeles BioMedical Research Institute, Torrance, CA, USA
Department of Urology, Justus-Liebig-University, Giessen, Germany
Central Manchester Healthcare NHS Trust, Manchester, UK

Available online 23 May 2005

Keywords: Guidelines; Hypogonadism; Aging Male

Androgen deficiency in the aging male has become a topic of increasing interest and debate throughout the world. The demographics clearly demonstrate the increasing percentage of the population that is in the older age groups. The data also support the concept that testosterone falls progressively with age and that a significant percentage of men over the age of 60 years have serum testosterone levels that are below the lower limits of young adult (age 20–30 years) men. The principal questions raised by these observations are whether older hypogonadal men will benefit from testosterone treatment and what will be the risks associated with such intervention. The past decade has brought evidence of benefit of androgen treatment of hypogonadal men on multiple target organs and recent studies show short term beneficial effects of testosterone in older men that are similar to those in younger men. Long term data on the effects of testosterone treatment in the older population are limited and specific risk data on the prostate and cardiovascular systems are needed.

Key questions of functional benefits that may retard frailty of the elderly are not yet available.

The recommendations described below were prepared for the International Society of Andrology (ISA) and the International Society for the Study of the Aging Male (ISSAM) following a panel discussion with active participation from the audience sponsored by the ISA on the topic at the 4th ISSAM Congress in Prague in February 2004. The ISA Member Societies were requested to comment on the draft guidelines. Representatives of the European Association of Urology (EAU) participated in the final draft of this document. This document is not intended to provide evidence for each recommendation as review of pertinent studies have recently been comprehensively summarized in the Clinical Research Directions on “Testosterone and Aging” by the Institute of Medicine (Washington 2004). The recommendations will be subject to revision as larger-scale and longer-term data become available.

In order to reach a large audience these recommendations are published in the International Journal of Andrology, the Journal of Andrology, The Aging Male and in European Urology.
Recommendation 1

Definition of late-onset hypogonadism (LOH): A clinical and biochemical syndrome associated with advancing age and characterized by typical symptoms and a deficiency in serum testosterone levels. It may result in significant detriment in the quality of life and adversely affect the function of multiple organ systems.

Recommendation 2

LOH is a syndrome characterized primarily by:

1. The easily recognized features of diminished sexual desire (libido) and erectile quality and frequency, particularly nocturnal erections,
2. Changes in mood with concomitant decreases in intellectual activity, cognitive functions, spatial orientation ability, fatigue, depressed mood and irritability,
3. Sleep disturbances,
4. Decrease in lean body mass with associated diminution in muscle volume and strength,
5. Increase in visceral fat,
6. Decrease in body hair and skin alterations,
7. Decreased bone mineral density resulting in osteopenia, osteoporosis and increased risk of bone fractures.

Recommendation 3

In patients at risk or suspected of hypogonadism in general and LOH in particular a thorough physical and biochemical work-up is mandatory and especially, the following biochemical investigations should be done:

1. A serum sample for total testosterone determination and sex hormone binding globulin (SHBG) should be obtained between 07.00 and 11.00 hours. The most widely accepted parameters to establish the presence of hypogonadism are the measurement of total testosterone and free testosterone calculated from measured total testosterone and SHBG or measured by a reliable free testosterone dialysis method.
2. There are no generally accepted lower limits of normal and it is unclear whether geographically different thresholds depend on ethnic differences or on the physicians’ perception. There is however general agreement that total testosterone levels above 12 nmol/L (346 ng/dL) or free testosterone below 180 pmol/L (52 pg/mL) require substitution. Similarly, based on the data of younger men, there is consensus that serum total testosterone levels below 8 nmol/L (231 ng/dL) or free testosterone below 180 pmol/L (52 pg/mL) require substitution. Since symptoms of testosterone deficiency become manifest between 12 and 8 nmol/L, trials of treatment can be considered in those in whom alternative causes of these symptoms have been excluded. (Since, there are variations in the reagents and normal ranges among laboratories, the cutoff values given for serum testosterone and free testosterone may have to be adjusted depending on the reference values given by each laboratory).
3. Salivary testosterone has been shown to be a reliable substitute for free testosterone measurements, but cannot be recommended at this time since the methodology has not been standardized and adult male ranges are not available in most hospital or reference laboratories.
4. If testosterone levels are below or at the lower limit of the accepted normal adult male values, it is recommended to perform a second determination together with assessment of serum luteinizing hormone (LH) and prolactin.

Recommendation 4

1. It is recognized that significant alterations in other endocrine systems occur in association with ageing but the significance of these changes is not well understood. In general terms, determinations of thyroid hormones, cortisol, DHEA, DHEA-S, melatonin, GH and IGF-I are not indicated in the uncomplicated evaluation of late-onset hypogonadism. If endocrine disorders are suspected, assessment of these and other hormones may be warranted.
2. Diabetes mellitus type 2 is a frequent disorder of aging men. It is unclear at the present time what effect testosterone has on blood sugar and insulin sensitivity; thus, until positive effects of testosterone on blood sugar control are definitively demonstrated, diabetes should be evaluated and treated before or simultaneously with testosterone substitution.
3. In aging men with the major complaint of erectile dysfunction lipids and the cardiovascular status should be assessed.

Recommendation 5

A clear indication based on a clinical picture together with biochemical evidence of low serum
testosterone should exist prior to the initiation of testosterone substitution.

**Recommendation 6**

1. Testosterone administration is absolutely contraindicated in men suspected or having carcinoma of the prostate or breast.
2. Men with significant polycythemia, untreated sleep apnea, severe heart failure, severe symptoms of lower urinary tract obstruction evident by high scores in the IPSS (International Prostate Symptom Score) or clinical findings of bladder outflow obstruction (increased post-micturition residual volume, decreased peak urinary flow, pathological pressure flow-studies) due to an enlarged, clinically benign prostate should not be treated with testosterone. Moderate obstruction represents a partial contraindication. After successful treatment of the obstruction, the contraindication is lifted.
3. In the absence of definite contraindications, age as such is not a contraindication to initiate testosterone substitution.

**Recommendation 7**

1. Preparations of natural testosterone should be used for substitution therapy. Currently available intramuscular, subdermal, transdermal, oral and buccal preparations of testosterone are safe and effective. The treating physician should have sufficient knowledge and adequate understanding of the pharmacokinetics as well as the advantages and drawbacks of each preparation. The selection of the preparation should be a joint decision of the patient and the physician.
2. Since the possible development of a contraindication during treatment (especially prostate carcinoma) requires rapid discontinuation of testosterone substitution short-acting (transdermal, oral, buccal) preparations should be preferred over long-acting (intramuscular, subdermal) depot-preparations in patients with LOH.
3. Inadequate data are available to determine the optimal serum testosterone level for efficacy and safety. For the present time mid to lower young adult male serum testosterone levels seem appropriate and should be the therapeutic goal. Supraphysiological levels must be avoided.

Although it may appear desirable, no evidence exists for or against the need to maintain the physiological circadian rhythm of serum testosterone levels.

**Recommendation 8**

1. Alkylated androgen preparations such as 17α-methyl testosterone are obsolete because of their potential liver toxicity and should no longer be prescribed.
2. There is not enough evidence to recommend a substitution with DHT in ageing men and there is no evidence to recommend other androgen preparations such as DHEA, DHEA-S, androstenediol or androstenedione for treatment.
3. hCG stimulates testosterone production of Leydig cells, albeit at a lower rate in older than in younger men. Since insufficient information exists about the effects and side effects of hCG treatment in older men this treatment cannot be recommended in LOH.

**Recommendation 9**

Improvement in signs and symptoms of testosterone deficiency should be sought and failure to benefit clinical manifestations should result in discontinuation of treatment. Further investigation for other causes is then mandatory.

**Recommendation 10**

Digital rectal examination (DRE) and determination of serum prostate-specific antigen (PSA) are mandatory in men over the age of 45 years as baseline measurements of prostate health prior to therapy with testosterone, at quarterly intervals for the first 12 months and yearly thereafter. Transrectal ultrasound-guided biopsies of the prostate are indicated only if the DRE or the serum PSA levels are abnormal.

**Recommendation 11**

Testosterone normally results in improvements in mood and well-being. The development of negative behavioural patterns during treatment calls for dose modifications or discontinuation of therapy.
Recommendation 12

Polycythemia occasionally develops during testosterone treatment. Periodic haematological assessment is indicated, i.e. before treatment, 3-monthly for one year and then annually. Dose adjustments may be necessary.

Recommendation 13

Bone density increases under testosterone substitution and fracture rates may be reduced. Therefore assessment of bone density at two-year intervals may be advisable (if available and affordable).

Recommendation 14

Some men with erectile dysfunction and low serum testosterone may not respond adequately under testosterone treatment alone. In these cases addition of phosphodiesterase 5-inhibitors may be indicated. Similarly, men with erectile dysfunction not responding to phosphodiesterase 5-inhibitors may have low serum testosterone and require testosterone substitution.

Recommendation 15

Men successfully treated for prostate cancer and suffering from confirmed symptomatic hypogonadism are candidates for testosterone substitution, after a prudent interval if there is no evidence of residual cancer. The risk and benefits must be clearly understood by the patient and the follow-up must be particularly careful. No reliable evidence exists in favour or against this recommendation. The clinicians must exercise good clinical judgement together with adequate knowledge of advantages and drawback of testosterone therapy in this situation.
Review—Overactive Bladder

The Effects of Antimuscarinic Treatments in Overactive Bladder: A Systematic Review and Meta-Analysis

Christopher Chapple\textsuperscript{a,}\textsuperscript{*}, Vik Khullar\textsuperscript{b}, Zahava Gabriel\textsuperscript{c}, Julie Ann Dooley\textsuperscript{d}

\textsuperscript{a}Sheffield Teaching Hospitals NHS Trust, Royal Hallamshire Hospital, Urology Research, J Floor Office, Glossop Road, Sheffield, S102JF, UK

\textsuperscript{b}Imperial College, St Mary’s Hospital, London, W2 1PG, UK

\textsuperscript{c}Heron Evidence Development Ltd, UK

\textsuperscript{d}Pfizer Ltd, UK

Accepted 24 February 2005

Available online 22 March 2005

Abstract

Objectives: To evaluate the tolerability, safety and efficacy of antimuscarinic drugs used to treat overactive bladder and to identify any differences between individual antimuscarinics.

Methods: Medline, Embase, CCTR and Cinahl databases were searched for published RCTs including an antimuscarinic agent from 1966 to August 2004. Data from included trials were extracted and meta-analysed where possible.

Results: Fifty-six trials were included. The antimuscarinics were found to be safe and efficacious. All antimuscarinics apart from oxybutynin IR were found to be well tolerated. Dry mouth was the most commonly reported adverse event and no drug was associated with an increase in any serious adverse event. There were significant differences between the antimuscarinics in rates of withdrawal and rates and range of adverse events and efficacy outcomes.

Conclusions: The antimuscarinics have different tolerability and safety profiles, which are clinically significant.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Antimuscarinic; Overactive bladder; Detrusor overactivity; Incontinence; Systematic review; Meta-analysis

Abbreviations: (A), trial published in abstract form only; AE, any adverse event; ANMF, the number of patients achieving normal micturition frequency; ASAE, any serious adverse event; BOO, bladder outlet obstruction; CCT, Controlled Clinical Trial; CCTR, Cochrane Controlled Trials Register; CI, confidence interval; CIE, mean change in incontinence episodes per 24 hours; CM, mean change in the number of micturitions per 24 hours; CUE, mean change in the number of urgency episodes per 24 hours; CVV, mean change in volume voided per micturition; dar, darifenacin; DO, detrusor overactivity; ER, extended release; ICI, International Consultation on Incontinence; ICS, International Continence Society; IIQ, Incontinence Impact Questionnaire; IR, immediate release; ITT, intention to treat; IUGA, International Urogynaecological Association; KHQ, King’s Health Questionnaire; LUTD, lower urinary tract disease; LUT, lower urinary tract; MI, mixed incontinence; n, number of patients included in the analysis; NR, not reported; OAB, overactive bladder; OBJECT, Overactive Bladder: Judging Effective Control and Treatment; OPERA, Overactive Bladder: Performance of Extended Release Agents; oxy, oxybutynin; P, placebo; PP, per protocol; pro, propiverine; PRO, patient reported outcome; QoL, quality of life; RC, the number of patients returned to continence; RCT, randomised controlled trial; RR, relative risk ratio; SF-36, Short-form 36; SF-12, Short-form 12; SIU, Société Internationale d’Urologie; sol, solifenacin; SUI, stress urinary incontinence; t, titrated dose; t (5), titrated from 5 mg/day; t (7.5), titrated from 7.5 mg/day; t (15), titrated from 15 mg/day; TDS, transdermal system; tol, tolterodine; tro, trospium; UDI, Urogenital Distress Inventory; UI, urinary incontinence; UTI, urinary tract infection; UUI, urge urinary incontinence; VAS, visual analog scale; WMD, weighted mean difference; WOCN, Wound Ostomy Continence Nurses’ Society; (X), trial was of cross-over design.

* Corresponding author. Tel. +44 114 2712559; Fax: +44 114 2798318.

E-mail address: c.r.chapple@sheffield.ac.uk (C. Chapple).
1. Introduction

Overactive bladder (OAB), otherwise known as the urgency frequency syndrome, is a symptom complex defined by the International Continence Society (ICS) as ‘urgency, with or without urge incontinence, usually with frequency and nocturia’ [1]. This is distinct from the urodynamic diagnosis of detrusor overactivity (DO), which refers to an involuntary rise in detrusor pressure during filling of the bladder in a laboratory situation in a conscious co-operative patient [1].

Non-surgical treatment is the mainstay of therapy for OAB and available options include bladder training, biofeedback, medication, and a combination of these options. The principal pharmacological treatment utilised to improve the symptoms of OAB is based on muscarinic receptor antagonism (antimuscarinics). To date no proof of concept studies for other oral pharmaco-therapeutic mechanisms have shown any significant efficacy. The mode of action of antimuscarinics, traditionally considered to be on muscarinic receptors lying within the detrusor muscle, has become increasingly controversial. At licensed doses, antimuscarinic treatments do not inhibit the normal voiding phase of the miciturition cycle, whilst they do alter bladder sensation during filling as evidenced by an improvement in filling symptoms (urgency, frequency, nocturia and incontinence) and bladder capacity. This has led to a recent hypothesis suggesting that antimuscarinic treatments may act via other mechanisms related to the afferent as opposed to the efferent system.

This systematic review was carried out to assess the safety, tolerability and efficacy of antimuscarinic treatments for OAB and DO. Further objectives of the review were to: (1) consider the effects of antimuscarinics on outcomes such as quality of life (QoL), which are important to patients and (2) assess whether there are differences between individual antimuscarinic drugs that are currently being used to treat OAB. These objectives were included to address criticism of a previous Cochrane review of pharmacological therapies for OAB [2].

The Cochrane review was criticised because the cover statement and conclusions do not appear to reflect the results of the review [3–5]. In particular, the outcome measures reported by Herbison and colleagues were ‘not necessarily the most pertinent outcomes to patients with OAB’ [6]. Although important factors such as QoL were mentioned in the review, these were not explored further in any detail. To address this criticism, we have analysed all reported QoL data in included trials and carried out meta-analyses of these data where possible. These analyses are described in detail in a separate publication [42].

In addition, the Cochrane review did not attempt to differentiate between individual antimuscarinic drugs. The authors chose to ‘lump’ the drugs together and evaluate the effects of the class, rather than to ‘split’ the drugs and assess any variation in effect between drugs. Due to the heterogeneity evident in the meta-analyses of some outcomes such as withdrawals and adverse events it was suggested that the drugs might have different profiles, yet potential differences were not explored further. In addition, a number of active controlled trials that have attempted to differentiate between OAB treatments have been published, but these were not evaluated by Herbison and colleagues [2]. In order to assess whether there are differences between individual antimuscarinic drugs, our methodology was distinctly different from that employed in the Cochrane review. We included active controlled trials in addition to placebo controlled trials and reviewed individual antimuscarinic drugs compared with either placebo or active controls. Our meta-analyses adopted a ‘splitting’ approach in order to assess any variation in effect between drugs.

2. Methods

2.1. Searching


A rigorous process was followed to minimise the risk of looking a publication. A team of reviewers independently determined the eligibility of each publication by applying a set of criteria (Table 1). Two different reviewers considered every publication and discrepancies were resolved through discussion. Cited references from included trials and reviews of similar trials were also searched. Many studies were reported in more than one publication and data from all such publications were included.

2.2. Data extraction

Reviewers extracted data from eligible publications in parallel. MS Access® was used to store extracted data and identify possible analyses. A third reviewer checked the resulting extractions and the team resolved any discrepancies.

2.3. Outcome measures

The primary outcome measures of the review were ‘total withdrawals’ and ‘any adverse event’. These outcomes, together with secondary outcomes, are shown in Table 2.
2.4. Quality assessment
We assessed the methodological quality of publications by recording the methods of generation of random allocation, concealment of allocation at randomisation, blinding of trial participants and investigators, completeness of treatment and follow-up and methods used to compensate for missing outcome data. Additionally, reviewers recorded whether the safety and efficacy analyses were carried out according to the ITT (intention to treat) or PP (per protocol) method and whether the trial was published as a full publication or an abstract.

2.5. Data analysis
Meta-analyses were carried out using MS Excel® and MetaView 5.0 to estimate the effect of each antimuscarinic. Data were

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Included</th>
<th>Excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>Age: ≥18 years</td>
<td>Age: &lt; 18 years</td>
</tr>
<tr>
<td>Race: any</td>
<td>Qualifying event/disease/factors:</td>
<td>Qualifying event/disease/factors:</td>
</tr>
<tr>
<td>Idiopathic OAB/UI/DO</td>
<td>&gt;50% of patients with DO consequent upon neurogenic pathology</td>
<td>&gt;50% of patients with DO consequent upon neurogenic pathology</td>
</tr>
<tr>
<td>MI (urge predominant incontinence)</td>
<td>UUI</td>
<td>SUI</td>
</tr>
<tr>
<td>≤50% of patients with BOO or previous LUTD surgery</td>
<td>Any severity of disease at baseline</td>
<td>MI (stress predominant incontinence)</td>
</tr>
<tr>
<td>≥50% of patients with BOO or previous LUTD surgery</td>
<td>UUI</td>
<td></td>
</tr>
<tr>
<td>Perspective of study</td>
<td>Prospective (Concurrent)</td>
<td>Retrospective (Non-concurrent, historical)</td>
</tr>
<tr>
<td>Type of study</td>
<td>RCT</td>
<td>Non-randomised CCT</td>
</tr>
<tr>
<td>(Blinded RCTs only for clinical efficacy data, open-label RCTs and blinded RCTs for safety and tolerability data)</td>
<td>Open-label follow-up of RCT</td>
<td></td>
</tr>
<tr>
<td>Cross-over trials with a wash-out period between treatments</td>
<td>Cohort</td>
<td></td>
</tr>
<tr>
<td>Language</td>
<td>All</td>
<td>Observational</td>
</tr>
<tr>
<td>Trial length</td>
<td>≥2 weeks</td>
<td>Case control</td>
</tr>
<tr>
<td>Sample size</td>
<td>Any</td>
<td>Case study</td>
</tr>
<tr>
<td>Intervention/treatments:</td>
<td>Oral monotherapy with antimuscarinics (UK licensed doses):</td>
<td>Oral monotherapy with antimuscarinics:</td>
</tr>
<tr>
<td>Darifenacin (Enablex®) [all doses]</td>
<td>Flavoxate (Urispas®)</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin IR (Cystrin®, Ditropan®) [2.5–5 mg bid, tid, 5 mg qid]</td>
<td>Fesoterodine</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin ER (Ditropan XL®) [5 mg od, 10 mg od, 15 mg od, 20 mg od]</td>
<td>Propantheline (Pro-Banthine®)</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin TDS/Oxytrol® [3.9 mg od]</td>
<td>All intravesical antimuscarinic formulations</td>
<td></td>
</tr>
<tr>
<td>Propiverine IR (Detrumorm®) [15 mg od, bid, tid, qid]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propiverine ER [all doses]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solifenacin (Vesicare®) [all doses]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolterodine IR (Detrusitol®, Detrol®)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1 mg bid, 2 mg bid]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolterodine ER (Detrusitol XL®, Detrol LA®) [2 mg od, 4 mg od]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trosprim (Regurin®) [20 mg bid]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination therapy with one of the included drugs and a non-pharmacological treatment if all the treatment arms receive the same non-pharmacological treatment.</td>
<td>Combination therapy with antimuscarinics, and an alpha-blocker.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control intervention/treatments:</td>
<td>Placebo or any of the included drugs</td>
<td>Combination therapy with one of the included drugs and a non-pharmacological treatment when not all the treatment arms receive the same non-pharmacological treatment.</td>
</tr>
<tr>
<td>Included trial outcomes</td>
<td>Any</td>
<td>Non-pharmacological treatment (bladder training, electronic stimulation, physiotherapy)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Usual care</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No intervention</td>
</tr>
<tr>
<td>BOO = bladder outlet obstruction, CCT = controlled clinical trial, DO = detrusor overactivity, ER = extended release, IR = immediate release, LA = extended release, LUTD = lower urinary tract disease, MI = mixed incontinence, OAB = overactive bladder, RCT = randomised controlled trial, SUI = stress urinary incontinence, TDS = transdermal system, UI = urinary incontinence, UUI = urge urinary incontinence, XL = extended release.</td>
<td></td>
<td>None</td>
</tr>
</tbody>
</table>
combined in the most appropriate statistical model (fixed or random effect) using relative risk for dichotomous outcomes and weighted mean differences for continuous outcomes. Further detail of these approaches can be found in the Cochrane Collaboration Handbook [7]. The method for combining data was applied not only to multiple trials, but also to single studies with a valid outcome measure. This approach was adopted due to the limited number of head-to-head studies included in the review. In such instances, trials reported as inadequately powered were noted. Each trial with more than two treatment arms was treated as three separate trials (e.g. intervention 1 versus placebo, intervention 2 versus placebo, intervention 1 versus intervention 2).

Four sensitivity analyses were planned a priori to investigate the effect of disease severity, type of disease, prior response to antimuscarinic therapy and crossover trials on treatment effect.

Statistically significant differences between interventions were extracted from all trial reports regardless of whether the data were suitable for meta-analysis or not. The data that were not suitable for meta-analysis were not ignored, but were compared with the results of the meta-analyses; any differences were reviewed.

### 3. Results

**Trial flow:** Of 11,663 references retrieved, 438 full text publications were ordered for more detailed evaluation (see Fig. 1). Fifty-six trials were included (see Appendix A) and 52 trials reported data suitable for meta-analysis.

**Trial characteristics:** A summary of the included trials is presented in Tables 3 and 4. Thirty-two trials were placebo controlled and twenty-four trials were active controlled. Placebo controlled trials for darifenacin, propiverine, oxybutynin IR and TDS, solifenacin, tolterodine IR and ER and trospium were included. No trials comparing oxybutynin ER with placebo met the inclusion criteria of the review. No trials comparing oxybutynin ER with placebo met the inclusion criteria of the review.

The large majority of trials were parallel in design, whereas, only six trials were crossover in design. Included trials ranged from two weeks to 18 months with the majority of trials being 4 or 12 weeks long. Trials included a minimum of 30 patients and a maximum of 1529 patients. Two trials included over 1000 patients. The majority of trials included patients with a mean age between 50 and 65 years (n = 50). Four trials included older patients with a mean age greater than 65 years [8–11]. Two trials included patients with a mean age less than 50 years [12,13]. Seventeen trials were published in abstract format only and 39 trials were published as full publications.

All trials were reported to be double blind apart from two trials [11,14]. Twenty-four trials used modified

<table>
<thead>
<tr>
<th>Tolerability</th>
<th>Total withdrawals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Withdrawals due to adverse events</td>
<td></td>
</tr>
<tr>
<td>Withdrawals due to death</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Safety</th>
<th>Any adverse event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blurred vision, confusion, dizziness, palpitations, tremor, vertigo</td>
<td></td>
</tr>
<tr>
<td>Constipation, diarrhoea, dry mouth (any severity), dry mouth (mild), dry mouth (moderate), dry mouth (severe), dry mouth (mild/moderate), dry mouth (moderate/severe), dyspepsia, nausea, vomiting</td>
<td></td>
</tr>
<tr>
<td>Fatigue, headache, insomnia, somnolence, increased sweating, urinary retention, UTI, erythema, pruritus</td>
<td></td>
</tr>
</tbody>
</table>

| Efficacy | |
| Change from baseline in the number of urgency episodes/24 hrs |
| Change from baseline in the number of pads used per day |
| Change from baseline in the number of daytime incontinent episodes/24 hrs |
| Change from baseline in the number of nocturnal incontinence episodes/24 hrs |
| Number of patients returned to continence at trial endpoint (recording no incontinence episodes on last voiding diary entry) |
| Change from baseline in the number of nocturnal awakenings related to overactive bladder/24 hrs |
| Number of patients achieving normal micturition frequency (≤7/8 micturitions/24 hrs) at trial endpoint |
| Change from baseline in the volume of urine voided per micturition (ml) |
| Change from baseline in the number of micturitions/24 hrs |
| Change from baseline in maximum cystometric capacity (ml) |

| QoL/PROs | |
| Generic: SF-36, SF-12 |
| Disease Specific: Incontinence Impact Questionnaire (IIQ), KHQ, UDI |
| Number of patients reporting improvement in disease |
| VAS scores of improvement in disease |

Primary outcome measures are in bold.

KHQ = King’s Health Questionnaire, UDI = Urogenital Distress Inventory, UTI = urinary tract infection, VAS = visual analog scale, SF-36 = Short-form 36, SF-12 = Short-form 12.
ITT analyses for efficacy data; 24 trials used the per-protocol method and eight trials did not describe the efficacy analysis methodology clearly. The majority of trials \((n = 32)\) included all randomised patients for the analysis of safety data. Sixteen trials used the PP method for safety analyses and eight trials did not report the methodology clearly.

### 3.1. Tolerability

#### 3.1.1. Total withdrawals (any cause)

All evaluated antimuscarinic formulations (darifenacin, propiverine IR and ER, solifenacin, tolterodine IR and ER, trospium) apart from oxybutynin IR were found to be well tolerated compared with placebo (Fig. 2, Table 5). Compared with patients receiving placebo, patients treated with oxybutynin IR (8.8–15 mg/day) have a 40% greater risk of withdrawing from treatment. Unexpectedly, there was a 29% lower incidence of total withdrawals in patients treated with tolterodine ER than in patients receiving placebo; this finding just reached statistical significance.

Statistically significant differences in active comparisons favoured oxybutynin ER, tolterodine IR and tolterodine ER compared with oxybutynin IR. For active comparisons of all other antimuscarinics, no other significant differences in total withdrawal rates were found (Table 6).

#### 3.1.2. Withdrawals due to adverse events and death

The only drugs associated with excess withdrawals due to adverse events were oxybutynin IR and oxybutynin TDS (see Tables 5 and 6). Tolterodine IR was found to cause fewer withdrawals due to adverse events than oxybutynin IR and tolterodine ER caused fewer than both oxybutynin IR and oxybutynin TDS (Table 6).
<table>
<thead>
<tr>
<th>Trial</th>
<th>Dosage</th>
<th>No. of randomised patients</th>
<th>Trial length (wks)</th>
<th>Baseline characteristics</th>
<th>Included population</th>
<th>Percentage of patients with prior therapy (% poor responders)</th>
<th>Mean number of incontinence episodes/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darifenacin</td>
<td>3.75 mg, 7.5 mg, 15 mg</td>
<td>561</td>
<td>12</td>
<td>DO (primarily idiopathic, neurogenic)</td>
<td>21% (NR)</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Haab 2004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hill 2004 (A)</td>
<td>7.5 mg, 15 mg, 30 mg</td>
<td>439</td>
<td>12</td>
<td>OAB</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Steers 2004 (A)</td>
<td>From 7.5 mg (titrated)</td>
<td>395</td>
<td>12</td>
<td>OAB (UI)</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Cardozo 2003b (A)</td>
<td>30 mg</td>
<td>72</td>
<td>2</td>
<td>OAB (UI)</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Propiverine</td>
<td>45 mg</td>
<td>107</td>
<td>4</td>
<td>Urgency or UUI or MI</td>
<td>NR</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Halaska 1994 (A)</td>
<td>45 mg</td>
<td>93</td>
<td>4</td>
<td>Urgency or UUI</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin IR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burgio 2001</td>
<td>7.5 mg</td>
<td>197</td>
<td>8</td>
<td>UUI</td>
<td>36% (NR)</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Miller 2002 (A)</td>
<td>7.5 mg</td>
<td>110</td>
<td>8</td>
<td>UUI</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Szonyi 1995 (A)</td>
<td>5 mg</td>
<td>60</td>
<td>6</td>
<td>DO</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Moore 1990 (X)</td>
<td>9 mg</td>
<td>53</td>
<td>Unclear</td>
<td>DO (idiopathic)</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Kirschner-Hermanns 1997 (A, X)</td>
<td>10 mg</td>
<td>36</td>
<td>3</td>
<td>Incontinence</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Brambila 2000 (X)</td>
<td>15 mg</td>
<td>44</td>
<td>6</td>
<td>Urgency and UUI</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Moisy 1980 (X)</td>
<td>15 mg</td>
<td>30</td>
<td>4</td>
<td>DO (idiopathic)</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Riva 1984 (X)</td>
<td>15 mg</td>
<td>30</td>
<td>3</td>
<td>UUI or OAB</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Tapp 1990 (X)</td>
<td>20 mg</td>
<td>37</td>
<td>2</td>
<td>DO</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Thuroff 1991†</td>
<td>15 mg</td>
<td>169</td>
<td>4</td>
<td>UUI</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin TDS Dmochowski 2002</td>
<td>3.9 mg</td>
<td>520</td>
<td>12</td>
<td>DO (primarily idiopathic, neurogenic) and UUI/MI</td>
<td>23% (NR)</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Solifenacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uchida 2002 (A)</td>
<td>2.5 mg, 5 mg, 10 mg, 20 mg</td>
<td>265</td>
<td>4</td>
<td>OAB</td>
<td>NR</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Cardozo 2003 (A)</td>
<td>5 mg, 10 mg</td>
<td>907</td>
<td>12</td>
<td>OAB</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Tolterodine ER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khullar 2004</td>
<td>4 mg</td>
<td>854</td>
<td>8</td>
<td>MI</td>
<td>34% (72%)</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Malone-Lee 2002 (A)</td>
<td>4 mg</td>
<td>308</td>
<td>12</td>
<td>DO</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Tolterodine IR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abrams 2001 (A)</td>
<td>4 mg</td>
<td>221</td>
<td>12</td>
<td>DO or BOO</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Jacquetin 2001</td>
<td>2 mg, 4 mg</td>
<td>251</td>
<td>4</td>
<td>DO</td>
<td>71% (72%)</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Jonas 1997</td>
<td>2 mg, 4 mg</td>
<td>242</td>
<td>4</td>
<td>DO</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Malone-Lee 2001</td>
<td>2 mg, 4 mg</td>
<td>177</td>
<td>4</td>
<td>OAB</td>
<td>70% (60%)</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Millard 1999</td>
<td>2 mg, 4 mg</td>
<td>316</td>
<td>12</td>
<td>DO</td>
<td>50% (61%)</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Rentzhog 1998</td>
<td>2 mg, 4 mg</td>
<td>81</td>
<td>2</td>
<td>DO</td>
<td>64% (NR)</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Tropium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alloussi 1998</td>
<td>40 mg</td>
<td>309</td>
<td>3</td>
<td>DO</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Cardozo 2000</td>
<td>40 mg</td>
<td>208</td>
<td>3</td>
<td>DO</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Chalihia 1998 (A)</td>
<td>40 mg</td>
<td>76</td>
<td>3</td>
<td>DO</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Rudy 2004 (A)</td>
<td>40 mg</td>
<td>658</td>
<td>12</td>
<td>OAB</td>
<td>NR</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Zimmer 2004</td>
<td>40 mg</td>
<td>523</td>
<td>12</td>
<td>OAB (UI)</td>
<td>54% (NR)</td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

(A) = trial published in abstract form only, (X) = trial was of cross-over design, P = placebo arm also present in the trial, OBJECT = Overactive Bladder: Judging Effective Control and Treatment, OPERA = Overactive Bladder: Performance of Extended Release Agents, ER = extended release, IR = immediate release, TDS = transdermal system, sol = solifenacin, tol = tolterodine, DO = detrusor overactivity, LUT = lower urinary tract, MI = mixed incontinence, OAB = overactive bladder, BOO = bladder outlet obstruction, UUI = urge urinary incontinence, NR = not reported.

* This trial had a second active treatment arm, propantheline 45 mg, not included in this review.

† The trial arm with the highest baseline value is stated.
### Table 4
Summary of included trials (active controlled trials)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Dosage</th>
<th>No. of randomised patients</th>
<th>Trial length (wks)</th>
<th>Baseline characteristics</th>
<th>Included population</th>
<th>Percentage of patients with prior therapy (% poor responders)</th>
<th>Mean number of incontinence episodes/day*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxybutynin ER, Oxybutynin IR</td>
<td>Anderson 1999</td>
<td>From 5 mg, from 5 mg (titrated)</td>
<td>105</td>
<td>2</td>
<td>UI or MI</td>
<td>100% (0%)</td>
<td>4.2</td>
</tr>
<tr>
<td>Oxybutynin ER, Oxybutynin IR</td>
<td>Barkin 2004</td>
<td>From 15 mg, from 15 mg (titrated)</td>
<td>125</td>
<td>6</td>
<td>UI</td>
<td>NR</td>
<td>3.5</td>
</tr>
<tr>
<td>Oxybutynin ER, Oxybutynin IR</td>
<td>Birns 2000</td>
<td>10 mg, 10 mg</td>
<td>130</td>
<td>4</td>
<td>DO (predominantly idiopathic)</td>
<td>100% (0%)</td>
<td>NR</td>
</tr>
<tr>
<td>Oxybutynin ER, Oxybutynin IR</td>
<td>Versi 2000</td>
<td>From 5 mg, from 5 mg (titrated)</td>
<td>226</td>
<td>Titration period + 1 wk</td>
<td>UUI</td>
<td>100% (0%)</td>
<td>3.2</td>
</tr>
<tr>
<td>Oxybutynin ER, Tolterodine ER</td>
<td>OPERA</td>
<td>10 mg, 4 mg</td>
<td>790</td>
<td>12</td>
<td>OAB</td>
<td>48% (NR)</td>
<td>6.2</td>
</tr>
<tr>
<td>Oxybutynin IR, Oxybutynin TDS</td>
<td>Davila 2001</td>
<td>From 5 mg, from 2.6 mg 2/week (titrated)</td>
<td>76</td>
<td>4</td>
<td>UI</td>
<td>100% (0%)</td>
<td>3.4</td>
</tr>
<tr>
<td>Oxybutynin IR, Trospium</td>
<td>Halaska 2003</td>
<td>10 mg, 40 mg</td>
<td>358</td>
<td>52</td>
<td>DO or UUI (primarily idiopathic, neurogenic) or MI</td>
<td>51% (NR)</td>
<td>2.1</td>
</tr>
<tr>
<td>Oxybutynin IR, Tolterodine ER, P</td>
<td>Homina 2003</td>
<td>9 mg, 4 mg</td>
<td>608</td>
<td>12</td>
<td>OAB</td>
<td>25% (53%)</td>
<td>3.1</td>
</tr>
<tr>
<td>Oxybutynin IR, Tolterodine IR</td>
<td>Lee 2002</td>
<td>10 mg, 4 mg</td>
<td>228</td>
<td>8</td>
<td>OAB</td>
<td>32% (47%)</td>
<td>2.6</td>
</tr>
<tr>
<td>Oxybutynin IR, Tolterodine IR</td>
<td>Leung 2002</td>
<td>10 mg, 4 mg</td>
<td>106</td>
<td>10</td>
<td>DO</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Oxybutynin IR, Tolterodine IR</td>
<td>Malone-Lee 2001b</td>
<td>10 mg, 4 mg</td>
<td>379</td>
<td>10</td>
<td>OAB</td>
<td>32% (72%)</td>
<td>2.9</td>
</tr>
<tr>
<td>Oxybutynin IR, Tolterodine IR, P</td>
<td>Abrams 1998</td>
<td>15 mg, 4 mg</td>
<td>293</td>
<td>12</td>
<td>DO (30% post LUT surgery)</td>
<td>60%</td>
<td>3.3</td>
</tr>
<tr>
<td>Oxybutynin TDS, Tolterodine ER, P</td>
<td>Dmochowski 2003</td>
<td>3.9 mg, 4 mg</td>
<td>361</td>
<td>12</td>
<td>OAB and UUI/MI</td>
<td>100% (0%)</td>
<td>5.4</td>
</tr>
<tr>
<td>Propiverine IR, Oxybutynin IR, P</td>
<td>Madersbacher 1999</td>
<td>45 mg, 10 mg</td>
<td>366</td>
<td>4</td>
<td>Urgency or UUI</td>
<td>33% (NR)</td>
<td>NR</td>
</tr>
<tr>
<td>Propiverine IR, Propiverine ER, P</td>
<td>Jünnemann 2004 (A)</td>
<td>30 mg, 30 mg</td>
<td>988</td>
<td>4.6</td>
<td>OAB (basis of diagnosis unclear)</td>
<td>NR</td>
<td>3.4</td>
</tr>
<tr>
<td>Propiverine IR, Tolterodine IR</td>
<td>Jünnemann 2003 (A)</td>
<td>30 mg, 4 mg</td>
<td>302</td>
<td>4</td>
<td>DO (idiopathic)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Solifenacin, Tolterodine IR, P</td>
<td>Chapple 2004</td>
<td>(sol) 2.5 mg, 5 mg, 10 mg, 20 mg, (tol) 4 mg</td>
<td>225</td>
<td>4</td>
<td>DO (idiopathic)</td>
<td>NR</td>
<td>1.7</td>
</tr>
<tr>
<td>Solifenacin, Tolterodine IR, P</td>
<td>Chapple 2004b</td>
<td>(sol) 5 mg, 10 mg, (tol) 4 mg</td>
<td>1081</td>
<td>12</td>
<td>OAB</td>
<td>40% (NR)</td>
<td>2.6</td>
</tr>
<tr>
<td>Tolterodine ER, Tolterodine IR, P</td>
<td>Van Kerrebroeck 2001</td>
<td>4 mg, 4 mg</td>
<td>1529</td>
<td>12</td>
<td>OAB</td>
<td>54% (38%)</td>
<td>3.3</td>
</tr>
<tr>
<td>Tolterodine, Tolterodine IR, P</td>
<td>Jünnemann 2000 (A)</td>
<td>40 mg, 4 mg</td>
<td>234</td>
<td>3</td>
<td>DO</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

(A) = trial published in abstract form only, (X) = trial was of cross-over design, P = placebo arm also present in the trial, OBJECT = Overactive Bladder: Judging Effective Control and Treatment, OPERA = Overactive Bladder: Performance of Extended Release Agents, ER = extended release, IR = immediate release, TDS = transdermal system, sol = solifenacin, tol = tolterodine, DO = detrusor overactivity, LUT = lower urinary tract, MI = mixed incontinence, OAB = overactive bladder, BOO = bladder outlet obstruction, UUI = urge urinary incontinence, NR = not reported.

*The trial arm with the highest baseline value is stated.
Table 5
Tolerability of antimuscarinics compared to placebo: results from meta-analyses

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Dar 7.5</th>
<th>Dar 15</th>
<th>Dar (7.5)</th>
<th>Dar IR 5-7.5</th>
<th>Dar IR 8.8-15</th>
<th>Pro IR 30</th>
<th>Pro IR 45</th>
<th>Pro ER 30</th>
<th>Sol 5</th>
<th>Sol 10</th>
<th>Tol IR 2</th>
<th>Tol IR 4</th>
<th>Tol ER 4</th>
<th>Tro 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total withdrawals</td>
<td>n=393</td>
<td>n=279</td>
<td>n=395</td>
<td>n=60</td>
<td>n=60</td>
<td>n=1242</td>
<td>n=221</td>
<td>n=1242</td>
<td>n=60</td>
<td>n=60</td>
<td>n=261</td>
<td>n=1363</td>
<td>n=1215</td>
<td>n=832</td>
</tr>
<tr>
<td>(0.26, 1.35)#</td>
<td>(0.47, 2.45)#</td>
<td>(0.54, 1.97)#</td>
<td>(0.59, 4.33)#</td>
<td>(1.06, 1.84)#</td>
<td>(0.58, 2.98)#</td>
<td>(0.50, 1.24)#</td>
<td>(0.41, 1.50)#</td>
<td>(0.56, 3.12)#</td>
<td>(0.71, 1.30)#</td>
<td>(0.51, 0.99)#</td>
<td>(0.73, 1.37)#</td>
<td>(0.55, 1.17)#</td>
<td>(0.66, 1.60)#</td>
<td></td>
</tr>
<tr>
<td>Withdrawals</td>
<td>n=393</td>
<td>n=60</td>
<td>n=100</td>
<td>n=60</td>
<td>n=60</td>
<td>n=956</td>
<td>n=597</td>
<td>n=593</td>
<td>n=60</td>
<td>n=60</td>
<td>n=261</td>
<td>n=1363</td>
<td>n=1215</td>
<td>n=832</td>
</tr>
<tr>
<td>(0.70, 8.09)#</td>
<td>(0.27, 6.1)#</td>
<td>(0.76, 8.09)#</td>
<td>(0.27, 6.1)#</td>
<td>(0.76, 8.09)#</td>
<td>(0.27, 6.1)#</td>
<td>(0.76, 8.09)#</td>
<td>(0.27, 6.1)#</td>
<td>(0.76, 8.09)#</td>
<td>(0.27, 6.1)#</td>
<td>(0.76, 8.09)#</td>
<td>(0.27, 6.1)#</td>
<td>(0.76, 8.09)#</td>
<td>(0.27, 6.1)#</td>
<td>(0.76, 8.09)#</td>
</tr>
<tr>
<td>Data included from a single trial.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data presented are RR (95% CI) compared with placebo where n = the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis, oxy = oxybutynin, tol = tolterodine, sol = solifenacin, pro = propiverine, tro = trospium, dar = darifenacin, IR = immediate release, ER = extended release, (t 7.5) = titrated dose from 7.5 mg/day.

Table 6
Tolerability of antimuscarinics compared to active control: results from meta-analyses

| Outcome               | Oxy IR 10 vs Tro 40 | Oxy IR 10 vs Pro IR 45 | Oxy IR 10 vs Oxy ER 10 | Oxy IR (t 15) vs Oxy ER (t 15) | Oxy IR (t) vs Oxy TDS (t) | Pro IR 30 vs Pro ER 30 | Pro IR 45 vs Pro ER 30 | Pro ER 30 vs Tol ER 4 | Tol IR 2 vs Oxy IR 10 | Tol IR 4 vs Oxy IR 10 | Tol IR 4 vs Tol ER 4 | Tol IR 4 vs Pro 30 | Tol IR 4 vs Sol 5 | Tol IR 4 vs Tol ER 4 | Tol IR 4 vs Tol ER 39 | Tol IR 4 vs Tol ER 39 |
|-----------------------|---------------------|------------------------|------------------------|-------------------------------|-------------------------|------------------------|------------------------|-----------------------|---------------------|---------------------|---------------------|------------------|---------------|----------------|------------------|------------------|------------------|
| Total withdrawals     | n=357  | n=294  | n=310  | n=125  | n=331  | n=76   | n=712  | n=712  | n=378  | n=619  | n=619  | n=483  | n=790  | n=244  | n=483  | n=790  | n=790  |
| (0.71, 1.95)#        | (0.46, 1.62)# | (0.34, 1.55)# | (0.53, 2.12)# | (0.56, 15.4)# | (0.53, 2.12)# | (0.56, 15.4)# | (0.53, 2.12)# | (0.56, 15.4)# | (0.53, 2.12)# | (0.56, 15.4)# | (0.53, 2.12)# | (0.56, 15.4)# | (0.53, 2.12)# | (0.56, 15.4)# | (0.53, 2.12)# | (0.56, 15.4)# | (0.53, 2.12)# | (0.56, 15.4)# |
| Withdrawals           | n=357  | n=294  | n=310  | n=125  | n=331  | n=76   | n=712  | n=712  | n=378  | n=619  | n=619  | n=483  | n=790  | n=244  | n=483  | n=790  | n=790  |
| (0.76, 3.65)#        | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# | (0.55, 2.47)# |
| Data included from a single trial. |

Data presented are RR (95% CI) compared with placebo where n = the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis, oxy = oxybutynin, tol = tolterodine, sol = solifenacin, pro = propiverine, tro = trospium, IR = immediate release, ER = extended release, TDS = transdermal system, (t) = titrated, (t 5) = titrated from 5 mg/day, (t 15) = titrated from 15 mg/day.

Data included from a single trial.

Data included from a single trial reported to be powered to show a significant difference between interventions.
Table 7
Adverse events of antimuscarinics compared with placebo: results from meta-analyses

<table>
<thead>
<tr>
<th>Intervention and Daily Dose (mg/day)</th>
<th>Dry Mouth</th>
<th>Urinary Urgency</th>
<th>Urinary Frequency</th>
<th>Urinary Incontinence</th>
<th>Dry Nocturia</th>
<th>UTI</th>
<th>elsewhere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate release (IR)</td>
<td>(0.23, 0.57)</td>
<td>(0.75, 0.91)</td>
<td>(0.60, 0.85)</td>
<td>(0.60, 0.82)</td>
<td>(0.53, 0.70)</td>
<td>(0.67, 0.81)</td>
<td>(0.60, 0.85)</td>
</tr>
<tr>
<td>Extended release (ER)</td>
<td>(0.23, 0.57)</td>
<td>(0.75, 0.91)</td>
<td>(0.60, 0.85)</td>
<td>(0.60, 0.82)</td>
<td>(0.53, 0.70)</td>
<td>(0.67, 0.81)</td>
<td>(0.60, 0.85)</td>
</tr>
<tr>
<td>Transdermal system (TDS)</td>
<td>(0.23, 0.57)</td>
<td>(0.75, 0.91)</td>
<td>(0.60, 0.85)</td>
<td>(0.60, 0.82)</td>
<td>(0.53, 0.70)</td>
<td>(0.67, 0.81)</td>
<td>(0.60, 0.85)</td>
</tr>
</tbody>
</table>

Data presented are relative risk ratios (95%CI) compared with placebo where n = the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis. * = trial definition. # = any adverse event, ASAE = any serious adverse event, UTI = urinary tract infection. No data suitable for meta-analysis for any intervention for tremor.

Data included from a single trial reported to be powered to show a significant difference between interventions.
Table 8

Adverse events of antimuscarinics compared to active control: results from meta-analyses

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Interventions and Daily Dose (mg/day)</th>
<th>Data presented are relative risk ratios (95%CI) compared with placebo where ( n ) = the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis. * = trial definition, AE = any adverse event, ASAE = any serious adverse event, UTI = urinary tract infection, oxy = oxybutynin, tol = tolterodine, sol = solifenacin, pro = propiverine, tro = trospium, IR = immediate release, ER = extended release, TDS = transdermal system, (t) = titrated dose, (t 5) = titrated from 5 mg/day, (t 15) = titrated from 15 mg/day. No data suitable for meta-analysis for confusion, pruritus, tremor, vertigo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac.*</td>
<td>n=357 vs 1.28 (95%CI: 1.52, 1.97) vs placebo</td>
<td>n=328 vs 1.06 (95%CI: 0.93, 1.35) vs placebo</td>
</tr>
<tr>
<td>AE*</td>
<td>n=328 (38.5%, 1.20) vs placebo</td>
<td>n=328 (38.5%, 1.20) vs placebo</td>
</tr>
<tr>
<td>Blurred Vision</td>
<td>n=357 vs 1.65 (95%CI: 0.57, 4.79) vs placebo</td>
<td>n=328 vs 0.70 (95%CI: 0.57, 4.79) vs placebo</td>
</tr>
<tr>
<td>Constipation</td>
<td>n=357 vs 1.30 (95%CI: 0.42, 4.04) vs placebo</td>
<td>n=328 vs 1.79 (95%CI: 0.42, 4.04) vs placebo</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>n=357 vs 1.36 (95%CI: 0.16, 1.27) vs placebo</td>
<td>n=328 vs 2.63 (95%CI: 0.16, 1.27) vs placebo</td>
</tr>
<tr>
<td>Dizziness</td>
<td>n=357 vs 1.53 (95%CI: 0.70, 4.58) vs placebo</td>
<td>n=328 vs 1.26 (95%CI: 0.70, 4.58) vs placebo</td>
</tr>
<tr>
<td>Dry Mouth</td>
<td>n=357 vs 1.35 (95%CI: 0.42, 3.25) vs placebo</td>
<td>n=328 vs 1.35 (95%CI: 0.42, 3.25) vs placebo</td>
</tr>
<tr>
<td>Dryness</td>
<td>n=357 vs 1.05 (95%CI: 0.35, 2.95) vs placebo</td>
<td>n=328 vs 1.05 (95%CI: 0.35, 2.95) vs placebo</td>
</tr>
<tr>
<td>Erythema</td>
<td>n=79 vs 0.94 (95%CI: 0.32, 1.60) vs placebo</td>
<td>n=79 vs 0.94 (95%CI: 0.32, 1.60) vs placebo</td>
</tr>
<tr>
<td>Fatigue</td>
<td>n=357 vs 1.26 (95%CI: 0.90, 2.20) vs placebo</td>
<td>n=328 vs 1.26 (95%CI: 0.90, 2.20) vs placebo</td>
</tr>
<tr>
<td>Headache</td>
<td>n=357 vs 1.17 (95%CI: 0.33, 1.29) vs placebo</td>
<td>n=328 vs 1.17 (95%CI: 0.33, 1.29) vs placebo</td>
</tr>
<tr>
<td>Increased Sweating</td>
<td>n=357 vs 0.59 (95%CI: 0.13, 2.60) vs placebo</td>
<td>n=328 vs 0.59 (95%CI: 0.13, 2.60) vs placebo</td>
</tr>
<tr>
<td>Insomnia</td>
<td>n=357 vs 1.65 (95%CI: 1.04, 2.51) vs placebo</td>
<td>n=328 vs 1.65 (95%CI: 1.04, 2.51) vs placebo</td>
</tr>
<tr>
<td>Irritation</td>
<td>n=357 vs 1.33 (95%CI: 0.41, 2.79) vs placebo</td>
<td>n=328 vs 1.33 (95%CI: 0.41, 2.79) vs placebo</td>
</tr>
<tr>
<td>Nausea</td>
<td>n=357 vs 1.44 (95%CI: 0.35, 2.45) vs placebo</td>
<td>n=328 vs 1.44 (95%CI: 0.35, 2.45) vs placebo</td>
</tr>
<tr>
<td>Palpitations</td>
<td>n=357 vs 1.17 (95%CI: 0.67, 2.07) vs placebo</td>
<td>n=328 vs 1.17 (95%CI: 0.67, 2.07) vs placebo</td>
</tr>
<tr>
<td>Somnolence</td>
<td>n=357 vs 1.05 (95%CI: 0.41, 2.07) vs placebo</td>
<td>n=328 vs 1.05 (95%CI: 0.41, 2.07) vs placebo</td>
</tr>
<tr>
<td>Urinary</td>
<td>n=357 vs 1.44 (95%CI: 0.70, 2.97) vs placebo</td>
<td>n=328 vs 1.44 (95%CI: 0.70, 2.97) vs placebo</td>
</tr>
<tr>
<td>Vomiting</td>
<td>n=357 vs 0.90 (95%CI: 0.46, 1.57) vs placebo</td>
<td>n=328 vs 0.90 (95%CI: 0.46, 1.57) vs placebo</td>
</tr>
</tbody>
</table>

Data presented are relative risk ratios (95%CI) compared with placebo where \( n \) = the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis. * = trial definition, AE = any adverse event, ASAE = any serious adverse event, UTI = urinary tract infection, oxy = oxybutynin, tol = tolterodine, sol = solifenacin, pro = propiverine, tro = trospium, IR = immediate release, ER = extended release, TDS = transdermal system, (t) = titrated dose, (t 5) = titrated from 5 mg/day, (t 15) = titrated from 15 mg/day. No data suitable for meta-analysis for confusion, pruritus, tremor, vertigo.

*Data included from a single trial.

Data included from a single trial reported to be powered to show a significant difference between interventions.
Although the finding just failed to reach statistical significance, propiverine IR 30 mg/day was associated with more than seven times the risk of withdrawal due to adverse events compared with placebo. No drug was associated with a significantly increased risk of death.

3.2. Safety
This review found the antimuscarinics as a class to be safe. Dry mouth was the most commonly reported adverse event and no drug was associated with an increase in any serious adverse event. There were differences between the antimuscarinics in the rate and type of adverse event.

3.2.1. Any adverse event
Each antimuscarinic was found to have a slightly different safety profile (Tables 7 and 8). Darifenacin (7.5 mg/day, 15 mg/day), and oxybutynin IR (8.8–15 mg/day) were associated with a greater incidence of any adverse event than placebo (Fig. 3). Propiverine IR 30 mg/day was associated with a greater incidence of any adverse event than placebo, but this difference was not found for propiverine IR at the higher dose of 45 mg/day. We did not find this difference for oxybutynin TDS, solifenacin, tolterodine IR, tolterodine ER and trospium.

Oxybutynin was associated with an excess of any adverse event compared with two other antimuscarinic formulations; an excess of any adverse event was found for oxybutynin ER (compared with tolterodine IR) and oxybutynin IR (compared with tolterodine IR and trospium) (Table 8).

3.2.2. Dry mouth
The most commonly reported adverse event for all the antimuscarinics was dry mouth. The majority of antimuscarinic formulations were found to cause a significant increase in the incidence of dry mouth compared with placebo (Table 9). Three antimuscarinic formulations (Oxybutynin TDS, oxybutynin IR 5–7.5 mg/day, propiverine IR 45 mg/day) were not associated with an increase in dry mouth compared with placebo, although the numbers of patients eligible for the analyses of the last two of these drugs were low (57 and 98 respectively). Oxybutynin IR was found to be associated with a greater incidence of dry mouth compared with oxybutynin ER, oxybutynin TDS, propiverine IR, tolterodine ER, tolterodine IR and trospium (Table 10).

---

**Fig. 3.** Forest plot of relative risk of any adverse event.

**ANY ADVERSE EVENT REPORTED IN PLACEBO CONTROLLED TRIALS**

<table>
<thead>
<tr>
<th>ANTIMUSCARINIC</th>
<th>ACTIVE</th>
<th>PLACEBO</th>
<th>RELATIVE RISK (95% CI)</th>
<th>RELATIVE RISK (95% CI)</th>
<th>MODEL (FIXED/RANDOM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darifenacin 7.5 mg/day</td>
<td>182/337</td>
<td>129/273</td>
<td>1.24 (1.05, 1.47)</td>
<td>Fixed</td>
<td></td>
</tr>
<tr>
<td>Darifenacin 15 mg/day</td>
<td>134/222</td>
<td>129/273</td>
<td>1.35 (1.14, 1.60)</td>
<td>Fixed</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin IR 8.8–15 mg/day</td>
<td>356/438</td>
<td>136/237</td>
<td>1.39 (1.12, 1.72)</td>
<td>Fixed</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin TDS 3.9 mg/day</td>
<td>23/121</td>
<td>14/117</td>
<td>1.59 (0.96, 2.53)</td>
<td>Fixed</td>
<td></td>
</tr>
<tr>
<td>Propiverine IR 30 mg/day</td>
<td>152/385</td>
<td>41/202</td>
<td>1.90 (1.40, 2.56)</td>
<td>Fixed</td>
<td></td>
</tr>
<tr>
<td>Propiverine IR 45 mg/day</td>
<td>97/198</td>
<td>38/121</td>
<td>0.72 (0.12, 4.32)</td>
<td>Random</td>
<td></td>
</tr>
<tr>
<td>Propiverine ER 30 mg/day</td>
<td>134/391</td>
<td>41/202</td>
<td>1.69 (1.24, 2.29)</td>
<td>Fixed</td>
<td></td>
</tr>
<tr>
<td>Solifenacin 5 mg/day</td>
<td>45/69</td>
<td>35/91</td>
<td>1.35 (0.81, 2.25)</td>
<td>Random</td>
<td></td>
</tr>
<tr>
<td>Solifenacin 10 mg/day</td>
<td>44/86</td>
<td>35/91</td>
<td>1.40 (0.76, 2.56)</td>
<td>Random</td>
<td></td>
</tr>
<tr>
<td>Tolterodine IR 2 mg/day</td>
<td>205/365</td>
<td>116/215</td>
<td>1.00 (0.87, 1.16)</td>
<td>Fixed</td>
<td></td>
</tr>
<tr>
<td>Tolterodine IR 4 mg/day</td>
<td>553/908</td>
<td>259/517</td>
<td>1.12 (0.98, 1.28)</td>
<td>Random</td>
<td></td>
</tr>
<tr>
<td>Tolterodine ER 4 mg/day</td>
<td>250/692</td>
<td>119/402</td>
<td>1.40 (0.84, 2.33)</td>
<td>Random</td>
<td></td>
</tr>
<tr>
<td>Trospium 40 mg/day</td>
<td>137/380</td>
<td>91/282</td>
<td>1.37 (0.91, 2.07)</td>
<td>Random</td>
<td></td>
</tr>
</tbody>
</table>
Table 9

| Intervention and Daily Dose | Dry mouth severity of antimuscarinics compared with placebo: results from meta-analyses | Data presented are relative risk ratios (95% CI) compared with placebo where \( n \) is the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis. oxy = oxybutynin, tol = tolterodine, sol = solifenacin, pro = propiverine, tro = trospium, dar = darifenacin, IR = immediate release, ER = extended release.

# Data included from a single trial.

## Data included from a single trial reported to be powered to show a significant difference between interventions.

Table 10

| Intervention and Daily Dose | Dry mouth severity of antimuscarinics compared to active control: results from meta-analyses | Data presented are relative risk ratios (95% CI) compared with placebo where \( n \) is the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis. oxy = oxybutynin, tol = tolterodine, sol = solifenacin, pro = propiverine, tro = trospium, IR = immediate release, ER = extended release, TDS = transdermal system, (t 7.5) = titrated from 7.5 mg/day.

# Data included from a single trial.

## Data included from a single trial reported to be powered to show a significant difference between interventions.
**Table 11**

Efficacy of antimuscarinics compared to placebo: results from meta-analyses

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Intervention and Daily Dose (mg/day)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dar 7.5</td>
<td>Dar 15</td>
<td>Dar (t 7.5)</td>
<td>Oxy JR 5-7.5</td>
<td>Oxy JR 8-15</td>
<td>Oxy TDS 3.9</td>
<td>Pro JR 30</td>
<td>Pro JR 45</td>
<td>Pro ER 30</td>
<td>Sol 5</td>
<td>Sol 10</td>
<td>Tad JR 2</td>
<td>Tad JR 4</td>
<td>Tad ER 4</td>
</tr>
<tr>
<td>CUE*</td>
<td>n=327-0.72</td>
<td>n=95-0.55</td>
<td>n=194-0.97</td>
<td>n=202-1.44</td>
<td>n=560-1.66</td>
<td>n=126-1.61</td>
<td>n=266-1.10</td>
<td>n=593-0.76</td>
<td>n=389-0.76</td>
<td>n=565-0.33</td>
<td>n=602-0.50</td>
<td>n=989-0.61</td>
<td>n=686-0.54</td>
<td>n=658-0.64</td>
</tr>
<tr>
<td>CIE*</td>
<td>n=294-0.56</td>
<td>n=111-0.69</td>
<td>n=192-0.99</td>
<td>n=153-1.20</td>
<td>n=323-0.89</td>
<td>n=323-0.93</td>
<td>n=277-0.81</td>
<td>n=523-0.81</td>
<td>n=324-0.47</td>
<td>n=640-0.30</td>
<td>n=391-0.40</td>
<td>n=275-0.68</td>
<td>n=497-0.66</td>
<td>n=297-0.73</td>
</tr>
<tr>
<td>CM*</td>
<td>n=340-0.80</td>
<td>n=94-0.56</td>
<td>n=162-0.62</td>
<td>n=136-1.62</td>
<td>n=76-1.39</td>
<td>n=76-1.39</td>
<td>n=136-1.62</td>
<td>n=76-1.39</td>
<td>n=76-1.39</td>
<td>n=76-1.39</td>
<td>n=76-1.39</td>
<td>n=76-1.39</td>
<td>n=76-1.39</td>
<td>n=76-1.39</td>
</tr>
<tr>
<td>RC**</td>
<td>n=110-3.53</td>
<td>(1.94, 6.41)</td>
<td>n=127-1.79</td>
<td>(0.82, 3.52)</td>
<td>n=238-1.75</td>
<td>(1.36, 2.62)</td>
<td>n=76-1.39</td>
<td>(0.96, 3.38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANMF**</td>
<td>n=965-1.70</td>
<td>(1.09, 2.42)</td>
<td>n=249-39.80</td>
<td>(28.0, 51.6)</td>
<td>n=228-23.00</td>
<td>(9.98, 31.1)</td>
<td>n=219-25.55</td>
<td>(18.2, 37.8)</td>
<td>n=257-3.88</td>
<td>(26.1, 39.3)</td>
<td>n=378-12.00</td>
<td>(12.7, 22.3)</td>
<td>n=394-17.41</td>
<td>(13.2, 21.6)</td>
</tr>
</tbody>
</table>

Data presented are RR or WMD (95% CI) compared with placebo where n = the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis. CUE = Mean change in urgency episodes per 24 hours, CIE = mean change in incontinent episodes per 24 hours, CM = mean change in micturitions per 24 hours, RC = the number of patients returned to continence, ANMF = the number of patients achieving normal micturition frequency. CVV = mean change in volume voided per micturition, oxy = oxybutynin, tol = tolterodine, sol = solifenacin, pro = propiverine, tro = trospium, dar = darifenacin, IR = immediate release, ER = extended release, TDS = transdermal system, (t 7.5) = titrated dose from 7.5mg/day.

*WMD (weighted mean difference).
**RR (relative risk ratio).
#Data included from a single trial.
##Data included from a single trial reported to be powered to show a significant difference between interventions.

**Table 12**

Efficacy of antimuscarinics compared to active control: results from meta-analyses

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Intervention and Daily Dose (mg/day)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxy JR 10 vs Oxy JR 40</td>
<td>Oxy JR 10 vs Pro JR 45</td>
<td>Oxy JR 10 vs Oxy ER 10</td>
<td>Oxy JR (E 15) vs Oxy ER (15)</td>
<td>Oxy JR (1.5) vs Oxy ER (1.5)</td>
<td>Oxy JR (T) vs Oxy ER (T)</td>
<td>Pro JR 30 vs Pro ER 30</td>
<td>Pro JR 45 vs Oxy ER 10</td>
<td>Sol 5 vs Tad JR 2</td>
<td>Sol 10 vs Tad JR 4</td>
<td>Tad JR 2 vs Tad JR 4</td>
<td>Tad ER 4 vs Tad ER 4</td>
<td>Tad ER 4 vs Oxy JR 10</td>
<td>Tad ER 4 vs Oxy ER 10</td>
<td>Tad ER 4 vs Oxy TDS 3.9</td>
</tr>
<tr>
<td>CUE*</td>
<td>n=1271.10</td>
<td>n=888-0.83</td>
<td>n=789-0.35</td>
<td>n=57-0.60</td>
<td>n=288-0.38</td>
<td>n=288-0.38</td>
<td>n=315-0.31</td>
<td>n=775-0.79</td>
<td>n=244-0.36</td>
<td>n=244-0.36</td>
<td>n=391-0.40</td>
<td>n=275-0.68</td>
<td>n=497-0.66</td>
<td>n=297-0.73</td>
<td>n=821-2.00</td>
</tr>
<tr>
<td>CIE*</td>
<td>n=1271.10</td>
<td>n=888-0.83</td>
<td>n=789-0.35</td>
<td>n=57-0.60</td>
<td>n=288-0.38</td>
<td>n=288-0.38</td>
<td>n=315-0.31</td>
<td>n=775-0.79</td>
<td>n=244-0.36</td>
<td>n=244-0.36</td>
<td>n=391-0.40</td>
<td>n=275-0.68</td>
<td>n=497-0.66</td>
<td>n=297-0.73</td>
<td>n=821-2.00</td>
</tr>
<tr>
<td>CM*</td>
<td>n=1271.10</td>
<td>n=888-0.83</td>
<td>n=789-0.35</td>
<td>n=57-0.60</td>
<td>n=288-0.38</td>
<td>n=288-0.38</td>
<td>n=315-0.31</td>
<td>n=775-0.79</td>
<td>n=244-0.36</td>
<td>n=244-0.36</td>
<td>n=391-0.40</td>
<td>n=275-0.68</td>
<td>n=497-0.66</td>
<td>n=297-0.73</td>
<td>n=821-2.00</td>
</tr>
<tr>
<td>RC**</td>
<td>n=110-3.53</td>
<td>(1.94, 6.41)</td>
<td>n=127-1.79</td>
<td>(0.82, 3.52)</td>
<td>n=238-1.75</td>
<td>(1.36, 2.62)</td>
<td>n=76-1.39</td>
<td>(0.96, 3.38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANMF**</td>
<td>n=965-1.70</td>
<td>(1.09, 2.42)</td>
<td>n=249-39.80</td>
<td>(28.0, 51.6)</td>
<td>n=228-23.00</td>
<td>(9.98, 31.1)</td>
<td>n=219-25.55</td>
<td>(18.2, 37.8)</td>
<td>n=257-3.88</td>
<td>(26.1, 39.3)</td>
<td>n=378-12.00</td>
<td>(12.7, 22.3)</td>
<td>n=394-17.41</td>
<td>(13.2, 21.6)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented are RR or WMD (95% CI) compared with placebo where n = the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis. CUE = mean change in urgency episodes per 24 hours, CIE = mean change in incontinent episodes per 24 hours, CM = mean change in micturitions per 24 hours, RC = the number of patients returned to continence, ANMF = the number of patients achieving normal micturition frequency. CVV = mean change in volume voided per micturition, oxy = oxybutynin, tol = tolterodine, sol = solifenacin, pro = propiverine, tro = trospium, dar = darifenacin, IR = immediate release, ER = extended release, TDS = transdermal system, (t 7.5) = titrated dose from 7.5mg/day, (t 15) = titrated from 15 mg/day.

*WMD (weighted mean difference).
**RR (relative risk ratio).
#Data included from a single trial.
##Data included from a single trial reported to be powered to show a significant difference between interventions.
Data on dry mouth severity was reported in 19 trials, although this data was limited to trials of oxybutynin ER, oxybutynin IR, tolterodine ER, tolterodine IR and propiverine IR (Tables 9 and 10). Not only was oxybutynin IR found to be associated with a higher incidence of dry mouth (any severity) than other treatments, but it was also found to cause more moderate/severe and severe dry mouth. Oxybutynin IR was associated with more severe dry mouth than three of the four other eligible active comparators: propiverine IR (twice the incidence), tolterodine IR (four times the incidence) and tolterodine ER (twenty times the incidence).

The data not suitable for meta-analysis support these results with the exception of one trial reporting conflicting data [15]. Leung reported comparable rates of dry mouth (any severity) in patients treated with tolterodine IR 4 mg/day and oxybutynin IR 10 mg/day, whereas we found a significantly lower incidence in favour of tolterodine IR from the meta-analysis [15].

3.2.3. Other adverse events

Other adverse events noted in placebo controlled trials were (Table 7): blurred vision (with oxybutynin IR, propiverine IR and solifenacin); constipation (with darifenacin, solifenacin and trospium); dyspepsia (with darifenacin and oxybutynin IR); erythema and pruritus (with oxybutynin TDS); and urinary retention (with oxybutynin IR).

A limited range of other adverse events was noted in active comparator trials: Solifenacin 10 mg/day was associated with more blurred vision than tolterodine IR 4 mg/day; the risk of constipation was reduced by using oxybutynin TDS in preference to oxybutynin IR, and tolterodine IR in preference to solifenacin; the incidence of dyspepsia was lower if tolterodine IR was used instead of oxybutynin IR; nausea was less in patients treated with oxybutynin ER compared with oxybutynin IR; and the risk of vomiting was less for oxybutynin ER compared with tolterodine ER.

These results were generally reflective of the data not suitable for meta-analysis.

3.3. Efficacy

3.3.1. Placebo controlled trials (Table 11)

All antimuscarinics apart from propiverine were found to be efficacious in one or more meta-analyses compared with placebo (Table 11). Some evidence of efficacy was available for propiverine, but was not reported in a format suitable for meta-analysis.

Urgency episodes were significantly reduced by over one episode per day in patients receiving solifenacin 5 mg/day, solifenacin 10 mg/day and tolterodine ER 4 mg/day compared with patients receiving placebo. Incontinence episodes were reduced by half an episode or more per day in patients receiving all analysed doses of oxybutynin (IR or TDS), solifenacin and tolterodine (IR or ER) compared with those on placebo. The frequency of micturition was reduced in patients receiving all analysed doses of solifenacin, tolterodine (IR or ER) and oxybutynin TDS compared with placebo. There was a greater number of patients returned to continence on oxybutynin IR 5–7.5 mg/day, oxybutynin TDS, tolterodine ER 4 mg/day and trospium compared with placebo. Darifenacin titrated from 7.5 mg/day helped more patients achieve normal micturition frequency compared with placebo. The volume voided per micturition was increased by an additional 13 to 40 ml by antimuscarinics (oxybutynin IR 8.8–15 mg/day, oxybutynin TDS 3.9 mg/day, solifenacin 5 mg/day, 10 mg/day, tolterodine IR 2 mg/day, 4 mg/day, tolterodine ER 4 mg/day) compared with placebo.

The following data was not suitable for meta-analysis, but provides further evidence of the efficacy of antimuscarinic formulations. Darifenacin was found to achieve greater reductions in urgency episodes, incontinence episodes and micturition frequency compared with placebo [16–18].

Oxybutynin IR 8.8–15 mg/day was found to reduce micturition frequency compared with placebo in three trials [19–21]. In addition, Burgio 2001 found a greater reduction in daily incontinence episodes in patients treated with oxybutynin IR 7.5 mg/day than in patients receiving placebo.

Propiverine IR 45 mg/day was found to reduce incontinence episodes [8]. Propiverine IR and ER (30 mg/day) were also found to reduce micturition frequency compared with placebo [22].

Trospium 40 mg/day was found to reduce daily incontinence and urgency episodes compared with placebo [23, 24]. Three trials also reported significantly greater reductions in micturition frequency in patients receiving trospium 40 mg/day compared with placebo [23–25].

3.3.2. Active controlled trials (Table 12)

Three antimuscarinic formulations (solifenacin, oxybutynin ER, oxybutynin IR) were found to have superior efficacy in direct comparison trials (Table 12). Patients receiving solifenacin 5 or 10 mg/day experienced up to one fewer urgency episode per day than those receiving tolterodine IR 4 mg/day. Patients receiving solifenacin 10 mg/day also experienced less frequent micturition than those receiving tolterodine IR 4 mg/day. Patients receiving oxybutynin ER 10 mg/day experience approximately two fewer incontinence episodes per week than patients receiving tolterodine ER.
4 mg/day. A greater number of patients treated with oxybutynin ER 10 mg/day were returned to continence than patients receiving tolterodine ER 4 mg/day. The increase in volume voided was greater in patients treated with oxybutynin IR 15 mg/day, solifenacin 5 mg/day and 10 mg/day than in patients receiving tolterodine IR 4 mg/day.

The OBJECT trial reported endpoint efficacy data that could not be incorporated in the meta-analysis [26]. This trial found a significantly greater reduction in daily incontinence episodes and micturition frequency in patients treated with oxybutynin ER 10 mg/day than in patients receiving tolterodine IR 4 mg/day.

### 3.4. Quality of life

Of 56 trials included in the review, 25 reported QoL findings. The most commonly employed instruments were the Incontinence Impact Questionnaire (IIQ), King’s Health Questionnaire (KHQ), Short-form 36 (SF-36), Gaudenz Appraisal Questionnaire and Urogenital Distress Inventory (UDI). The Contilife questionnaire and the Basle Subjective Well-Being Survey were also used infrequently.

Statistically significant differences in QoL compared to placebo were reported for tolterodine IR and ER, trospium, solifenacin, propiverine IR and oxybutynin TDS. These data were limited by inconsistency in the instruments used, a small number of assessed patients and few reported domains. A pooled analysis of three RCTs presented QoL data for darifenacin, but this paper did not meet the inclusion criteria of the review. Included trials reported significant differences between antimuscarinics and placebo for global and disease-specific domains. The data indicate that antimuscarinics improve several areas of QoL ranging from physical activities, sleep and energy to emotions and relationships (Table 13).

Meta-analysis of QoL data demonstrated that patients receiving antimuscarinics have greater improvements in QoL than patients on placebo (Table 14). Improvements were noted for oxybutynin TDS, trospium, tolterodine IR, and tolterodine ER. Limited analyses of direct comparisons found no significant differences between interventions.

These results are described in detail in a separate publication focusing on the effects of antimuscarinic treatments on QoL [42].

### 3.5. Sensitivity analyses

The sensitivity analyses altered few results, indicating that the meta-analyses were robust to pertinent study and patient characteristics. A limited number of tolerability and safety results changed after removing

<table>
<thead>
<tr>
<th>Global QoL domains</th>
<th>Significant impact reported for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall QoL</td>
<td>Solifenacin</td>
</tr>
<tr>
<td>Contilife: Overall QoL</td>
<td>Oxybutynin TDS, Tolterodine ER</td>
</tr>
<tr>
<td>IIQ: Overall QoL</td>
<td>Oxybutynin TDS, Tolterodine ER</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Daily activities</th>
<th>Contilife: Daily activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solifenacin, Tolterodine IR</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physical limitations</th>
<th>KHQ: Physical limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine IR, ER</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Travel</th>
<th>IIQ: Travel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxybutynin TDS, Tolterodine ER, Trospium</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sleep and energy</th>
<th>KHQ: Sleep and energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine IR, ER</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Basle: Tired-fresh</th>
<th>Self-image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propiverine IR</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Emotions</th>
<th>Contilife: Emotional consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solifenacin</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KHQ: Emotional problems</th>
<th>IIQ: Feelings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine IR, ER</td>
<td>Tropium</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relationships</th>
<th>KHQ: Relationships</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine IR, ER</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KHQ: Personal relationships</th>
<th>Social limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine ER</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KHQ: Severity (Coping)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine IR, ER</td>
</tr>
</tbody>
</table>

### Table 13

Summary of the effects of antimuscarinics on QoL (all significant differences between antimuscarinics and placebo reported in included trials)

<table>
<thead>
<tr>
<th>Disease specific domains</th>
<th>Significant impact reported for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom severity</td>
<td>Tolterodine IR, ER</td>
</tr>
<tr>
<td>KHQ: Symptom severity</td>
<td>Oxybutynin TDS, Tolterodine ER</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incontinence</th>
<th>KHQ: Incontinence impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine IR, ER</td>
<td></td>
</tr>
</tbody>
</table>

| Gaudenz: Stress score    | Propiverine IR                  |

<table>
<thead>
<tr>
<th>Urgency</th>
<th>Gaudenz: Urge score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propiverine IR</td>
<td></td>
</tr>
</tbody>
</table>

QoL = quality of life, Contilife = Quality of Life Assessment Questionnaire Concerning Urinary Incontinence, IIQ = Incontinence Impact Questionnaire, KHQ = King’s Health Questionnaire, Basle = Basle Subjective Well-Being Survey, Gaudenz = Gaudenz Appraisal Questionnaire, UDI = Urinary Distress Inventory, IR = immediate release, ER = extended release, TDS = transdermal system.
trials including patients with mild or severe incontinence at baseline and varying disease type. These results were not considered to be clinically significant and have not been reported here.

The only sensitivity analysis that changed efficacy outcome results was for oxybutynin ER and oxybutynin TDS and was related to the inclusion of patients with prior response to treatment. The results of the meta-analyses for oxybutynin ER and oxybutynin TDS should be treated with caution when considering treatment naïve patients or prior treatment failures. Three out of four oxybutynin ER trials included 100% prior responders to antimuscarinic treatment [27–29]. In the Birns 2000 trial, only patients with prior response in efficacy and proven tolerability to treatment with oxybutynin IR were included. Only one included trial provided data for the efficacy, safety and tolerability of oxybutynin ER (titrated from 15 mg/day) in treatment naïve patients [30]. One of two trials comparing oxybutynin TDS with placebo also included 100% prior responders to antimuscarinic treatment [31]. When this trial was removed from the meta-analyses, reductions in daily incontinence episodes and micturition frequency in patients receiving oxybutynin TDS compared with patients on placebo were no longer found to be significantly in favour of active treatment. If the Dmochowski 2003 trial is excluded from the meta-analyses, oxybutynin TDS only results in significantly greater efficacy for one outcome compared with placebo (return to continence) and this meta-analysis only includes data from a single trial [32]. Removing trials including patients with mild or severe incontinence at baseline and varying disease type had no effect on the efficacy results, neither did removing cross-over trials.

4. Discussion

This systematic review suggests that many antimuscarinics are well tolerated and have a predictable adverse event profile with proven efficacy in the treatment of OAB and DO. There appear to be differences in the profile of the individual antimuscarinic treatments. The 'splitting' approach (assessing any variation in effect between drugs) that has been adopted has resulted in very different findings from the 'lumping' approach (pooling all drugs together) adopted in the earlier review by Herbison [2]. The results reported here suggest there is quantifiable benefit conferred by antimuscarinics, including some evidence that the drugs can return patients to continence. The results also suggest that antimuscarinics confer a significant quality of life benefit to patients. In contrast, Herbison concluded that 'the benefits [of the drugs] are of limited clinical significance’. The findings reviewed here suggest that there are differences between the drugs, whereas the sensitivity analysis conducted by Herbison, ‘did not show any differences in the results for type of drug’. The larger numbers of trials and the meta-analyses of active comparator trials included in this review may partially explain the differences in the findings between these two reviews.

Table 14

<table>
<thead>
<tr>
<th>Quality of Life of antimuscarinics compared with placebo: results from all possible meta-analyses from included trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intervention and Daily Dose (mg/day)</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>GAD n=238 -9.00</td>
</tr>
<tr>
<td>(16.8, -1.22)</td>
</tr>
<tr>
<td>IIO n=228 -25.0</td>
</tr>
<tr>
<td>(47.5, -2.91)</td>
</tr>
<tr>
<td>T n=238 -12.0</td>
</tr>
<tr>
<td>(-19.0, -4.97)</td>
</tr>
<tr>
<td>PA n=238 -7.50</td>
</tr>
<tr>
<td>(-7.21, 2.21)</td>
</tr>
<tr>
<td>F n=472-4.00</td>
</tr>
<tr>
<td>(-8.39, -0.61)</td>
</tr>
<tr>
<td>R n=472-4.00</td>
</tr>
<tr>
<td>(-8.39, -0.61)</td>
</tr>
<tr>
<td>KHQ GH n=982</td>
</tr>
<tr>
<td>(2.56, 0.99)</td>
</tr>
<tr>
<td>II n=982-6.66</td>
</tr>
<tr>
<td>(12.1, -5.22)</td>
</tr>
<tr>
<td>S (C) n=982-4.28</td>
</tr>
<tr>
<td>(6.89, -1.76)</td>
</tr>
<tr>
<td>SS n=982-1.50</td>
</tr>
<tr>
<td>(2.01, -0.99)</td>
</tr>
<tr>
<td>RL n=982-6.55</td>
</tr>
<tr>
<td>(-10.3, -2.92)</td>
</tr>
<tr>
<td>PL n=982-5.19</td>
</tr>
<tr>
<td>(-8.89, -1.58)</td>
</tr>
<tr>
<td>SL n=982-1.58</td>
</tr>
<tr>
<td>(-4.39, 1.23)</td>
</tr>
<tr>
<td>PR n=982-1.26</td>
</tr>
<tr>
<td>(-4.12, -1.63)</td>
</tr>
<tr>
<td>EP n=982-4.67</td>
</tr>
<tr>
<td>(-7.64, -1.70)</td>
</tr>
<tr>
<td>SE n=982-3.68</td>
</tr>
<tr>
<td>(-6.52, -1.08)</td>
</tr>
<tr>
<td>SF-36 PCS n=1022-2.33</td>
</tr>
<tr>
<td>(-0.96, 0.70)</td>
</tr>
<tr>
<td>MICS n=1022-2.33</td>
</tr>
<tr>
<td>(-0.65, 1.47)</td>
</tr>
<tr>
<td>UDI IS n=238-7.00</td>
</tr>
<tr>
<td>(-13.4, -0.63)</td>
</tr>
</tbody>
</table>

Data presented are weighted mean differences (95% CI) compared with placebo where n = the number of patients included in the analysis. Statistically significant differences are presented in boxes. Blank cells indicate comparisons for which no data was suitable for meta-analysis, Oxy = oxybutynin, Tol = tolterodine, Tro = trospium, IR = immediate release, ER = extended release, TDS = transdermal system, GAD = Global assessment of disease, IIQ (Incontinence Impact Questionnaire) domains: QoL = Overall QoL, T = Travel, PA = Physical Activities, F = Feelings, R = Relationships, KHQ (King’s Health Questionnaire) domains: GH = General Health, II = Incontinence Impact, RL = Role Limitations, PL = Physical Limitations, SL = Social Limitations, PR = Personal Relationships, EP = Emotional Problems, SE = Sleep and Energy, S (C) = Severity (Coping), SS = Symptom Severity, SF-36 (Short-form 36) domains: PCS = Physical Component Summary, MCS = Mental Component Summary, UDI = Urinary Distress Inventory domains: IS = Irritative Symptoms. No QoL data for the following interventions (compared with placebo) were suitable for meta-analysis: darifenacin, oxybutynin IR, propiverine IR, ER, solifenacin, tolterodine IR 2 mg/day.
A number of the concerns expressed by Herbison are valid, such as the variable quality of trial reporting, inappropriate choice of comparator (especially in older trials), and inappropriate outcome measure; these have been reported in many systematic reviews. Herbison pointed out the lack of QoL evidence and although this review has identified further QoL data, this data is still limited. There is also a lack of comparative and adjunctive trials of bladder retraining.

**Tolerability:** We found evidence that the antimuscarinics have different tolerability profiles, which may well be of clinical significance. Tolterodine ER was found to have the most favourable tolerability profile of all the antimuscarinics. Tolterodine ER was the only antimuscarinic found to be associated with significantly fewer all-cause withdrawals compared with placebo. All other antimuscarinics apart from oxybutynin IR were found to occupy the middle ground, causing similar all-cause withdrawals as placebo. However, it is promising to note that there were non-significant results with respect to darifenacin (7.5 mg/day) and solifenacin (5 mg/day, 10 mg/day) suggesting that these drugs may also cause fewer all-cause withdrawals than placebo. Oxybutynin IR was the least well tolerated antimuscarinic and, although data was limited for oxybutynin TDS, the transdermal delivery system was associated with over six times the rate of withdrawals due to adverse events compared with tolterodine ER.

**Adverse events:** The adverse event profiles of the antimuscarinics were safe and predictable; there was some evidence of differences between the profiles of drugs.

Based on significant results of meta-analyses compared with placebo, the drugs with the most favourable profile were oxybutynin IR 5–7.5 mg/day and tolterodine (IR, ER). The finding for oxybutynin IR 5–7.5 mg/day should be treated with caution as it was based on data from a single study of 57 patients.

There were only two instances where tolterodine ER 4 mg/day was associated with excess adverse events; a greater incidence of the well recognised antimuscarinic adverse event dry mouth compared with placebo; and vomiting compared with oxybutynin ER in the OPERA trial. Given that vomiting was not reported in placebo controlled trials of tolterodine ER and the rates of nausea reported in these trials were similar to placebo, we were unable to explain the findings for vomiting. Tolterodine IR and ER have comparable profiles, although tolterodine ER was associated with less dry mouth than the IR formulation.

Although oxybutynin ER was associated with more dry mouth than tolterodine ER, it will be worth investigating this further in the future. In a single meta-analysis oxybutynin ER was associated with lower rates of dry mouth compared with the IR formulation.

Darifenacin, oxybutynin IR at doses above 7.5 mg/day, oxybutynin TDS, propiverine (30 mg/day IR, ER), solifenacin (5 mg/day, 10 mg/day) and trospium (40 mg/day) were all significantly associated with multiple adverse events. Oxybutynin TDS, based on limited data, appears to have overcome the dry mouth problem associated with oxybutynin IR, but at the expense of application site reaction and an increase in withdrawals due to adverse events (found in a comparison with tolterodine ER only).

Oxybutynin IR was consistently associated with high rates of adverse events. In thirteen out of the twenty significant differences in adverse event rates found in active comparator analyses, oxybutynin IR was found to cause higher rates of adverse events than other antimuscarinic formulations. Oxybutynin IR was also associated with a notably high rate of moderate and severe dry mouth, and was the only drug found to increase rates of urinary retention.

**Efficacy:** We found clear evidence that every antimuscarinic was effective based on one or more of the outcome measures we included in the review. This publication focuses on the efficacy of antimuscarinics measured using clinical outcomes. The results of the analysis of QoL data are described in detail in a separate publication and the efficacy results presented in this publication should therefore be considered in the context of the effects of antimuscarinics on QoL [42].

We could not conclude that the drugs had similar effects, however: solifenacin caused significantly greater reductions in urgency episodes and micturition frequency compared with tolterodine IR; and oxybutynin ER caused greater reductions in incontinence episodes and a larger number of patients returned to continence compared with tolterodine ER.

### 4.1. Limitations of the evidence

As this review only included randomised controlled trials, the results may not be fully representative of clinical practice. We identified seven potential caveats to our findings: choice of outcome measure, trial length; limited active comparison trials, patient population; the placebo effect; treatment naïve patients; and economic consequences.

#### 4.1.1. Choice of outcome measure

A fundamental aim of treatment for OAB is to achieve continence, normal micturition frequency and to improve QoL [33]. These factors are highly
important and have not received enough attention in the past. Only 13 trials reported the number of patients returned to continence at trial endpoint; just two trials reported the number of patients achieving normal micturition frequency [18,34] and approximately half the trials \((n = 25)\) reported QoL findings. All of these infrequently reported outcomes have great relevance to the patient.

### 4.1.2. Trial length

The included data is limited in terms of generalisability to clinical practice due to the short length of included trials in contrast with the chronic treatment required by most patients with OAB. The majority of included trials were less than 12 weeks long. Eighteen trials were of 12 weeks duration. Despite the paucity of long-term randomised controlled trials of antimuscarinics, evidence from a single long-term randomised controlled trial, and several long-term non-randomised trials, support the long-term use of antimuscarinics [14,35–38].

Only one included trial clearly reported a relatively long-term (52-week) follow-up [14]. Halaska 2003 concluded that the incidence of side effects was no greater than the incidence reported in short-term trials. This indicates that the adverse event profile of the antimuscarinics reported in short-term trials is probably representative of longer-term treatment.

A limited number of longer-term, non-randomised studies evaluating the efficacy and tolerability of antimuscarinics are available although they failed to meet the inclusion criteria for this systematic review. We have identified four such studies [35–38]. Data from these studies support the findings of this review concerning the differing tolerability profiles of oxybutynin and tolterodine and support the long-term use of antimuscarinics.

### 4.1.3. Active controlled trials

A concern is the limited number of studies that have been conducted with an active comparator. Approximately half the trials included more than one antimuscarinic treatment arm enabling the direct comparison of interventions. However, even active comparator trials published in the last two years failed to include the newer ER formulations of tolterodine and oxybutynin and oxybutynin TDS. Only one trial compared oxybutynin ER and tolterodine ER [39] and only one trial compared oxybutynin TDS and oxybutynin ER [32].

Due to the limited number of active controlled trials, analyses included data from single trials and in certain cases these trials did not report powering to detect a difference between the interventions.

### 4.1.4. Restricted population

We have only included trials of patients within a narrow disease specification. Trials had to include greater than 50% of patients with idiopathic OAB/DO or urge predominant mixed incontinence. The results of this review are therefore not generalisable to patients with DO consequent upon neurogenic pathology or patients with stress predominant mixed incontinence.

Although OAB symptoms are particularly prevalent in the older population [40], few of the included trials researched the effects of antimuscarinic treatment in older patients. Four trials included patients with a mean age greater than 65 years, but only two of these included patients with a mean age greater than 70 years [10,11]. An open-label observational study including 2,250 OAB patients treated with tolterodine IR found that advancing age was associated with decreased responsiveness to treatment [41]. These data indicate that the efficacy of antimuscarinics may vary according to the patient’s age. The results of this review may therefore not be generalisable to older patients suffering from OAB. It is very clear that since many of the patients treated with antimuscarinics are elderly this should be the focus of future studies, in particular investigating the importance of this class of drugs on cognitive dysfunction [40].

This problem is not unique to this review as all controlled studies using strict inclusion and exclusion criteria provide high internal but low external validity. Extrapolation of results such as these to clinical practice must therefore be undertaken with appropriate caution.

### 4.1.5. The placebo effect

Herbison documented the substantial placebo effect experienced by patients with OAB. The placebo rates reported by included trials in this review confirm this effect within the OAB patient population. The results of this review do suggest that there are differences between antimuscarinic treatments and placebo if one looks specifically at individual components of tolerability, safety, efficacy and QoL outcomes, despite the magnitude of the placebo effect. Further research is required to help to explain the variation in placebo rates between trials evaluating antimuscarinic treatments for OAB.

### 4.1.6. Treatment naïve patients

The only sensitivity analysis conducted that generated a consequential finding was for prior responders to
treatment found for oxybutynin ER and oxybutynin TDS. The efficacy of both products was less certain for treatment naïve patients and prior treatment failures.

4.1.7. Economic consequences

Evidence relating to cost effectiveness of antimuscarinics needs to be considered in the future, particularly as this is becoming an increasingly important criteria to healthcare providers. We did not include cost-effectiveness studies of the antimuscarinics in our review, although the economic consequences of drug choice should be an important criterion to decision makers.

4.2. Implications for practice

It is clear from the variation in withdrawal, adverse event and efficacy rates between antimuscarinics, that differences could be anticipated in longer-term adherence between the drugs.

4.3. Implications for research

There are four areas that we consider would benefit from additional research: new and recently licensed products should be assessed in head to head trials with the most relevant established interventions (including adjunctive bladder retraining or biofeedback); study design should include outcome measures that are more meaningful to patients such as return to continent, achievement of normal micturition frequency and quality of life; variation in persistence rates between the drugs needs to be evaluated; and more studies of the effects of antimuscarinics are required in older patients.

Appendix A. References of included trials


[7] Birns J, Lekkari E, Malone-Lee JG. A randomised controlled trial comparing the efficacy of controlled-release oxybutynin tablets (10 mg once daily) with conventional oxybutynin tablets (5 mg twice daily) in patients whose symptoms were stabilized on 5 mg twice daily of oxybutynin. BJU Int 2000;85(7):793–8.


[21] Drutz HP, Appell RA, Gleason D, Klimberg I, Radomska S. Clinical efficacy and safety of tolterodine compared to oxybutynin and placebo


References


[27] Birns J, Lukkari E, Malone-Lee JG. A randomized controlled trial comparing the efficacy of controlled-release oxybutynin tablets (10 mg once daily) with conventional oxybutynin tablets (5 mg twice daily) in patients whose symptoms were stabilized on 5 mg twice daily of oxybutynin. BJU Int 2000;85(7):793–8.


Dietary Intake of Calcium, Vitamin D, Phosphorus and the Risk of Prostate Cancer

Alessandra Tavani, Paola Bertuccio, Cristina Bosetti, Renato Talamini, Eva Negri, Silvia Franceschi, Maurizio Montella, Carlo La Vecchia

Abstract

Objectives: A relation of prostate cancer risk with calcium, vitamin D and phosphorus has been suggested, but remains controversial.

Methods: A case-control study was conducted in Italy in 1991–2002. Cases were 1294 men with incident prostate cancer, and controls were 1451 men admitted to hospital for acute non-neoplastic diseases. Odds ratios (OR) and the corresponding 95% confidence intervals (CI) were estimated using unconditional multiple logistic regression.

Results: Compared to the lowest one quintile of dietary calcium intake the OR was 1.18 for the highest, 1.01 for an increment of 622 mg/day of calcium, and 1.29 (95% CI 0.78–2.13) for 2000 mg/day or more of calcium. The OR of prostate cancer for the highest quintile of dietary intake of vitamin D and phosphorus were 1.33 and 1.20 respectively.

Conclusions: This study shows no material association of dietary intake of calcium, vitamin D and phosphorus with prostate cancer risk.

Keywords: Calcium; Case-control study; Phosphorus; Prostate cancer; Risk factors; Vitamin D

1. Introduction

A positive association between milk and dairy product intake and the risk of prostate cancer has been reported in ecological, case-control and cohort studies [1,2]. This has been related to the influence on prostate carcinogenesis exerted by either their high fat content, or the balance between calcium and vitamin D, although these hypotheses are not mutually exclusive [3]. In several studies the association with milk and dairy products was apparently stronger than that with other sources of animal fat, including meat [3], suggesting that intake is not sufficient to explain the increased prostate cancer risk. On the other hand, laboratory evidence indicates that high circulating levels of vitamin D and its active metabolite 1,25(OH)2-vitamin D (1,25(OH)2D) (500- to 1000-fold more active than vitamin D) inhibits prostate carcinogenesis in vitro by reducing prostate cellular proliferation and enhancing cellular differentiation [4].

The balance of circulating levels of calcium, phosphorus, fructose and animal protein, with vitamin D and 1,25(OH)2D is complex. Measures of vitamin D levels are difficult, since only part of biologically available vitamin D comes from dietary sources, part
being produced in the skin through sun exposure [3]. Within a range of typical intakes, calcium modulates 1,25(OH)₂D levels; on the other hand, low dietary calcium transiently reduces plasma calcium, which, through the action of parathyroid hormone, increases the conversion rate of 1,25(OH)₂D from vitamin D [3,5], in turn enhancing intestinal absorption of calcium. Another important regulator of 1,25(OH)₂D levels is phosphorus, as low blood phosphate levels increase 1,25(OH)₂D production and blood levels [6], and high phosphate levels are relatively minor inhibitors of 1,25(OH)₂D synthesis.

Thus, a proposed hypothesis is that a diet low in calcium and phosphorus increases vitamin D and 1,25(OH)₂D circulating levels and consequently decreases the risk of prostate cancer [3]. However, epidemiological evidence on the impact of dietary calcium and phosphorus on 1,25(OH)₂D levels is unclear, and their role on prostate carcinogenesis too.

In a case-control study conducted in Italy, we observed a direct association between milk and dairy product consumption and prostate cancer risk, but no relation with cheese and meat intake [7]. In the same study we found no association of protein, total fats and saturated fatty acid intake with prostate cancer risk [8]. It is therefore unclear which ingredient of dairy products may be specifically related to prostate cancer risk. We analysed the data of this study to assess the relation between calcium, phosphorus and vitamin D dietary intake and prostate cancer risk, taking into account the potential confounding effect of several covariates.

2. Subjects and methods

The data were derived from a case-control study of prostate cancer, conducted between 1991 and 2002 in four Italian areas: greater Milan (northern Italy), the provinces of Pordenone and Gorizia (North-eastern Italy), the province of Latina (central Italy) and the urban area of Naples (southern Italy) [7,8]. Cases were 1294 men (median age 66, range 46 to 74 years), with incident, histologically confirmed prostate cancer, admitted to the major teaching and general hospitals in the areas under surveillance. Controls were 1451 men (median age 63, range 46 to 74 years), residing in the same geographical areas and admitted to the same network of hospitals of cases for a wide spectrum of acute conditions unrelated to known or likely risk factors for prostate cancer. Among controls, 21% had traumatic conditions, 32% non-traumatic orthopedic disorders, 17% acute surgical conditions and 29% miscellaneous other illnesses (such as eye, ear, nose, throat and dental disorders). All interviews for cases and controls were conducted in hospital by trained interviewers using a structured questionnaire; less than 5% of cases and controls approached refused interview, and the response rates did not vary across hospitals and geographic areas.

The structured questionnaire included information on socio-demographic factors, self-reported anthropometric variables, general lifestyle habits, such as smoking, alcohol and coffee consumption, frequency of intake of selected food items, personal medical history and family history of cancer. Information on diet referred to the two years before the onset of the disease that led to hospital admission and included the frequency of consumption of 78 foods, food groups, or dishes, including major dietary sources of calcium in the Italian diet (milk, cheese, yoghurt), and foods with the highest content of vitamin D (fish, eggs, milk) and phosphorus (milk, cheese, eggs). Specific questions on intake of milk (full fat, skimmed) and all types of cheese (including hard and soft cheese and portion size) were asked to patients, together with other questions on minor dietary sources of calcium [9]. The detailed questionnaire allowed to estimate the intake of selected nutrients and of total energy, using Italian food composition databases [9].

2.1. Data analysis

Odds ratios (OR) and the corresponding 95% confidence intervals (CI) were estimated using unconditional multiple logistic regression models [10], including terms for quinquennia of age, centre, education, body mass index (kg/m²), tobacco smoking, physical activity, total energy, and family history of prostate cancer in first degree relatives. The nutrients of interest were entered in the models either in quintiles or in continuous. In the latter case, the measurement unit was set to the difference in intake between the upper cut-point of the forth quintile and that of the first one.

3. Results

The relation between the intake of calcium, vitamin D and phosphorus and the risk of prostate cancer is reported in Table 1. Compared to the lowest quintile of calcium intake, the multivariate OR for increasing quintiles of intake were 1.14, 1.01, 0.94 and 1.18, with no significant trend in risk; the OR was 1.01 for an increment of 622 mg/day of calcium, approximately corresponding to an intake of 60–100 g of cheese or to 100–150 ml of milk, and the OR for calcium intake of 2000 mg/day or more was 1.29 (95% CI 0.78–2.13). The OR for increasing quintiles of vitamin D intake were 1.22, 1.05, 1.27 and 1.32, with no significant trend in risk, and the OR for an increment of 2.1 μg/day was 1.06. The OR for increasing quintiles of phosphorus intake were 1.04, 0.83, 1.11 and 1.20, with no significant trend in risk, and the OR for an increment of 692 mg/day was 1.05.

The risk estimates for calcium intake were not heterogeneous in strata of age, education, body mass index, total energy intake, and family history of prostate cancer (Table 2). The OR was 1.00 (95% CI 0.92–1.10) for the highest quintile of calcium intake for prostate cancer cases with a Gleason score 2–6 (based on 538 cases), and 1.03 (95% CI 0.93–1.15) for those with a Gleason score ≥7 (based on 384 cases).
Table 1
Distribution of 1294 cases of prostate cancer and 1451 controls, and corresponding odds ratios (OR) with 95% confidence intervals (CI), according to intake of calcium, vitamin D and phosphorus, Italy, 1991–2002

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Quintile of intake</th>
<th>Continuous OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>χ&lt;sup&gt;2&lt;/sup&gt;&lt;sub&gt;trend&lt;/sub&gt; (&lt;i&gt;p&lt;/i&gt; value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases:controls</td>
<td>Upper cut-point (mg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>195.290</td>
<td>263.291</td>
<td>254.290</td>
</tr>
<tr>
<td>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</td>
<td>1.14 (0.88–1.49)</td>
<td>1.01 (0.77–1.32)</td>
<td>0.94 (0.70–1.25)</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Upper cut-point (μg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>199.290</td>
<td>260.290</td>
<td>234.291</td>
</tr>
<tr>
<td>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</td>
<td>1.22 (0.94–1.59)</td>
<td>1.05 (0.80–1.38)</td>
<td>1.27 (0.97–1.66)</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Upper cut-point (mg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>198.290</td>
<td>248.291</td>
<td>244.289</td>
</tr>
<tr>
<td>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</td>
<td>1.04 (0.78–1.38)</td>
<td>0.83 (0.60–1.16)</td>
<td>1.11 (0.77–1.59)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Estimates from multiple logistic regression models including terms for age, centre, education, body mass index, tobacco smoking, physical activity, total energy, and family history of prostate cancer.

<sup>b</sup> OR for a difference in intake equal to the difference between the upper cut-point of the fourth quintile and that of the first (622 mg/day for calcium, 2.1 μg/day for vitamin D, and 692 mg/day for phosphorus).

<sup>c</sup> Reference category.

4. Comment

This study found no consistent association between calcium, vitamin D and phosphorus dietary intake and prostate cancer risk, at least within the range of intakes observed in this population, as all the point estimates for subsequent levels were close to unity and there was no significant trend in risk. Epidemiological studies on the relation of calcium, vitamin D and phosphorus with prostate cancer risk

Table 2
Odds ratio (OR) of prostate cancer, and corresponding 95% confidence intervals (CI), according to intake of calcium in strata of selected covariates, Italy, 1991–2002

<table>
<thead>
<tr>
<th>Cases:controls</th>
<th>Quintile of calcium intake, OR&lt;sup&gt;a&lt;/sup&gt; (95% CI)</th>
<th>Continuous OR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>χ&lt;sup&gt;2&lt;/sup&gt;&lt;sub&gt;trend&lt;/sub&gt; (&lt;i&gt;p&lt;/i&gt; value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>529:790</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.05 (0.71–1.65)</td>
</tr>
<tr>
<td>≥65</td>
<td>765:661</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.24 (0.87–1.77)</td>
</tr>
<tr>
<td>Education (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>636:844</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.06 (0.74–1.51)</td>
</tr>
<tr>
<td>≥7</td>
<td>658:607</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.20 (0.81–1.78)</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;26</td>
<td>318:678</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.99 (0.68–1.44)</td>
</tr>
<tr>
<td>≥26</td>
<td>672:768</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.30 (0.89–1.88)</td>
</tr>
<tr>
<td>Total energy intake (kcal/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2487.07</td>
<td>618:754</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.32 (0.98–1.77)</td>
</tr>
<tr>
<td>≥2487.07</td>
<td>676:697</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.17 (0.67–2.04)</td>
</tr>
<tr>
<td>Family history of prostate cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>204:1423</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.08 (0.83–1.41)</td>
</tr>
<tr>
<td>Yes</td>
<td>90:28</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.10 (0.67–97.86)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Estimates from multiple logistic regression models including terms for age, centre, education, body mass index, tobacco smoking, physical activity, total energy and family history of prostate cancer.

<sup>b</sup> OR for an increment of 622 mg/day (the difference in intake equal to the difference between the upper cut-point of the fourth quintile and that of the first).

<sup>c</sup> Reference category.
have produced conflicting results. The results of most relevant studies, ten cohort [11–20] and eleven case-control studies [21–31], are summarized in Table 3. The relation with calcium intake was considered in at least twelve studies [14,19,21,22,25,26,28,29]: of these, seven reported risk estimates above unity [14,16,17,19,22,26,28], significant in four [14,17,19,22]; four studies reported risk estimates around unity [15,18,25,29], and one study reported estimates significantly below unity [21]. Various

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium, phosphorus and vitamin D intake and prostate cancer risk: main results from selected studies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author, year, reference</th>
<th>Country</th>
<th>Cases</th>
<th>Variable</th>
<th>Relative risk (95% confidence interval)</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohort studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corder et al., 1993 [11]</td>
<td>USA</td>
<td>181</td>
<td>25(OH)D 1,25(OH)2D</td>
<td>0.92 (0.56–1.50) highest quartile</td>
<td>Kaiser Permanente Medical Care program.</td>
</tr>
<tr>
<td>Braun et al., 1995 [12]</td>
<td>USA</td>
<td>61</td>
<td>25(OH)D 1,25(OH)2D</td>
<td>0.88 (0.53–1.45) highest quartile</td>
<td>Population based campaign to collect serum in Washington County.</td>
</tr>
<tr>
<td>Gann et al., 1996 [13]</td>
<td>USA</td>
<td>232</td>
<td>Plasma levels</td>
<td>1.09 (0.79–1.50) highest quartile</td>
<td>Physicians’ Health study.</td>
</tr>
<tr>
<td>Giovannucci et al., 1998 [14]</td>
<td>USA</td>
<td>1792</td>
<td>Total calcium Dietary intake</td>
<td>1.49 (1.04–1.26) &gt;600 mg/die</td>
<td>Health Professional follow-up study. No trend in risk for calcium.</td>
</tr>
<tr>
<td>Rodriguez et al., 1999 [15]</td>
<td>The Netherlands</td>
<td>642</td>
<td>Calcium Dietary intake</td>
<td>1.00 (0.78–1.15) highest quartile</td>
<td>The Netherlands cohort study.</td>
</tr>
<tr>
<td>Chan et al., 2000 [16]</td>
<td>Finland</td>
<td>184</td>
<td>Calcium Dietary intake</td>
<td>1.69 (0.83–3.40) highest quartile</td>
<td>Alpha-Tocopherol Beta-Carotene cancer prevention (ATBC) study.</td>
</tr>
<tr>
<td>Chan et al., 2001 [17]</td>
<td>USA</td>
<td>1012</td>
<td>Calcium Dietary intake</td>
<td>0.80 (0.53–1.23) highest quartile</td>
<td>Physicians’ Health study. Baltimore longitudinal study of aging.</td>
</tr>
<tr>
<td>Berndt et al., 2002 [18]</td>
<td>USA</td>
<td>69</td>
<td>Calcium Dietary intake</td>
<td>0.81 (0.47–1.39) highest quartile</td>
<td>Cancer prevention study II nutrition cohort.</td>
</tr>
<tr>
<td>Rodriguez et al., 2003 [19]</td>
<td>USA</td>
<td>3811</td>
<td>Calcium Dietary intake</td>
<td>1.19 (0.79–1.79) highest quartile</td>
<td>Health professional follow-up study.</td>
</tr>
<tr>
<td>Platz et al., 2004 [20]</td>
<td>USA</td>
<td>460</td>
<td>Calcium Dietary intake</td>
<td>1.25 (0.82–1.90) highest quartile</td>
<td>Cancer prevention study II nutrition cohort.</td>
</tr>
<tr>
<td><strong>Case-control studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vlajinac et al., 1997 [21]</td>
<td>Serbia</td>
<td>101</td>
<td>Calcium Dietary intake</td>
<td>0.37 (0.14–0.94) highest tertile</td>
<td>Hospital-based; significant linear trend for calcium intake.</td>
</tr>
<tr>
<td>Chan et al., 1998 [22]</td>
<td>Sweden</td>
<td>526</td>
<td>Calcium Dietary intake</td>
<td>1.69 (0.44–4.63) highest tertile</td>
<td>Population-based; significant linear trend.</td>
</tr>
<tr>
<td>Nomura et al., 1998 [23]</td>
<td>USA</td>
<td>136</td>
<td>25(OH)D Dietary intake</td>
<td>0.80 (0.44–1.83) highest quartile</td>
<td>Nested in a cohort of Japanese men living in the Hawaii.</td>
</tr>
<tr>
<td>Denne-Pellegrini et al., 1999 [24]</td>
<td>Uruguay</td>
<td>175</td>
<td>Vitamin D Dietary intake</td>
<td>0.70 (0.41–1.23) highest quartile</td>
<td>Hospital-based.</td>
</tr>
<tr>
<td>Hayes et al., 1999 [25]</td>
<td>USA</td>
<td>932</td>
<td>Calcium Dietary intake</td>
<td>0.80 (0.44–1.83) highest quartile</td>
<td>Hospital-based.</td>
</tr>
<tr>
<td>Kristal et al., 1999 [26]</td>
<td>USA</td>
<td>697</td>
<td>Calcium Dietary intake</td>
<td>1.25 (0.73–2.17) users</td>
<td>Population-based.</td>
</tr>
<tr>
<td>Ahonen et al., 2000 [27]</td>
<td>Finland</td>
<td>149</td>
<td>25(OH)D Serum levels</td>
<td>0.60 (0.21–1.0) highest quartile</td>
<td>Population-based. Nested in the Helsinki heart study.</td>
</tr>
<tr>
<td>Tavani et al., 2001 [28]</td>
<td>Italy</td>
<td>288</td>
<td>Calcium Dietary intake</td>
<td>1.12 (0.67–1.88) highest quintile</td>
<td>Hospital-based. Population-based.</td>
</tr>
<tr>
<td>Kristal et al., 2002 [29]</td>
<td>USA</td>
<td>605</td>
<td>Calcium Dietary intake</td>
<td>1.06 (0.66–1.70) highest quintile</td>
<td>Population-based.</td>
</tr>
<tr>
<td>Jacobs et al., 2004 [31]</td>
<td>USA</td>
<td>83</td>
<td>25(OH)D Serum levels</td>
<td>0.75 (0.29–1.91) highest tertile</td>
<td>Nested in the nutritional prevention of cancer (NCP) trial.</td>
</tr>
</tbody>
</table>

\*25(OH) vitamin D = 25(OH)D; 1,25(OH)2-vitamin D = 1,25(OH)2D.
\*Computed from published data.
measure of vitamin D (vitamin D intake, 25(OH)D and 1,25(OH)2D circulating levels) were obtained in at least fourteen studies [11–14,16,18,20,21,23,24,27,29–31]: four studies reported non-significant direct relations [12,14,18,20]; five studies inverse relations [13,16,21,24,27], four studies reported risks around unity [11,23,29,31], none significant, and one study reported an U-shaped curve [30]. Of the four studies measuring phosphorus intake [14,16,18,21], two reported risk estimates above unity [18,21] and the other two studies below unity [14,16], none significant.

An association between calcium and prostate cancer has been mainly observed for more advanced disease [14] or for very high intakes [14,17,19]. However, we found no different risk for patients with Gleason score below and over 7, and no increased risk for patients reporting an intake of more than 2000 mg/day of calcium. This observation, however, does not exclude that calcium could cause tumors to progress independently of grade.

In this analysis, calcium, phosphorus and vitamin D intake was computed by a food frequency questionnaire tested for validity [32] and reproducibility [33]: the Pearson correlation coefficient for validity of calcium intake was 0.54 [32]. Milk and cheese, in the Italian diet, account for about 70% of total calcium intake [34], and phosphorus is plentiful in diary products and meat and, at normal intakes, it is also well absorbed by the gut [6]. In this population less than 15% of dietary calcium derived from milk and over 50% from cheese [34]. This may explain the different findings for calcium intake and milk, moderately related to prostate cancer risk in this population (OR 1.15 for the highest quintile of intake) [7]. A potential explanation is that the bioavailability of calcium from cheese may be reduced by concomitant intake of other foods (particularly those with high fibre content) compared to milk, which in Italy is consumed mostly alone or with carbohydrates at breakfast and not during meals. Other explanations include the higher concentration in cheese of several micronutrients and dietary correlates of milk and cheese intake. Other sources of calcium in Italy, such as yoghurt, citrus fruit or green salad, accounted only for 2–3% of calcium intake and were included in the computing of calcium intake [9,34]. There was no information on the use of calcium supplements, but their use was extremely limited in Italy, i.e. less than 5% of the population [9,35].

Vitamin D dietary intake does not represent circulating levels, as it is also synthesized in the skin in a reaction catalyzed by ultraviolet light, and it undergoes several well regulated hydroxylation reactions to become biologically active [3]. Thus, given the complex biological regulation of vitamin D levels, which involves also fructose and animal protein intake, our results on vitamin D dietary intake should be taken with caution. Moreover, vitamin D intake was very low and would not be expected to influence circulating 25(OH)D by more than 1–2 ng/ml, while the typical observable range is about ten times higher, and it would be likely that any association observed with vitamin D would be confounded by the source of vitamin D. There are differences in vitamin D expression in prostate cancer, and this might also explain, at least in part, the lack of association between prostate cancer and nutrients that may influence 1,25(OH)2D levels.

A potential difficulty in these studies is to disentangle the individual effect of dietary calcium, phosphorus and vitamin D, as milk and dairy products are the major sources of dietary calcium and phosphorus and an important source of dietary vitamin D, too [9]. A limitation of this study is also that a number of undetected prostate cancer cases might be present in the control group, since case diagnosis by PSA may have been more common in the case group than in the control group, and that subjects undergoing PSA might be more health-conscious and may have healthier life-style, including diet. Screening for prostate cancer is not widespread in Italy, although occasional case finding through PSA testing is increasingly common. However, high education is strongly related to PSA utilization and allowance for this variable has been accurately done.

Although we used hospital controls, patients admitted for conditions potentially influencing dietary habits were specifically excluded from the comparison group; cases and controls were drawn from the same catchment areas, their interviews were conducted in the same hospital setting, their participation was almost complete. The food frequency questionnaire was satisfactorily valid [32] and reproducible [33] and allowance for several confounding factors, including fruit consumption (the major source of fructose, another modulator of vitamin D levels, together with animal proteins) did not notably modify the relative risk estimates. The OR for calcium consumption after further allowance for phosphorus intake were 1.14, 0.99, 0.84, 1.00 for increasing quintiles of intake, compared to the lowest one, thus materially different from those presented in Table 1. The OR for the highest quintile of intake, after allowance for age, centre and total energy intake were 1.24 (95% CI 0.95–1.61) for calcium, 1.37 (95% CI 0.91–2.07) for phosphorus and 1.57 (95% CI 1.20–2.05) for vitamin D, not materially different from the fully adjusted ones. The exclusion from the control group of subjects admitted for traumas or for orthopedic non traumatic conditions (which may
have different calcium/vitamin D levels) did not materially change the risk estimates.

5. Conclusions

In this case-control study there is no evidence that dietary calcium and phosphorus, at levels consumed by this population, may exert unfavourable effects on prostate carcinogenesis. Thus, our results do not support the hypothesis that milk and cheese consumption influence prostate cancer risk through their high cal-
cium or phosphorus content, even at intakes higher than 1300 mg/day of calcium and higher than 1897 mg/day of phosphorus. However, additional prospective studies in this field are needed.

Acknowledgments

Supported by the Italian Association for Research on Cancer, the Italian League Against Cancer, and the Italian Ministry of Education (COFIN 2003). The authors thank M.P. Bonifacino for editorial assistance.

References

[6] Portale AA, Halloran BP, Murphy MM, Morris Jr RC. Oral intake of phosphorus can determine the serum concentration of 1, 25-dihydroxy-
[15] Schuurman AG, van den Brandt PA, Dorant E, Goldbohm RA. Animal products, calcium and protein and prostate cancer risk in the Nether-
[19] Rodriguez C, McCullough ML, Mondul AM, Jacobs EJ, Fakhraabadi-
[31] Tuohimaa P, Tenkanen L, Ahonen M, Lumme S, Jellum E, Hallmans G, et al. Both high and low levels of blood vitamin D are associated...


**Prostate Cancer**

**Insulin-like Growth Factor 1, Chromogranin A and Prostate Specific Antigen Serum Levels in Prostate Cancer Patients and Controls**

Martin Marszalek\(^a,b\), Johann Wachter\(^a,b\), Anton Ponholzer\(^a,b\), Thomas Leitha\(^c\), Michael Rauchenwald\(^a,b\), Stephan Madersbacher\(^a,b,*\)

\(^a\)Department of Urology and Andrology, Donauspital, Langobardenstrasse 122, A-1220 Vienna, Austria

\(^b\)Ludwig Boltzmann Institute for Urological Oncology, Vienna, Austria

\(^c\)Department of Nuclear Medicine, Donauspital, Vienna, Austria

Accepted 14 March 2005

Available online 2 April 2005

**Abstract**

**Objective:** Insulin-like growth factor 1 (IGF-1) and chromogranin A (CGA) are currently discussed as supplemental serum markers for prostate cancer (PC) diagnosis. To address this issue we determined serum levels of IGF-1, CGA and PSA in men with newly diagnosed PC and controls.

**Methods:** A consecutive series of 156 men (median age: 67 yrs) with newly diagnosed, untreated PC and 271 controls (69 yrs) were recruited. The diagnosis of PC was made by transrectal ultrasound guided biopsies only. In controls, the presence of PC was excluded by digito-rectal examination, serum prostate specific antigen (PSA) levels by using age-specific reference values and–if indicated–by transrectal ultrasound guided 12-core biopsies. Serum levels of IGF-1, CGA and PSA were compared between cases and controls and correlated to histopathological findings and age.

**Results:** Serum PSA-levels were significantly higher in men with PC (49.6 ± 13.9 ng/ml, mean ± standard error of the mean; median: 7.0 ng/ml) than in controls (2.6 ± 0.2 ng/ml; median: 1.3 ng/ml) (\(p < 0.001\)). In contrast, serum levels of IGF-1 (PC: 166 ± 6.1 ng/ml, median: 155 ng/ml; controls: 159 ± 4.5 ng/ml, 153 ng/ml) and CGA (PC: 92 ± 7.4 U/l, median: 67 U/l; controls: 117 ± 12.0 U/l; median: 74 U/l) were identical in both groups (\(p > 0.05\)). Serum levels of IGF-1 and CGA revealed no correlation to serum PSA, Gleason score and number of positive biopsy cores. In the PC-cohort all three serum markers did not correlate with age. In controls, PSA (\(p = 0.018\)) and CGA (\(p < 0.001\)) correlated positively and IGF-1 (\(p < 0.001\)) negatively with age.

**Conclusion:** Our data suggest that quantification of IGF-1 and CGA-serum levels provides no useful information in the diagnosis of PC.

© 2005 Elsevier B.V. All rights reserved.

**Keywords:** Insulin-like growth factor; Chromogranin A; Tumour marker; Prostate

---

**1. Introduction**

Insulin-like growth factor 1 (IGF-1) is a growth hormone dependent polypeptide related to cell growth and differentiation [1]. IGF-1 is mainly produced in the liver in response to growth hormone secretion and regulates cell proliferation, differentiation and apoptosis in the prostate [2,3]. IGF-1 secretion seems to be modulated by prostate specific antigen (PSA) [4,5]. While prospective studies provided evidence for a relationship between circulating levels of both IGF-1 and IGF binding protein (IGFBP-3) and the risk for...
developing PC, the role of IGF-1 in the diagnosis of PC is controversially discussed [6–8].

Chromogranin A (CGA) is increasingly accepted as a serum marker for neuroendocrine differentiation of malignant tumours of various origins. PC-cells can undergo neuroendocrine differentiation resulting in a release of CGA as a paracrine factor [9]. These cells are usually distinct from prostate basal and secretory cells and produce numerous hormonal factors besides CGA including serotonin, histamine, somatostatin and vascular endothelial growth factor. In a recent study, human prostate neuroendocrine cells were found to represent a cell lineage of their own, being of neuro- genic origin and therefore distinct from the urogenital sinus-derived prostate secretory and basal cells [10,11]. Neuroendocrine cells invade the urogenital sinus around the 10th week of embryonal development [10,11]. Malignant differentiated neuroendocrine cell should be distinguished from normal prostatic neuroendocrine cells due to differences of cellular processes and morphologic features resembling adjacent cancer cells [10,11]. Studies on the role of CGA as a serum marker for PC are scant [12–14].

Aim of our study was to assess the role of serum levels of CGA and IgF-1 in the diagnosis of PC. We therefore determined IGF-1 and CGA serum levels in men with newly diagnosed PC and controls and correlated these values to serum PSA-levels, histopathological findings and age.

2. Materials and methods

2.1. Patients

Men with newly diagnosed, untreated PC and, as controls, men without PC were included to this study. Diagnosis of PC was made by transrectal ultrasound guided 12-core biopsies in all patients. Tumour grading was determined by the Gleason score and tumours were categorized into well (Gleason score 2 to 5), moderately (Gleason score 6–7) and poorly differentiated (Gleason score 8–10) tumours. In controls, the presence of PC was excluded by a negative digito-rectal examination (DRE) and serum PSA-level using age-specific reference values: 40–49 yrs: 2.5 ng/ml, 50–59: 3.5 ng/ml, 60–69 yrs: 4.5 ng/ml, 70–79 yrs: 6.5 ng/ml [15]. If indicated a 12-core transrectal ultrasound guided biopsy of the prostate was performed. Overall, 13.5% of controls (n = 37) underwent a prostate biopsy prior study inclusion. Institutional review board approval was obtained and patients/controls gave informed consent.

2.2. Methods

All serum samples of PC-patients were obtained before prostate biopsy. Total and free PSA serum levels were assessed using PSA Total EIA II and PSA Free EIA assay (both manufactured by Roche Diagnostics GmbH; D-68298 Mannheim, Germany): Total PSA: intraassay variation: 1.8–2.7%, interassay variation: 2.9–5.7%; free PSA: intraassay variation: 1.2–3.2%; interassay variation: 1.3–2.2%. IGF-1 serum levels were determined using the DSL-5600 assay (Diagnostic Systems Laboratories Inc, TX-77598 Webster, Texas, USA); intraassay variation: 1.5–3.4%; interassay variation: 1.5–8.2%. CGA serum levels were quantified with the CGA-RIA CT assay (CIS bio international, F-91192 Gif/Yvette Cedex, France): intraassay variation: 2.2–6.0%; interassay variation: 5.3–8.5%.

2.3. Statistical analysis

All statistical analyses were conducted using Statistical Package for Social Sciences, version 8.00 (SPSS Inc., Chicago, IL). We used Spearman correlation coefficient and Kruskal Wallis H test to assess differences in marker serum levels between groups and correlation of marker serum levels with Gleason scores and age. All statistical tests were two sided and statistical significance was set as p < 0.05.

3. Results

3.1. Patient characteristics

A total of 427 men (66.9 ± 10.3 yrs; median 68 yrs) entered this study, 156 men (66.7 ± 7.7 yrs; median 67 yrs) with newly diagnosed PC and 271 controls (67.1 ± 11.6 yrs; median 69 yrs) (Table 1). Serum PSA levels were significantly higher in the PC-group (49.6 ± 13.9 ng/ml; range: 41325.4 ng/ml; median 7.0 ng/ml) as compared to controls (2.6 ± 0.2 ng/ml; range: 0.1–18.7 ng/ml; median 1.3 ng/ml; p < 0.001) (Table 1, Fig. 1). In the PC-group, serum PSA levels of less than 10 ng/ml were present in 66.0%, 10.1–20 ng/ml in 12.8% and above 20 ng/ml in 21.2% of patients (Table 1). PSA levels above the age-specific ranges were present in 14% of controls and in 76.2% of PC-patients. Free to total PSA ratio was significantly lower in the PC-group (12.6 ± 0.6%; range: 2.8–36.0%; median 11.4%) as compared to controls (20.7 ± 1.1%; range: 2.1–47.9%; median 19.5%; p < 0.001). Initial therapy

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal patient characteristics</td>
</tr>
<tr>
<td>PC-patients</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Age [yrs]</td>
</tr>
<tr>
<td>PSA [ng/ml]</td>
</tr>
<tr>
<td>&lt;10 ng/ml</td>
</tr>
<tr>
<td>10.1–20 ng/ml</td>
</tr>
<tr>
<td>&gt;20 ng/ml</td>
</tr>
<tr>
<td>CGA [U/l]</td>
</tr>
<tr>
<td>IGF-1 [ng/ml]</td>
</tr>
</tbody>
</table>

Gleason score (biopsy results)

| 2–5 | 19 (15.6%) |
| 6–7 | 79 (64.8%) |
| 8–10 | 24 (19.6%) |

Median values and standard errors of the mean are given.
was radical prostatectomy in 122 (78.2%) patients, brachytherapy with Palladium103 in one (0.6%) and androgen deprivation therapy with LHRH-antagonists in 33 patients (21.2%).

### 3.2. IGF-1 and CGA serum levels

Serum IGF-1 levels averaged at 167 ± 6.0 ng/ml (range: 10.0–448.0 ng/ml; median 154.5 ng/ml) in the PC-group and at 159 ± 4.5 ng/ml (range: 5.0–530.0 ng/ml; median 153.0 ng/ml) in controls ($p = 0.33$) (Fig. 1). CGA serum levels were also comparable between PC-patients (92 ± 7.4 U/l; range: 17.1–423.6 U/l; median 67.4 U/l) and controls (117 ± 12.0 U/l; range: 17.0–1161.8 U/l; median 73.8 U/l; $p = 0.12$) (Fig. 1).

### 3.3. Impact of age on PSA, fPSA, IGF-1 and CGA serum levels

The impact of age on PSA, fPSA, IGF-1 and CGA serum levels was analysed separately for PC-patients and controls (Fig. 2). In PC-patients, there was no correlation ($p > 0.05$) between patient age and PSA, CGA or IGF-1 (Fig. 2). In controls, however, PSA ($p = 0.018$) and CGA ($p < 0.001$) correlated positively and IGF-1 ($p < 0.001$) negatively with age (Fig. 2). Free PSA serum levels were positively correlated to age in cases ($p = 0.002$) and controls ($p = 0.045$).

### 3.4. Impact of tumour grading on PSA, IGF-1 and CGA serum levels

To minimize grading errors, only radical prostatectomy specimens ($n = 122$) were used for subsequent analyses: well defined tumours (Gleason score 2–5) were present in 19 (15.6%), moderately defined tumours (Gleason score 6–7) in 79 (64.8%) and poorly defined tumours (Gleason score 8–10) in 24 (19.6%) (Table 1). Serum-PSA increased from $5.5 ± 2.1$ ng/ml (median ± standard error of the mean) in those with highly differentiated tumours to $13.8 ± 10.5$ ng/ml in those with poorly differentiated tumours (Gleason score 8–10) (Fig. 3). In contrast, neither IGF-1 nor CGA-serum levels were correlated to the Gleason score on radical prostatectomy specimens (Fig. 3). Because of the discrepancy between highly significant differences in PSA serum levels between cases and controls and non significant differences of IGF-1 and CGA serum levels, we abstained from calculating ratios of these different serum markers.

### 4. Discussion

The interest in the insulin-like growth factor axis, in particular of IGF-1 and IGF binding protein 3 (IGFBP-3), for PC was primarily triggered by prospective studies providing evidence for a relationship between IGF-1 and IGFBP-3 serum levels and the risk of developing PC [7,16,17]. Wolk et al. reported on an association between serum levels of IGF-1 and the risk of PC [18]. In a nested case-control study, Chan et al. concluded that circulating levels of IGF-1 and IGFBP-3 may predict the risk of developing advanced-stage PC, but their utility for screening patients with early stage disease may be limited [7]. Oliver et al. investigated 176 cases and 324 matched controls selected out of a cohort of 7,383 men and observed that the risk of PC increased across quartiles of IGF-1 and IGF-2 [19]. Associations between IGFs and cancer risk were stronger for advanced cases [19]. Woodson et al. challenged these encouraging reports in a prospective Finnish study that observed no evidence to support a causal association between serum IGF-1 or IGFBP-3 and the risk of PC [20]. Nevertheless, the majority of studies support the role of IGF-1 as a predictor for PC-development. Recently, Janssen et al. have shown that measurement of serum levels of IGF-1 and/or IGFBP-3 in addition to PSA does not improve the identification of men at high risk to develop early stages of prostate cancer [21]. The authors concluded that the endocrine IGF-1 system is not directly involved in the growth of early stages of prostate cancer [21].
The role of IGF-1 in the diagnosis of PC is more controversially discussed. The negative findings of our study are in line with the majority of series suggesting that IGF-1 serum levels provide no relevant information for the diagnosis of PC. Cutting et al. analysed 94 consecutive patients undergoing TRUS-guided 6-core prostate biopsies [22]. The authors observed no statistical significant difference of serum IGF-1 levels between PC-patients and controls [22]. Finne et al. quantified IGF-I and IGFBP-3 serum levels in 665 consecutive men undergoing prostate biopsies [23]. After adjustment for prostate volume, the negative association between serum IGF-1 and PC-risk was no longer significant [23]. The authors concluded that serum IGF-1 is not a useful diagnostic test for PC [23]. Ismail et al. investigated 652 men undergoing prostate biopsies and concluded that serum IGF-1 and IGFBP-3 do not predict the results of prostate biopsy [8]. In a subsequent study, serum IGF-1 and IGFBP-3 did not correlate with tumour volume or Gleason score [24]. Again these data are in line with our study as we also observed—in contrast to PSA—no correlation between IGF-1 serum levels and Gleason score (Fig. 3). Djavan et al. investigated 245 consecutive men with PSA-levels between 2.5–15 ng/ml undergoing octant biopsies [17]. Although IGF-1 and IGF-1 density were

---

Fig. 2. Impact of age on serum levels of PSA, IGF1 and CGA in PC-patients and controls. In PC-patients, there was no correlation between PSA, IGF1 and CGA and age. In contrast, all three markers were correlated to age in the control group.

Fig. 3. Correlation of PSA, IGF-1 and CGA with Gleason-score. Columns indicate median values.
unable to enhance the performance of PSA, the IGF-1/PSA ratio significantly improved PC-detection over the use of PSA alone [17]. Nam et al. studied 1,031 consecutive men undergoing prostate biopsy because of elevated serum PSA-levels [25]. In their study IGF-1 levels were lower in cases than in controls \( (p = 0.05) \) yet not predictive for the presence of PC [25]. Similar findings were reported by Baffa et al. [26]. In summary, the majority of studies failed to demonstrate a role of IGF-1 for diagnosis of PC.

One potential limitation of our approach is the exclusion of PC in our control group. Although all individuals with PSA-values above the age-specific ranges underwent a 12-core ultrasound guided biopsy, we can not fully exclude PC in these controls (such as in those with normal PSA and DRE). It is worth to note that the median PSA-value in our control group was 1.3 ng/ml.

The role of CGA in PC is not well documented [11–13]. Though CGA appears to follow serum levels of the dominant tumour marker our data suggest that this is not the case in PC as we observed no correlation between CGA and PSA. Furthermore, CGA-serum levels did not correlate to the Gleason score (Fig. 3). These data are in contrast to a recent series of Sciarra et al. who observed an association between CGA and pathological stage in 83 patients undergoing radical prostatectomy [27]. There is accumulating evidence, however, that CGA might play a role as a marker for advanced PC, particularly under androgen deprivation and during the hormone refractory phase of the disease [12,13,28,29]. The pathomechanism leading to the age-related increase of CGA in our control population are unclear (Fig. 2). One can hypothesize that neuroendocrine differentiated cells increase in number with benign prostatic enlargement and lead—in parallel to PSA—to an age-related increase in those without PC.

In conclusion our data indicate that quantification of IGF-1 and CGA-serum levels provides no useful information in the diagnosis of PC.

References


Abstract

**Objectives:** To assess the factors effecting PSA bounce and to identify any possible relationship with biochemical control after 3-D conformal radiotherapy (3D-CRT) and total androgen deprivation (TAD) for prostate cancer by evaluating four previously described PSA bounce definitions.

**Methods:** Between January 1998 and January 2001, 83 consecutive patients with clinically localized prostate cancer were treated by 3D-CRT with neoadjuvant 3 months and/or 6 months adjuvant TAD. All patients had a pretreatment PSA level, at least eight post-external beam radiotherapy (EBRT) PSA and testosterone levels and minimum two years of follow-up. Total radiotherapy dose was 73.6 Gy at ICRU reference point. Four previous definitions of PSA bounce were used: Critz definition (≥0.1 ng/mL), Cavanagh definition (≥0.2 ng/mL), Hanlon definition (≥0.4 ng/mL) and Rosser definition (≥0.5 ng/mL) according to original methodology performed to report PSA bounce. Biochemical failure was defined in accordance with the ASTRO consensus guidelines.

**Results:** The median follow-up time was 40 months. PSA bounce was recorded as follows: Critz definition, 33 patients (40%); Cavanagh definition, 21 patients (25%); Hanlon definition, 11 patients (13%); and Rosser definition, 7 patients (8%). In multivariate analysis, pre-EBRT PSA level and the duration of TAD for Critz definition; age, pre-EBRT PSA and the duration of TAD for Cavanagh definition; age and duration of TAD for Hanlon definition; age and pre-biopsy PSA for Rosser definition were significant independent prognostic factors determining PSA bounce. A significant increase of mean testosterone level in bouncers was detected at the 6th–9th and 18th–21st months. PSA bounce did not predict for PSA failure in multivariate analysis.

**Conclusions:** We observed no correlation between biochemical failure and PSA bounce. The longer duration of TAD and older age were found to be inversely proportional with PSA bouncing in this cohort. Notably, recovery of testosterone might cause PSA bouncing.

© 2005 Elsevier B.V. All rights reserved.

**Keywords:** Prostate cancer; Radiotherapy; Androgen deprivation; PSA bounce

1. Introduction

PSA levels are expected to decrease after external beam radiation therapy (EBRT) to nadir, but usually remain detectable. The issue of an appropriate definition of prostate specific antigen (PSA) failure (biochemical failure) after EBRT has been determined with ASTRO consensus of three consecutive increases [1]; however a single rise in post-EBRT PSA level continues to be a source of considerable anxiety due to the intriguing uncertainty of relationship between PSA bouncing and disease relapse.
Various definitions of PSA bounce have been used in the literature. Critz et al. [2] used a PSA bounce as a rise of $\geq 0.1$ ng/mL after BRT and EBRT. Cavanagh et al. [3] defined an increase of $\geq 0.2$ ng/mL after BRT. Hanlon et al. [4] described a PSA bounce after EBRT as a rise of $\geq 0.4$ ng/mL, while Rosser et al. [5] recently used an increase of $\geq 0.5$ ng/mL as PSA bounce definition. A PSA bounce phenomenon was not previously reported after 3-dimensional conformal radiotherapy (3D-CRT) and total androgen deprivation (TAD). The aim of this study was to evaluate the frequency of bouncing, the possible factors effecting PSA bounce and to identify any correlation between bouncing and biochemical control (bNED) following 3D-CRT combined with short-term AD (STAD) for prostate cancer by evaluating four previously described PSA bounce definitions.

2. Materials and methods

2.1. Patient characteristics

We analyzed 83 patients with clinical stage T2-T3 prostate adenocarcinoma without radiological evidence of lymph node metastasis and distant spread, who were treated between January 1998 and January 2002 in our institutional protocol. The latter date was chosen to have at least 24 months of follow up. The median age was 68 years (range, 53–79). Patients were staged according to the American Joint Committee for Cancer Staging System (AJCC) 1997 [6]. T stages and Gleason scores of patients are shown in Table 1.

2.2. 3-D Conformal radiotherapy

Clinical target volume (CTV) was prostate and seminal vesicles. Seven 6 MV photon beams (anterior, right and left lateral, right and left anterior oblique, right and left posterior oblique) which were equally weighted were used. A total dose of 70 Gy with daily fraction dose of 2 Gy was prescribed to planning target volume (PTV) in all patients regardless of risk group. ICRU reference point (isocenter) dose was 73.6 Gy.

2.3. Short-term androgen deprivation (STAD)

Extracapsular extension (stage T3a-b), PSA $\geq 10$ ng/dl and Gleason Score (GS) 7 and above were considered as high risk criteria and in case of even one positive factor, the patient was considered in the high risk group. If all above factors were negative, patients were treated in the low risk group. All patients were administered neoadjuvant TAD with either triptoreline acetate or goserelin acetate and ciproterone acetate for 3 months and thereafter were given 3D-CRT. Total androgen deprivation has been stopped during EBRT. In high risk patients, same TAD regimen was continued for further 6 months after the completion of EBRT. Thus, seventeen patients were in the low and 66 were in the high risk group in our series.

2.4. Biochemical failure and PSA bounce definition

Biochemical relapse failure was determined according to American Society for Therapeutic Radiology and Oncology (ASTRO) consensus definition that is 3 consecutive increases in post treatment PSA after achieving a nadir [1].

Four previous definitions were used according to their original methodology to report PSA bounce [2–5]. Critz definition: PSA bounce is defined by a PSA increase of 0.1 ng/ml or greater above the level before bounce followed by a subsequent decrease to or below that level with PSA 0.2 ng/ml as the floor. Hanlon definition: A minimal rise of 0.4 ng/ml over a 6 month follow up period, i.e., an increase with a slope $\geq 0.07$, followed by a drop in PSA level of any magnitude. The drop in PSA may be immediate or following subsequent maintenance and/or increase in PSA. Additionally, the time lapse between two PSA levels used for slope calculations was required to be at least 30 days. Rosser definition: An initial PSA increase of at least 0.5 ng/ml, followed by a decrease to pre-bounce baseline serum PSA value no more than 60 months after EBRT. Cavanagh definition: A PSA increase of $\geq 0.2$ ng/ml, followed by a drop in PSA level of any magnitude.

We have also analyzed the correlation of the kinetic of testosterone (3 months interval changes) with the occurrence of PSA bouncing as well as the correlation with biochemical failure.

2.5. Follow-up

Patients were seen in every 3 months for the first 2 years, 4 months for the 3rd and 4th year every 6 months thereafter. In each visit, total serum PSA, free PSA, total testosterone levels, and prostate volumes were measured. All patients had a pretreatment PSA level and at least eight post-EBRT PSA levels in order to assess PSA bounce.

2.6. Statistical analysis

Biochemical relapse failure date was calculated according to ASTRO consensus definition [1]. The freedom from biochemical failure (bNED) was calculated from the end of 3D-CRT for low risk group or adjuvant TAD for high risk group. Differences in percentages for categorical variables according to bouncing were evaluated using the $\chi^2$-test. Mann-Whitney U test was used for the comparison of differences between the means of continuous variables. Logistic regression analysis was performed to assess the independent predictive factors for PSA bounce. Kaplan-Meier method was used for survival estimates. The Log Rank test was used to evaluate differences between subgroups. Cox proportional hazards regression model was performed for multivariate prognostic factor analysis of bNED. Statistical significance was assigned to $p$ values of 0.05 or less. All statistical analyses were performed by SPSS 12.0 (SPSS Inc., Chicago, IL).

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T stage and Gleason scores of patients</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AJCC Stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2a</td>
<td>31</td>
<td>36</td>
</tr>
<tr>
<td>T2b</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>T3a</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>T3b</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td><strong>Gleason Score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–6</td>
<td>44</td>
<td>65</td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>8–10</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

Abbreviations: N = number of patients, AJCC = American Joint Committee for Cancer.
3. Results

The median follow-up time was 40 months (range, 24–88 months); 46 months for the low risk, and 39 months for the high risk groups. PSA failure was detected in 19 out of 83 patients. PSA bounce was recorded as follows: Critz definition, 33 patients (40%); Cavanagh definition, 21 patients (25%); Hanlon definition, 11 patients (13%); and Rosser definition, 7 patients (8%).

Univariate analyses are summarized in Table 2. Patients with a recorded PSA bounce had significantly lower pre-biopsy (according to Critz, Cavanagh and Rosser definitions), and pre-EBRT PSA levels (according to Critz and Cavanagh definitions). Older patients less likely experienced a PSA bounce (according to Cavanagh, Hanlon and Rosser definitions). Furthermore, patients receiving only neoadjuvant TAD had significantly higher incidence of PSA bounce in comparison with neoadjuvant plus adjuvant TAD (according to Critz, Cavanagh, and Hanlon definitions). Logistic regression model for multivariate analysis including age, T stage, Gleason score pre-biopsy PSA, pre-RT PSA, prostate volume, testosterone level and duration of TAD showed that pre-EBRT PSA level and duration of TAD (Critz definition); age, pre-EBRT PSA and duration of TAD (Cavanagh definition); age and duration of TAD (Hanlon definition); age and pre-biopsy PSA (Rosser definition) were significant independent prognostic factors determining PSA bounce (Table 3).

The relationship between testosterone kinetics and PSA bounce is summarized in Table 4. We have found a significant increase in the mean testosterone level of the bouncers at the 6th–9th months according to Critz, Cavanagh, and Hanlon definitions. Similarly, a significant increase in the mean testosterone level of the bouncers was detected according to Critz and Cavanagh definitions at the 18th–21st months. We have not detected any correlation between biochemical failure and the kinetic of testosterone.

To determine whether a PSA bounce was a predictor of biochemical failure, the effect of bounce on bNED was tested. Among four definitions, only Critz definition was found to have a significant impact on bNED in univariate analysis (74% for bouncers and 43% for non-bouncers).

### Table 2
Effect of different factors on developing a PSA bounce in univariate analyses

<table>
<thead>
<tr>
<th></th>
<th>Bounce presence</th>
<th>Critz definition</th>
<th>Cavanagh definition</th>
<th>Hanlon definition</th>
<th>Rosser definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>–</td>
<td>68</td>
<td>0.2</td>
<td>68</td>
<td>0.03</td>
</tr>
<tr>
<td>+</td>
<td>66</td>
<td>64</td>
<td>0.007</td>
<td>22</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean prebiopsy-PSA (ng/mL)</td>
<td>–</td>
<td>22</td>
<td>0.01</td>
<td>2</td>
<td>0.004</td>
</tr>
<tr>
<td>+</td>
<td>16</td>
<td>13</td>
<td>0.07</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Mean Prostate volume (cc)</td>
<td>–</td>
<td>42</td>
<td>0.07</td>
<td>42</td>
<td>0.1</td>
</tr>
<tr>
<td>+</td>
<td>38</td>
<td>38</td>
<td>0.6</td>
<td>77</td>
<td>0.4</td>
</tr>
<tr>
<td>Mean testosterone level (ng/dl)</td>
<td>–</td>
<td>86</td>
<td>0.6</td>
<td>77</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>109</td>
<td>154</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Categorical variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2a-b</td>
<td>–</td>
<td>28</td>
<td>0.1</td>
<td>37</td>
<td>0.2</td>
</tr>
<tr>
<td>+</td>
<td>25</td>
<td>16</td>
<td>0.2</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>T3a-b</td>
<td>–</td>
<td>22</td>
<td>0.2</td>
<td>25</td>
<td>0.2</td>
</tr>
<tr>
<td>+</td>
<td>8</td>
<td>5</td>
<td>0.2</td>
<td>5</td>
<td>0.2</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–6</td>
<td>–</td>
<td>31</td>
<td>0.2</td>
<td>37</td>
<td>0.08</td>
</tr>
<tr>
<td>+</td>
<td>23</td>
<td>17</td>
<td>0.2</td>
<td>17</td>
<td>0.2</td>
</tr>
<tr>
<td>7–10</td>
<td>–</td>
<td>19</td>
<td>0.2</td>
<td>25</td>
<td>0.2</td>
</tr>
<tr>
<td>+</td>
<td>10</td>
<td>4</td>
<td>0.2</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td>Duration of TAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>–</td>
<td>5</td>
<td>0.004</td>
<td>7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>+</td>
<td>12</td>
<td>10</td>
<td>0.004</td>
<td>10</td>
<td>0.03</td>
</tr>
<tr>
<td>9 months</td>
<td>–</td>
<td>45</td>
<td>0.5</td>
<td>55</td>
<td>0.6</td>
</tr>
<tr>
<td>+</td>
<td>21</td>
<td>11</td>
<td>0.5</td>
<td>11</td>
<td>0.6</td>
</tr>
</tbody>
</table>

TAD = total androgen deprivation.
* Figures represent the number of patients.
non-bouncers, \( p = 0.04 \). However, PSA bounce of Critz definition did not predict for PSA failure in multivariate analysis.

### 4. Discussion

PSA is expected to decrease slowly to reach nadir levels after a successful radiotherapy [7–9]. ASTRO has defined three consecutive PSA rises as a biochemical failure [1]. However, transient increase in PSA level followed by a decrease to preceding value called as PSA bounce is a significant source of anxiety for both clinicians and patients. There are various definitions of PSA bounce after a variety of radiation techniques with or without AD [2–5]. Thus, we performed a study to evaluate PSA bounce phenomena in our patients with prostate cancer via four previously reported definitions. To the best of our knowledge, this is the first study assessing the PSA bounce phenomena in patients treated with 3D-CRT and STAD, as well as its relationship with the kinetics of testosterone.

The previous series evaluating PSA bounce were not consistent in terms of dose, technique or hormonotherapy [2–5,10–12]. In contrast, our data consisted of patients who were uniformly treated in terms of radiation technique (3D-CRT) and dose (73.4 Gy at ICRU point). As all patients were administered STAD, only the duration of AD differed; 3 months for low risk and 9 months for high risk groups.

It can be argued that if the radiobiology of EBRT can be directly compared to that of brachytherapy; this hint is indeed pressing when one compares their PSA bouncing curves. But our methodology of comparing EBRT with BRT is not the first in literature. Similar method was used by others [12]. Stock et al. implied that PSA bounce after brachytherapy may be different from the bounce seen after EBRT [12]. Stock et al. also stated that PSA bounce after EBRT may represent an early manifestation of PSA failure in a certain percentage of patients and the phenomena seen after brachytherapy may seem to have little effect on PSA failure developing; however our data contrasts with their proposal that we found no association between bNED and PSA bounce regardless of the definition used.

Among four definitions, only Hanlon et al. [4] showed a significant relationship of bouncing to bNED control, with bouncers and non-bouncers having 5-year rates of 52% and 69% respectively. They also showed that statistical significance was demonstrated only for patients presenting with pre-biopsy PSA levels <10 ng/ml after stratifying the bouncers with pretreatment PSA level. In current series, we had only 24 patients with pre-biopsy PSA level of 10 and below and among them there were only 3 patients with PSA relapse. Thus, we could not make further subgroup analysis in order to test this issue due to small sample size. Nevertheless Hanlon et al. concluded that bouncing activity should not be used as a sole measure of

### Table 3
Factors significantly associated with PSA bounce in logistic regression analyses

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>p</th>
<th>Exp(B)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critz definition (PSA ( \geq 0.1 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-EBRT PSA</td>
<td>–0.52</td>
<td>0.05</td>
<td>0.59</td>
<td>0.36 0.987</td>
</tr>
<tr>
<td>Duration of TAD</td>
<td>–1.31</td>
<td>0.04</td>
<td>0.27</td>
<td>0.079 0.914</td>
</tr>
<tr>
<td>Cavanagh definition (PSA ( \geq 0.2 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>–0.16</td>
<td>0.008</td>
<td>0.85</td>
<td>0.75 0.96</td>
</tr>
<tr>
<td>Pre-EBRT PSA</td>
<td>–1</td>
<td>0.04</td>
<td>0.37</td>
<td>0.14 0.95</td>
</tr>
<tr>
<td>Duration of TAD</td>
<td>–2.36</td>
<td>0.003</td>
<td>0.09</td>
<td>0.02 0.45</td>
</tr>
<tr>
<td>Hanlon definition (PSA ( \geq 0.4 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>–0.24</td>
<td>0.004</td>
<td>0.78</td>
<td>0.67 0.92</td>
</tr>
<tr>
<td>Duration of TAD</td>
<td>–2.89</td>
<td>0.005</td>
<td>0.057</td>
<td>0.007 0.42</td>
</tr>
<tr>
<td>Rosser definition (PSA ( \geq 0.5 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>–0.20</td>
<td>0.02</td>
<td>0.82</td>
<td>0.69 0.97</td>
</tr>
<tr>
<td>Pre-biopsy PSA</td>
<td>–0.19</td>
<td>0.05</td>
<td>0.82</td>
<td>0.68 0.99</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval, PSA = prostate specific antigen, TAD = total androgen deprivation.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>p</th>
<th>Exp(B)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critz definition (PSA ( \geq 0.1 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-EBRT PSA</td>
<td>–0.52</td>
<td>0.05</td>
<td>0.59</td>
<td>0.36 0.987</td>
</tr>
<tr>
<td>Duration of TAD</td>
<td>–1.31</td>
<td>0.04</td>
<td>0.27</td>
<td>0.079 0.914</td>
</tr>
<tr>
<td>Cavanagh definition (PSA ( \geq 0.2 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>–0.16</td>
<td>0.008</td>
<td>0.85</td>
<td>0.75 0.96</td>
</tr>
<tr>
<td>Pre-EBRT PSA</td>
<td>–1</td>
<td>0.04</td>
<td>0.37</td>
<td>0.14 0.95</td>
</tr>
<tr>
<td>Duration of TAD</td>
<td>–2.36</td>
<td>0.003</td>
<td>0.09</td>
<td>0.02 0.45</td>
</tr>
<tr>
<td>Hanlon definition (PSA ( \geq 0.4 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>–0.24</td>
<td>0.004</td>
<td>0.78</td>
<td>0.67 0.92</td>
</tr>
<tr>
<td>Duration of TAD</td>
<td>–2.89</td>
<td>0.005</td>
<td>0.057</td>
<td>0.007 0.42</td>
</tr>
<tr>
<td>Rosser definition (PSA ( \geq 0.5 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>–0.20</td>
<td>0.02</td>
<td>0.82</td>
<td>0.69 0.97</td>
</tr>
<tr>
<td>Pre-biopsy PSA</td>
<td>–0.19</td>
<td>0.05</td>
<td>0.82</td>
<td>0.68 0.99</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval, PSA = prostate specific antigen, TAD = total androgen deprivation.

## Table 4
The relationship between the kinetics of testosterone and PSA bouncing

<table>
<thead>
<tr>
<th></th>
<th>Bounce presence</th>
<th>N</th>
<th>Mean testosterone increase at the 6th–9th months (ng/dL)</th>
<th>p</th>
<th>Mean Testosterone increase at the 18th–21th months (ng/dL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critz definition (PSA ( \geq 0.1 ))</td>
<td>–</td>
<td>50</td>
<td>2</td>
<td>0.02</td>
<td>32</td>
<td>0.05</td>
</tr>
<tr>
<td>+</td>
<td>33</td>
<td>193</td>
<td>0.02</td>
<td>108</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Cavanagh definition (PSA ( \geq 0.2 ))</td>
<td>–</td>
<td>62</td>
<td>3</td>
<td>0.03</td>
<td>160</td>
<td>0.06</td>
</tr>
<tr>
<td>+</td>
<td>21</td>
<td>268</td>
<td>0.03</td>
<td>47</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Hanlon definition (PSA ( \geq 0.4 ))</td>
<td>–</td>
<td>72</td>
<td>29</td>
<td>0.03</td>
<td>47</td>
<td>0.06</td>
</tr>
<tr>
<td>+</td>
<td>11</td>
<td>230</td>
<td>0.03</td>
<td>47</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Rosser definition (PSA ( \geq 0.5 ))</td>
<td>–</td>
<td>76</td>
<td>26</td>
<td>0.08</td>
<td>56</td>
<td>0.07</td>
</tr>
<tr>
<td>+</td>
<td>7</td>
<td>159</td>
<td>0.08</td>
<td>56</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

\( N = \) number of patients.
disease relapse and more importantly, as an indicator for the institution of salvage therapies since half of their patients with PSA bounce were free of biochemical failure.

Evident data related with PSA bounce phenomena in case of AD and radiotherapy is limited. Patel et al. reported that the use of AD did not affect the incidence of PSA bounce using 0.2 ng/ml as PSA increase cut-off in his series of 295 patients treated with BRT and 6 months neoadjuvant AD (62% of cohort) [10]. Similarly, Sengoz et al. also could not detect any PSA bounce difference among 72 patients with prostate cancer receiving EBRT + AD (82% of cohort) and EBRT alone [11]. They used AD as adjuvant, and the median duration was 6 months (range 4 to 54 months). TAD has a suppressive effect on follow-up PSA levels as expected. Thus, we allowed at least 24 months of follow up for the recovery of testosterone levels. In our series, 13% of patients with TAD according to Hanlon definition showed PSA bounce compared to 17% of patients with AD in Sengoz series which used the same definition. Interestingly, we found that the duration of TAD was inversely proportional with PSA bounce incidence. This finding can not be truly compared with Patel and Sengoz series, because all of our patients received TAD whereas other two series had comparison of their patients between AD and non-AD groups. However we suggest that prolongation of TAD seems to decrease the incidence of PSA bounce. Longer hormonotherapy may prevent bouncing as testosterone often does not rise for several months, sometimes a year or more, following completion of LHRH-agonist therapy due to a marked decrease in the number of Leydig cells as well as peritubular thickening and fibrosis in the testis after longer term use of LHRH agonists [13,14]. Therefore, we ensured at least two years of follow-up in our series to allow sufficient period of time for the recovery of androgen levels and its possible influence on PSA levels.

We have detected a higher bounce frequency in younger men compared to older men according to Cavanagh, Hanlon and Rosser definitions in our data while this is an incidental observation; surprisingly it seems to be supported in at least three studies in Critz, Stock and Patel series [10,12,15]. The effect seen with age was not significant but close to significance, possibly due to the small number of bouncers with increasing threshold for PSA bounce. The previous prospective studies showed that the median time to normalization of testosterone in majority of patients took 6–18 months after withdrawal of androgen deprivation therapy [16–21]. In addition, delay in recovery of testosterone after more prolonged periods of androgen suppression can be anticipated; especially with 3 months depot preparations of luteinizing hormone releasing agonists, like we used [16]. Accordingly in our cohort, the first testosterone increase correlated with PSA bounce signify 9 months after the end of neoadjuvant hormonotherapy in low risk patients and the second increase was 12 months after the end of adjuvant hormonotherapy in high risk patients.

Scattered radiation dose to the testicles above 1 Gy may also depress Leydig cell function [18,22–26]. This observation was mainly based on data using large pelvic fields, which would have generated larger scattered radiation doses to the testis [23–26]. Additionally, the effects of radiation on testicles and the luteinizing hormone suppression may have an additive or synergistic effect on Leydig cell dysfunction [18,25]. Consequently, recovery of Leydig cells following the radiotherapy itself may cause a PSA bounce, just as recovery after hormonal therapy. It is also likely that recovery of Leydig cells after combined hormonotherapy and radiotherapy decreases with age [25]. This may explain the negative association between increasing age and occurrence of PSA bounce. Another explanation could be that elderly men are more often treated for high-risk prostate cancer and, hence, are more likely to receive adjuvant hormonal therapy. Furthermore, a direct effect of radiotherapy on the prostate can also be hypothesized. Miller et al. demonstrated a statistically significant increase in serum testosterone levels in 63 patients who had undergone radical prostatectomy [27]. They suggested that there was the production of substance(s) by the prostate that can affect the feedback mechanisms of gonadotrophin secretion. However whether radiotherapy could have a similar effect is not known. If so, PSA bouncing in patients receiving brachytherapy or EBRT without AD may be due to this mechanism of testosterone increase. There were also other hypothesis regarding sexual activity [10,15], or delayed apoptotic event [10] which we did not evaluate in the current study. An evidence based explanation of all those issues is still lacking, therefore needs to be investigated in further trials.
5. Conclusions

PSA bouncing occurs 8–40% of patients treated with 3D-CRT and STAD depending on the bounce definition. We observed no correlation between biochemical failure and PSA bounce. The longer duration of TAD and older age were found to be inversely proportional with PSA bouncing in this cohort. Notably, recovery of testosterone after EBRT+AD seems to cause PSA bouncing.

References

[27] Critz FA. Time to achieve a prostate specific antigen nadir of 0.2 ng/ml after simultaneous irradiation for prostate cancer. J Urol 2002;168:2434–8.
Abstract

Objective: We examined distribution and numbers of extramural ganglion cells in the male pelvis, classifying them as sympathetic or parasympathetic.

Methods: Specimens were obtained from 14 formalin-fixed donated male cadavers. Semiserial sections were processed for histologic examination, and for immunohistochemistry using anti-tyrosine hydroxylase (TH) or anti-peptide histidine isoleucine (PHI).

Results: Like those along the sacral sympathetic trunk, most other pelvic ganglion cells were located in and along nerve components. Yet the ganglion cell clusters attached to pelvic viscera accounted for 22% to 38% of ganglion cells. These were seen at the dorsal aspect of the bladder, the bladder/prostate junction, the dorsal aspect of the seminal vesicle, and along the prostate, but not along the extrapelvic pudendal nerve, cavernous tissues including the penile hilum, the rhabdosphincter, retropubic fat or recto-urethral muscle. Two fold interindividual variation was seen for total ganglion cell number (3044 to 6522) in the pelvis. TH-positive and PHI-positive cells intermingled at various ratio in every ganglion cell cluster. Sympathetic TH-positive proportions tended to be site-specific.

Conclusions: Pelvic autonomic cells exist not only in nerve components but also along viscera. Even nerve-sparing radical prostatectomy can compromise visceral ganglia. Simple classification of pelvic nerve components as sympathetic or parasympathetic would seem misleading given coexistence of both cell types in a ganglion.

Keywords: Ganglion; Male pelvis; Immunohistochemistry; Radical prostatectomy

1. Introduction

In nerve-sparing surgery in the male pelvis, major structures designated for preservation include nerve bundles such as the cavernous nerve and hypogastric nerve. In contrast, pelvic autonomic ganglion cells have received little consideration in nerve-sparing strategies. However, surgical damage to ganglion cells would seem likely to have far worse consequences than nerve bundle injury since, unlike nerve fibers, ganglion cells lack capacity for repair [1].

According to previous knowledge concerning distribution of pelvic autonomic ganglia, parasympathetic
ganglia are located near or along the pelvic viscera, while sympathetic ganglia along the lumbar and sacral sympathetic nerve trunks [2,3]. Thus, one would expect that parasympathetic ganglion cells should remain intact after surgery if the visceral nerve target such as the bladder is successfully preserved. Moreover, the sympathetic trunk generally is located far dorsally, out of the surgical field. If these generalizations are true, little or no specific attention need be given for preservation of pelvic ganglion cells during surgery. We are not aware of a previous study specifically focused on qualitative and quantitative delineation of human pelvic ganglion distribution. The aims of this preliminary work were to describe distribution and number of ganglion cells at each site in the male pelvis using semiserial sections and to classify these cells as sympathetic or parasympathetic.

2. Materials and methods

Histologic specimens were obtained from 14 donated male cadavers (72 to 85 years old at death), that had been fixed by arterial injection of 10% formalin solution. The cadavers harbored no macroscopic tumor in thoracoabdominal regions. To facilitate sectioning and identification of structures, intrapelvic soft tissues usually were divided into three or four large blocks including the hypogastric nerve and distal ureter; the bladder, prostate, seminal vesicle, and rectum; the membranous urethra, penile hilum, and levator ani; and, for half of the cadavers, the pelvic splanchnic nerves. To isolate these nerves, we separated the rectum slightly from the presacral space to obtain a sheetlike specimen (fourth block) including nearly the entire course of the pelvic splanchnic nerves.

After routine processing for paraffin embedding, semiserial sections 6 to 10 μm in thickness were cut at 1-mm intervals. For each section stained with hematoxylin and eosin (H and E), two or three adjacent sections were processed for immunohistochemistry at sites chosen according to observations in H and E sections. The primary antibody used to identify sympathetic neurons was polyclonal rabbit anti-tyrosine hydroxylase (TH; Chemicon International, Temecula, CA; 1:400 dilution in phosphate-buffered saline, or PBS). TH is expressed by noradrenergic sympathetic neuron [4,5]. To identify parasympathetic neurons, we used rabbit anti-peptide histidine isoleucine (PHI) anti-serum (Yanaihara Institute, Fujinomiya, Japan; 1:200 dilution in PBS). PHI is considered to colocalize with vasoactive intestinal peptide (VIP), a major marker for parasympathetic nerve cells [6]. PHI- and VIP-immunoreactive nerves were similarly distributed in the human and experimental animal tissues [7,8].

3. Results

3.1. Routine histology

Pelvic ganglion cells displayed a homogeneous morphology, appearing large and eosinophilic, with a diameter of 25 to 30 μm. These cells were found along nerve fiber bundles, in enlargements of nerve bundles, and in round or oval, grossly identifiable ganglia surrounded by a thick connective tissue capsule (Figs. 1–3). However, because of numerous intermediate morphologies between a macroscopically typical ganglion and enlargements along a nerve, and because
cell numbers found in a ganglion varied significantly between sites and individuals, strict definition of a “ganglion” proved to be difficult. We therefore adopted the term, “ganglion cell cluster” (GCC) for the present description. GCC were seen in or along nerve components such as the pelvic splanchnic, cavernous, and hypogastric nerves, and the pelvic plexus, as well as near or along pelvic visceral surfaces (Table 1, Fig. 4).

In and along macroscopically identified nerve components, many ganglion cells could be found (Table 1, upper portion). Along pelvic splanchnic nerves, ganglion cells usually were distributed distally, 15 to 30 mm ventral from the anterior sacral foramina (Fig. 3). However, in some individuals, these cells were found along nearly the entire nerve course, including a site immediately ventral to the anterior sacral foramen. Great interindividual differences in cell numbers were evident along splanchnic nerves (84 to 1262 cells). Because the cavernous nerve runs near or along the levator ani, GCC sometimes were located upon the fascia covering this muscle. The hypogastric nerve, previously believed to contain mainly sympathetic nerve fibers, also contained GCC, even a large ganglion-like mass, along its distal course near the distal ureter at levels caudal to S2 (not illustrated). The pelvic plexus along the lateral aspect of the seminal vesicle also contained many ganglion cells (250 to 1113). GCC were not attached to the seminal vesicle, but were separated from it by at least 1.0 mm (Fig. 1C). Macroscopically, grayish ganglia found along the sacral sympathetic trunk numbered 2 to 8 per pelvis. However, the size of these ganglia (1 to 6 mm in maximum length) and cell numbers (249 to 1566) varied widely between individuals. In addition, we sometimes found
ganglion cells along nerves passing through the levator ani [9] (Fig. 1D).

Along the pelvic visceral surface GCC were not distributed evenly, but rather at limited sites located on the dorsal aspect of the bladder, the bladder/prostate junction (Fig. 1A), the dorsal aspect of the seminal vesicle, and along the prostate (Table 1, lower portion). At these sites, one to five cell clusters were found per section, each composed of 5 to 65 ganglion cells. These visceral GCC were attached to the prostatic capsule or vesical smooth muscle, or even embedded within the capsule or smooth muscle. In particular, the posterolateral aspect of the prostate, which is identified as the neurovascular bundle in the surgical field, contained many ganglion cells (66 to 908; Figs. 1B and 2).

Comparatively large numbers of ganglion cells were present even near the prostatic apex, the most peripheral portion of the pelvis. In relation to the ventral aspect of the prostate, we found a few GCC in only two specimens; these clusters were located ventrally but close to the apex. No ganglion cells were found along the extrapelvic pudendal nerve, in or around cavernous tissues including the penile hilum, in the area of the rhabdosphincter, in retropubic fat, or in well-developed recto-urethral muscle.

Those results, including interindividual differences, are summarized in Table 1 for seven cadavers in which essentially all sites in the pelvis were investigated. Total number of cells and cell numbers at each site displayed significant interindividual variation (total, 3044 to 6522 cells). The ratio of viscerally located ganglion cells to all ganglion cells was 22% to 38%.

### 3.2. Immunohistochemistry

In general, TH immunoreactivity was consistently strong, while staining for PHI sometimes was weak. Some doubly negative cells were consistently present. We found no significant difference in size or shape between TH-positive and PHI-positive cells. Notably, in every section in which immunohistochemistry was performed, every GCC contained TH-positive cells coexisting with cells stained for PHI, even in ganglia along the sacral sympathetic trunk. These two cell types sometimes tended to cluster apart from one another (Fig. 2), but more often the types were closely intermingled (Fig. 3B). Although TH-positive cells sometimes appeared singly within a cell cluster.

---

**Table 1** Numbers and distribution of extramural ganglion cells in the male hemipelvis: An evaluation using semiserial sections at 1-mm intervals

<table>
<thead>
<tr>
<th>Specimen</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>M7</th>
<th>Average No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NERVE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypogastric nerve</td>
<td>604</td>
<td>945</td>
<td>276</td>
<td>–</td>
<td>248</td>
<td>–</td>
<td>825</td>
<td>579.6 ± 314.8</td>
</tr>
<tr>
<td>Pelvic splanchnic nerves</td>
<td>1262</td>
<td>396</td>
<td>223</td>
<td>84</td>
<td>853</td>
<td>285</td>
<td>765</td>
<td>552.6 ± 420.9</td>
</tr>
<tr>
<td>Pelvic plexus</td>
<td>1113</td>
<td>332</td>
<td>250</td>
<td>–</td>
<td>534</td>
<td>411</td>
<td>575</td>
<td>535.8 ± 307.7</td>
</tr>
<tr>
<td>Sacral sym. ganglion</td>
<td>1566</td>
<td>687</td>
<td>–</td>
<td>249</td>
<td>1092</td>
<td>849</td>
<td>1884</td>
<td>1054.5 ± 596.2</td>
</tr>
<tr>
<td><strong>VISCERA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder, dorsal aspect</td>
<td>172</td>
<td>48</td>
<td>44</td>
<td>0</td>
<td>10</td>
<td>162</td>
<td>567</td>
<td>143.3 ± 199.1</td>
</tr>
<tr>
<td>B/P junction</td>
<td>78</td>
<td>280</td>
<td>135</td>
<td>53</td>
<td>50</td>
<td>232</td>
<td>211</td>
<td>148.4 ± 93.2</td>
</tr>
<tr>
<td>SV, dorsal aspect</td>
<td>163</td>
<td>25</td>
<td>78</td>
<td>–</td>
<td>212</td>
<td>45</td>
<td>273</td>
<td>132.7 ± 99.2</td>
</tr>
<tr>
<td>Prostate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal aspect</td>
<td>155</td>
<td>101</td>
<td>0</td>
<td>65</td>
<td>230</td>
<td>15</td>
<td>535</td>
<td>157.3 ± 184.7</td>
</tr>
<tr>
<td>Ventral aspect</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>–</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2.2 ± 4.0</td>
</tr>
<tr>
<td>Near the apex</td>
<td>15</td>
<td>0</td>
<td>10</td>
<td>109</td>
<td>177</td>
<td>104</td>
<td>387</td>
<td>114.6 ± 136.8</td>
</tr>
<tr>
<td>NVB</td>
<td>698</td>
<td>230</td>
<td>448</td>
<td>66</td>
<td>908</td>
<td>96</td>
<td>500</td>
<td>420.9 ± 313.3</td>
</tr>
<tr>
<td>In levator ani</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>16</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3.2 ± 6.4</td>
</tr>
<tr>
<td>Total numbers of cells</td>
<td>5826</td>
<td>3044</td>
<td>1474</td>
<td>642</td>
<td>4317</td>
<td>2202</td>
<td>6522</td>
<td></td>
</tr>
<tr>
<td>% in VISCERA</td>
<td>22</td>
<td>22</td>
<td>–</td>
<td>37</td>
<td>–</td>
<td>–</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

* a Along the hypogastric nerve and distal ureter.
* b From 0 to 30 mm from the anterior sacral foramina.
* c Macroscopically Sacral sympathetic ganglion.
* d Bladder-prostate junction.
* e Seminal vesicle.
* f Histologically, the seminal vesicle completely overlapped the upper half of the prostate. Thus, the “upper half of the prostate” in typical surgical views was included in these sections.
* g Within 10 mm from the apex, not including the rhabdosphincter area.
* h Neurovascular bundle, posterolateral aspect of the prostate.
* i (Number of cells in VISCERA/total cell number) x 100.
Fig. 3D, proportions of the types tended to be site-specific (Table 2), with TH-positive cells predominating in ganglia along the sacral sympathetic trunk and appearing relatively dominant in GCC along the hypogastric nerve near the distal ureter. Nevertheless, the proportion was different, sometimes by a factor of 10 or more (Fig. 3B and D), between adjacent or nearby clusters of ganglion cells.

4. Discussion

Urologists who perform nerve-sparing radical prostatectomy pay careful attention to the course of the cavernous nerve, but seldom if ever consider the distribution of ganglion cells. Using a rat model, Kato et al. [1] recently reported difficulty in regeneration of injured ganglion cells, even following simple damage to nerve fibers. Accordingly, we need to know the distribution, number, and character of ganglion cells at each site in the male pelvis. In this study we demonstrated three features of human pelvic autonomic ganglia: relative scattering of ganglion cells, significant interindividual differences in cell numbers, and co-existence of sympathetic and parasympathetic cells within a GCC.

The present study demonstrated a site-specific distribution of pelvic autonomic ganglion cells. Even when the large numbers of cells found along the sacral sympathetic trunk were excluded, most remaining cells still were located in and along macroscopically identified nerve components, especially in parts of the pelvic plexus near the seminal vesicle and along the proximal course of the cavernous nerve. The latter corresponds to the most caudal portion of the pelvic splanchnic nerve [10]. GCC attached to the pelvic viscera were

![Diagram](image_url)

**Fig. 4.** Localization of the ganglion cells in the male pelvis. Panel A displays the ganglion localization described in Table 1. The 4 sites along the macroscopically identified nerves, those are shown in the upper part of Table 1 and contain more than half of the ganglion cells, correspond to the hypogastric nerve, pelvic splanchnic nerve, pelvic plexus and sacral sympathetic ganglia. In addition, as shown in the lower major part of Table 1, some parts of the ganglion cell population are present immediately around the the dorsal aspect of bladder, bladder/prostate junction, apex of the prostate, dorsal aspect of the prostate, seminal vesicle, neurovascular bundle. Panel B demonstrates the distribution of the ganglion cells (stars) immediately around the seminal vesicle and prostate (posterior view). These stars show the average numbers of ganglion cells at each sites. Even in the nerve-sparing retropubic radical prostatectomy, the ganglion cells at the dorsal aspect of seminal vesicle, bladder/prostate junction, and along the prostate must be excised, due to the location close to the visceral capsules. These visceral ganglion cells are attached to the prostatic capsule or vesical smooth muscle, or even embedded within the capsule or smooth muscle. By contrast with these sites, the ganglion cells at the pelvic plexus might be preserved because these are separated from the seminal vesicle by at least 1.0 mm. AP: apex of the prostate; VA: ventral aspect of the prostate, DA; AP: apex of the prostate, VA: ventral aspect of the prostate, DA; BL: dorsal aspect of the bladder, B/P: bladder/prostate junction, SV: dorsal aspect of the seminal vesicle, UR: ureter, HGN; hypogastric nerve, SSG; sacral sympathetic ganglion, PSN; pelvic splanchnic nerve, PP; pelvic plexus, LA; levator ani, NVB; neurovascular bundle, DEN; Denonvilliers’ fascia, REC; rectum. A large and small star show a hundred and ten ganglion cells, respectively.

<table>
<thead>
<tr>
<th>TH-positive ratio per one cadaver % (mean %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sacral sympathetic trunk</td>
</tr>
<tr>
<td>Along the hypogastric nerve</td>
</tr>
<tr>
<td>Pelvic plexus</td>
</tr>
<tr>
<td>NVB</td>
</tr>
<tr>
<td>Along the PSN</td>
</tr>
</tbody>
</table>

NVB, neurovascular bundle; PSN, pelvis splanchnic nerve; TH, tyrosine hydroxylase.
more limited in number (22% to 38% of all ganglion cells). Visceral GCC included those on the dorsal aspect of the bladder, at the bladder/prostate junction, and at the dorsal aspect of the seminal vesicle and along the prostate. No GCC were found along the extrapelvic pudendal nerve, in or around the cavernous tissues including the penile hilum, in the area of the rhabdosphincter, in retropubic fatty tissue, or in the rectourethral muscle. Nevertheless, human pelvic ganglion cells were distributed over a large area at various sites, in contrast to pelvic ganglion cells in experimental animals, where ganglion cells appear largely limited to major pelvic ganglia [11]. This is consistent in part with interindividual variation in cavernous nerve course [12]. In nerve-sparing radical prostatectomy, urologists are likely to believe that parasympathetic ganglion cells are essentially preserved if the neurovascular bundle also preserved. However, visceral GCC often are attached to the prostatic capsule or even embedded in the capsule (Fig. 1B), and may be removed with the prostate. Additionally, when we separate the seminal vesicle from the dorsal aspect of the bladder, or cut between the bladder and prostate with ordinary procedures using an electric scalpel, we may inadvertently injure many ganglion cells (Fig. 4B). Taguchi et al. [13] carried out whole-mount staining of fresh human pelvic viscera using acetylcholineesterase enzyme histochemistry, dividing parasympathetic ganglia into primary and secondary. The former were located along the inputs to the pelvic plexus, while the latter connected to postganglionic fibers, apparently acting as interneurons. Because secondary ganglia tended to be located along and near pelvic viscera, they seemed more vulnerable to injury during radical prostatectomy than primary ganglia. Thus, we can not preserve all extramural GCC in the male pelvis even in the nerve-sparing surgery.

The present study suggested great interindividual differences in ganglion cell numbers, more than twofold in the whole pelvis and more than fifteenfold in the pelvic splanchnic nerve, although we examined only semiserial sections. For comparison, counts of the total number of autonomic ganglion cells in experimental animals were 540 to 1080 in the rat pulmonary nerve plexus [14] and 5000 to 7000 in the rat cardiac nerve plexus [15]. This interindividual variation in cell numbers and GCC ratio attached to the pelvic viscera appears likely to influence functional outcome after surgery. Although much further study is required, we suspect that greater numbers of cells, especially an abundance of secondary ganglion cells, may confer greater resistance to dysfunction from surgical injuries.

If TH-positive cells are sympathetic and PHI-positive cells are parasympathetic, the present study indicated that both kinds of cells intermingled and co-existed in individual ganglion cell clusters in the male pelvis. Notably the hypogastric nerve, believed to convey sympathetic input to pelvic viscera [3], included cells that stained as parasympathetic ganglion cells. These ganglion cells appear to give rise to ascending nerve fibers to the ureter and descending colon [16]. In fact some studies showed the colocalization of TH and VIP in the nerve fibers of human ureter [17,18]. Thus, damage to parasympathetic rather than sympathetic elements in the hypogastric nerve would appear to result in dysfunction of these organs. Likewise, the pelvic splanchnic nerve appeared to contain sympathetic fibers as well as parasympathetic fibers. Co-existence or intermingling of the two types of ganglion cells has been described in experimental animals [19] and also in human cadavers [4,20,21]. Our result confirmed the previous studies. Consequently, the usual simple classification of pelvic nerve components as “sympathetic hypogastric nerve” or “parasympathetic pelvic splanchnic nerve,” appears to distort understanding of autonomic physiology, and could impede evaluation of nerve damage during surgery.

5. Conclusions

Human pelvic autonomic ganglia existed not only in nerve components but to a lesser extent along visceral surfaces. The usual simple designation of grossly identified pelvic nerve components as either sympathetic or parasympathetic is likely to cause misunderstanding of pelvis physiology. Distribution, numbers, proportions of sympathetic and parasympathetic pelvic ganglion cells are likely to influence outcome after nerve-sparing surgery.

Acknowledgments

We thank Mr. Seiji Ohtani and Ms. Mami Yamaguchi in the biomedical laboratory center of Sapporo Medical University for their assistance in immunohistochemistry, and Ms. Yoko Yoshida and Ms. Kazumi Wakabayashi in the microscopic center of Kawasaki Medical School for their assistance in sectioning. We are also grateful to Professor Toshihiko Iwanaga of Hokkaido University and Professor Ken Asamoto of Aichi Medical University for their helpful technical comments in the beginning of this study.
References


Editorial Comment
Nicolas Mottet, Saint Etienne, France nnmottet@mutualite-loire.com

Many of us believe that the neuro-anatomy of the autonomous system is relatively simple, with two clearly different pathways: a sympathetic and a parasympathetic ones, both with clear courses. This knowledge is important as the nerve preservation has a major impact for the functional results in pelvic surgery. But knowing this and in the best hands, it is surprising that the obtained results are not so reproducible from one patient to another. The paper form Takenaka and all could partly explain why.

This study based on an immunohistochemistry analysis of the sympathetic and parasympathetic pelvic gangliaions add several important points to our knowledge concerning this system.
Bladder Cancer

Cystectomy in Patients with High Risk Superficial Bladder Tumors Who Fail Intravesical BCG Therapy: Pre-Cystectomy Prostate Involvement as a Prognostic Factor

J. Huguet\textsuperscript{a,*}, M. Crego\textsuperscript{a}, S. Sabate\textsuperscript{b}, J. Salvador\textsuperscript{a}, J. Palou\textsuperscript{a}, H. Villavicencio\textsuperscript{a}

\textsuperscript{a}Urology Service, Fundacio Puigvert, C/Cartagena, 340, 08025 Barcelona, Spain
\textsuperscript{b}Anaesthesiology Service, Fundacio Puigvert, Barcelona, Spain

Accepted 16 March 2005
Available online 7 April 2005

Abstract

Purpose: To review understaging and outcome of patients who underwent radical cystectomy (RC) for high risk superficial bladder cancer after bacillus Calmette-Guérin (BCG) failure.

Patients and methods: We carried out a retrospective study of 62 cases in which RC was indicated for clinical stage Tis, Ta, T1 transitional cell bladder tumors that failed transurethral resection (TUR) and BCG treatment. We used BCG (81 mg/Connaught BCG) in patients with superficial grade 3 tumors and CIS. We considered BCG failure a high-grade recurrence at 3 months of the first BCG course or after 2 courses. RC indications, correlation between their clinical and pathological stage and the ensuing progress were analyzed. We assessed the existence of any pre-cystectomy clinical or pathological factor related to understaging and survival.

Results: RC was performed in 22 patients with carcinoma in situ (CIS) (35%), 7 with Ta (11,2%), 31 with T1 (50%), and 2 with Tx tumors (3%). All 62 but one were high-grade tumors (grade 3 and/or CIS). Tumor was clinically understaged with stages pT2 or greater on the RC specimen in 17 patients (27%). The presence of tumor in the prostatic urethra at the moment of endoscopic staging before RC was the only factor associated with clinical understaging ($p = 0.003$) and shorter survival ($p < 0.0002$).

Five-year disease-specific survival rate was significantly lower in understaged (38%) as compared with not-understaged patients (90%) after a median follow-up of 40-months (range 1–142) ($p = 0.006$). Overall five-year disease-specific survival was 79%.

Conclusions: RC should be performed prior to progression in high risk superficial tumors that fail after TUR and BCG. In patients with clinical and pathological nonmuscle invasive disease, RC provides an excellent disease-free survival. One third of patients with HRSBT who underwent RC after BCG failure were understaged and had a shorter survival. Tumor in the prostatic urethra at endoscopic staging was the only factor associated to understaging and shorter survival.

Copyright © 2005 Elsevier B.V. All rights reserved.

Keywords: Superficial bladder cancer; Cystectomy; BCG failure

1. Introduction

High grade stage Ta, T1 and CIS have been associated with increased risk of recurrence and progression and are considered high-risk superficial bladder tumors (HRSBT) [1]. Transurethral resection (TUR)
and adjuvant bacillus Calmette-Guérin (BCG) therapy is accepted as the optimal treatment for most patients with HRSBT [2]. BCG has proved effective in lowering the risk of recurrence and progression especially when maintenance is used [3]. Radical cystectomy (RC) may be considered as initial therapy if the tumors are large, if they are located in a poorly accessible site for complete resection, and if the patient has symptomatic diffuse disease [4,5].

Ten to 15% of BCG-treated HRSBTs progress, but in series with a long-term follow-up progression and mortality the percentage may be as high as 53% and 34% respectively [3,6]. RC performed in HRSBT that progressed showed extravesical disease in 40–60% of cases [7,8], and survival was lower than 30% at 15 years [9]. Thus, tumor progression should not be awaited to undertake radical treatment in HRSBT that fails BCG [7,9,10].

The controversy lies on what do we consider BCG failure and when to perform a RC for HRSBT that recurs after BCG but without progressing. In general, RC is recommended for patients with carcinoma in situ (CIS) or high-grade T1 that persisted or recurred after initial BCG treatment [4]. Other authors consider RC if CIS or high grade tumor recurs less than 3–6 months after BCG [11,12]. Some patients may benefit from a second course of BCG, but when this fails a RC should be considered [13].

We report 62 patients who underwent RC for non-muscle invasive urothelial carcinoma that recurred after BCG treatment. Our study provides a review of the indications, rates and factors regarding understaging and outcome in these patients.

2. Material and methods

Eight hundred and sixty-four RCs were performed at our Center between January 1989 and May 2002 for transitional cell bladder cancer. We carried out a retrospective study of 62 (7%) RCs for clinical stage Tis, Ta, and T1 tumors that failed both TUR and BCG treatment. All patients received at least one course of endovesical instillations of BCG (81 mg/Connaught BCG/weekly/six weeks). Our patients did not receive maintenance BCG. We used BCG in patients with superficial grade 3 disease and CIS in the bladder or prostatic urethra. We did not perform a restaging TUR in T1 tumors before BCG treatment.

Follow-up was performed by means of cystoscopy every 3 months over the first 2 years, and subsequently every 6 months. After BCG, all patients with previous CIS underwent multiple random cold cup biopsies including prostatic urethra.

We first considered BCG failure a high-grade recurrence (grade 3 or CIS) after 2 BCG courses. Intravesical Mytomycin C was administered in low grade recurrences. Over the last years we also established that high-grade recurrence 3 months after the first BCG course was also a failure. Large tumor size, solid appearance, multifocal disease, local toxicity after BCG, severe micciturional syndrome due to multiple TUR were also contributory to the RC indications, especially after 1 BCG course.

The study did not include RC for superficial tumors without a previous BCG treatment, patients treated with BCG and clinical diagnosis of invasive disease (≥T2) and retracted bladders (pT0).

To study the correlation between clinical (T) and pathological (P) stage we compared the histology of the last TUR prior to RC with the histology of the RC specimen.

Clinical endoscopic staging was performed by means of cystoscopy, TUR of the tumor, and 6 cold cup biopsies of normal-looking mucosa, one of them including prostatic urethra near the verumontanum. If there were any macroscopic tumor in the prostatic urethra, we performed TUR and the histologic study was made separately from the bladder. Four possibilities of prostatic tumor involvement were considered: superficial papillary tumor, CIS, prostatic duct involvement (superficial prostatic involvement) and stromal invasion (invasive prostatic involvement). Those cases with infiltration of prostatic stroma in the clinical stage were excluded. Two patients were included who had no muscularis propria in the transurethral resection specimen (Tx).

The median interval between the last TUR (clinical endoscopic stage) and cystectomy was 80 days (range 25–240).

We considered understaged patients with tumors in clinical stages Tis, Ta and T1 who presented with infiltrating bladder tumor (≥pT2) or with prostatic stromal invasion in the RC specimen. We did not classify stromal invasion as intraurethral or extravesical.

Post-cystectomy follow-up was performed every 4 months over the first year, and subsequently every 6 months. Physical exploration, blood tests, abdominal ultrasound scan and chest x-ray were performed. The upper urinary tract was evaluated by urography or loopography 4 months postoperatively and then annually. CT and bone gammography were carried out during follow-up, particularly when local recidivation or metastatic disease was suspected.

We evaluated pre-cystectomy clinical and pathological prognostic factors related to understaging and survival. Clinical factors: sex, early BCG failure (recurrence 3 months after treatment), failure of one or more BCG courses, number of TURs prior to RC (more or less than three), interval between tumor diagnosis and RC, interval between BCG treatment initiation and RC, time between initial presentation and second course of BCG, time between the last TUR and RC. Pathological factors: single or multiple tumor, tumor size (larger or smaller than 3 cm), macroscopic appearance of the tumor (papillary or solid), tumor grade, stage, presence of CIS, single or multiple CIS, tumor location in the bladder neck or in the prostatic urethra.

The statistical analysis was carried out with SPSS 11.5 (SPSS Inc.).

The understage dichotomous variable was used as a dependent variable. Bivariate analysis was performed using X2 test for categorical independent variables, and the logistic regression test for continuous numerical independent variables. p < 0.05 values were considered significant.

A multivariate analysis was also carried out through a forward stepwise logistic regression analysis. The most significant variables in the bivariate analysis were incorporated into the multivariate study. The independent variables were included in the model when p was <0.05 based on the probability of a log likelihood test ratio. The variables were excluded when p > 0.10.
The survival analysis was performed with the Kaplan–Meyer test.

3. Results

Sixty-two RCs for clinical stages Tis, Ta and T1 transitional bladder tumor were performed in 56 men and 6 women with a mean age of 65 (range 46–79). The median interval between the first evidence of tumor and RC was 31 months (range 5–156). During this time a mean 3.7 TURs (range 2–18, median 3) were performed per patient. The median interval between BCG treatment and RC was 16 months (range 4–125).

Histology of last TUR prior to RC was CIS in 22 patients (35%), Ta in 7 (11.2%), T1 in 31 (50%) and Tx in 2 (3%). All of the tumors but one were high-grade tumors (grade 3 and/or CIS). RC was indicated in 29 cases (46%) following failure of 2 BCG courses, and in 28 (45%) following one course. In 15 cases (53%) of the last group the indication was early BCG failure. Only 5 patients (8%) received more than 2 BCG courses. (Table 1).

No tumor was identified in 13 (21%) RC specimens (pT0). The clinical stage was the same as the pathologic stage in 29% of the cases, and in 32% the clinical stage was higher. Seventeen patients (27%) were understaged, they presented with infiltrating bladder tumor (≥pT2) or with prostatic stromal invasion in the RC specimen (Table 2).

Four patients (6%) had lymph node metastasis.

The only factor associated with clinical understaging both at the bivariate and the multivariate study was the presence of tumor at the prostatic urethra at the moment of endoscopic staging (p = 0.003). (Table 3).

Understaging was verified in 7 (53%) out of 13 cases with prostatic urethra tumor at endoscopic staging. Their five-year disease-specific survival was lower (20%) than in patients without prostatic urethra tumor (78%) (p < 0.0002). Five out of 9 patients with macroscopic tumor and superficial prostatic involvement after TUR, had stromal invasion in the RC specimen. Cold cup biopsies in normally appearing prostatic

### Table 1
Number of BCG courses administered and histology of last TUR prior cystectomy

<table>
<thead>
<tr>
<th>Stage</th>
<th>Grade</th>
<th>N°</th>
<th>BCG failure</th>
<th>Other cystectomy indications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 Course</td>
<td>2 Courses</td>
</tr>
<tr>
<td>CIS</td>
<td></td>
<td>22</td>
<td>8 (3)</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>G1 + Cis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>G3 + Cis</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Ta</td>
<td>G2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G2 + Cis</td>
<td>4</td>
<td>2 (1)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>19</td>
<td>13 (8)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>G3 + Cis</td>
<td>7</td>
<td>4 (3)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>G3</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Tx</td>
<td></td>
<td></td>
<td>62</td>
<td>28 (15)</td>
</tr>
</tbody>
</table>

(*) BCG early failure. SMS: Severe Micturitional Syndrome. BCG Int: Intolerance of BCG.

### Table 2
Correlation between clinical stage (TUR prior to cystectomy) and pathological stage (cystectomy specimen)

<table>
<thead>
<tr>
<th>Clinical stage (TUR prior to cystectomy)</th>
<th>Pathological stage (cystectomy specimen)</th>
<th>Total N°</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pTo pTa pT1 pT2-T4 N+</td>
<td></td>
</tr>
<tr>
<td>Tis</td>
<td>5 9 1 3 4 22</td>
<td></td>
</tr>
<tr>
<td>Ta</td>
<td>– 1 1 2 4 7</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>8 5 2 8 8 31</td>
<td></td>
</tr>
<tr>
<td>Tx</td>
<td>– 1 – 1 2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13 15 4 13 17 62</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3
Multivariable analysis of clinical-pathological factors related to understaging

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor in prostatic urethra</td>
<td>12.2</td>
<td>2.2–65.5</td>
<td>0.003</td>
</tr>
<tr>
<td>No tumor</td>
<td>0.4</td>
<td>0.07–2.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Size</td>
<td>2.3</td>
<td>0.4–12.01</td>
<td>0.3</td>
</tr>
<tr>
<td>Grade</td>
<td>0.7</td>
<td>0.1–3.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Presence of CIS</td>
<td>0.3</td>
<td>0.08–1.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Sex</td>
<td>0.1</td>
<td>0.01–1.5</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Only the most significant variables in the bivariate analysis are included.
urethra detected 4 CIS, and 2 of them had stromal invasion in the RC specimen, and 6 cases with prostatic tumor at RC were not detected, 3 of them with stromal invasion. (Table 4).

Six of these last 10 cases with prostatic urethra involvement and without macroscopic tumor had multifocal disease, and 6 had bladder CIS. Six patients had previous tumor in the bladder neck or trigone, and 4 had previous CIS in the prostatic urethra.

Table 5 shows the characteristics of the understaged cases. Prostatic stroma invasion was found in 10 (58%) of the 17 understaged cases.

Five-year disease-specific survival rate was significantly lower in patients who presented with understaging (38%) as compared with not-understaged patients (90%) after a 40-month follow-up median (range 1–142) (p = 0.006, Fig. 1). Overall five-year disease-specific survival was 79%.

Table 4
Correlation between clinical stage (TUR prior to cystectomy) and pathological stage (cystectomy specimen) in patients with prostatic involvement

<table>
<thead>
<tr>
<th>Endoscopic clinical prostatic stage prior cystectomy</th>
<th>Pathological prostatic stage (cystectomy specimen)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stromal invasion</td>
</tr>
<tr>
<td>Macroscopic tumor (TUR) Ta-T1</td>
<td>6</td>
</tr>
<tr>
<td>Ductal inv</td>
<td>3</td>
</tr>
<tr>
<td>No macroscopic tumor C1</td>
<td>4</td>
</tr>
<tr>
<td>Cold cup biopsy No tumor</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>56</td>
</tr>
</tbody>
</table>


Table 5
Tumor characteristics of understaged patients

<table>
<thead>
<tr>
<th>TUR (n=8)</th>
<th>BCG courses (n=8)</th>
<th>Time BCG - cystectomy (months)</th>
<th>Clinical stage (TURB prior to cystectomy) Bladder/prostatic urethra (pu)</th>
<th>Pathological stage (cystectomy specimen)</th>
<th>Progress at follow up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>4</td>
<td>Cis</td>
<td>G3 pT3b + CIS</td>
<td>Alive (33)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>20</td>
<td>Cis</td>
<td>G3 pT2b/pu: Cis</td>
<td>Alive (83)</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>27</td>
<td>G3 Tx</td>
<td>G3 pT2b + CIS</td>
<td>Alive (43)</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>110</td>
<td>G3 Ta + CIS</td>
<td>G3 pTa + CIS + N1</td>
<td>Dead (36)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>9</td>
<td>G3 T1</td>
<td>G3 pT2b</td>
<td>Alive* (40)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>18</td>
<td>G3 T1</td>
<td>G3 pT2b</td>
<td>Alive (24)</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>15</td>
<td>G3 T1 + CIS</td>
<td>G3 pT2a + CIS</td>
<td>Alive (36)</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>6</td>
<td>Cis</td>
<td>G3 stromal inv + CIS</td>
<td>Alive (35)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>9</td>
<td>G3 T1</td>
<td>G3 stromal inv + CIS + N1</td>
<td>Dead (17)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>6</td>
<td>G3 T1</td>
<td>G3 stromal inv</td>
<td>Alive (2)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>50</td>
<td>pu: Cis</td>
<td>G3 stromal inv + CIS</td>
<td>Dead (70)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>22</td>
<td>G3 Ta/pu: Cis</td>
<td>G3 stromal inv + CIS</td>
<td>Alive (16)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>12</td>
<td>Cis/pu: G3 Ta</td>
<td>G3 stromal inv + CIS</td>
<td>Dead (9)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>12</td>
<td>G3 T1/pu: G3 T1</td>
<td>G3 stromal inv + N1</td>
<td>Dead (6)</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>9</td>
<td>G2 T1/pu: G2 ducts</td>
<td>G2 stromal inv + N2</td>
<td>Dead (2)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>40</td>
<td>G3 T1/pu: ducts</td>
<td>G3 stromal inv + CIS</td>
<td>Alive (44)</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>9</td>
<td>G3 Ta/pu: ducts</td>
<td>G3 stromal inv</td>
<td>Alive (31)</td>
</tr>
</tbody>
</table>

Tumors in clinical stages Tis, Ta, T1 that presented with infiltrating bladder tumor (>pT2) or with prostatic stromal invasion on the cystectomy specimen. TUR: Transurethral resection, Pu: prostatic urethra, Stromal inv: Prostatic stromal invasion.

* Alive with disease.
4. Discussion

It is advisable to perform RC in HRSBT unresponsive to BCG before progression [7,9,10]. Which patients to operate and the optimum timing of surgery are controversial. The risk of over-aggressive treatment must be balanced against the potential to offer timely treatment with curative intent [14]. Even though our patients did not receive maintenance BCG, indications for RC followed the accepted guidelines in this group of patients [4,5]. Most were carried out on high-grade tumors that had failed 1 or 2 BCG courses. Following those indications, 5-year survival of patients with clinical and pathological nonmuscle invasive disease reached 90%, an outcome similar to others [7,8,15–17]. However, 17 cases (27%) were understaged. Those were tumors whose progression was detected neither during follow-up, nor at the time of clinical staging with TUR. Five-year survival in these patients was 38%, implying that in several instances RC was performed too late.

Understaging and progression, occasionally subclinical, of HRSBTs, are the factors responsible for BCG-treated patients to present with an unexpectedly poor outcome [18].

Understaging has been observed after a second TUR for primary bladder cancer in 4.7–29% of cases [19,20] and also in RC specimens of patients with Tis, Ta and T1 tumors (24 to 40%) [7,14–17]. Understaging tends to be higher in stage T1 tumors and for this reason some authors perform restaging TUR before BCG therapy [19–21]. In our series a 27% understaging after BCG failure is a low percentage in such a high risk population study and would amount to 13% not counting the understaged cases at a prostatic urethra level. To improve the understaging error we currently perform restaging TUR in TxG3 and in large and solid T1G3 tumors.

In 78 RCs for clinical stages T1 or less, Dutta et al. found more severe understaging in cases with suspicious radiography and with absence on the biopsy specimen [14]. Freeman et al. could identify no clinical characteristics predictive of understaging in 182 RC carried out in superficial bladder tumors [7].

We found that tumor involvement of the prostatic urethra at endoscopic staging was the only factor associated to understaging. TUR and cold cup biopsies were useful to diagnose the presence of a tumor at a prostatic urethra level, but not to establish a correct staging.

Series assessing prostatic involvement before RC have identified transurethral lateromontanal biopsy as the most accurate method of determination. Wood et al. were 90% accurate in demonstrating the presence of prostatic tumor involvement, but only 2 of 5 cases (40%) of stromal involvement were detected [22]. Donat et al. detected prostatic involvement in 80 of 99 patients (81%) suffering from prostatic tumor but the specificity (77%), sensitivity (53%) and positive predictive value (45%) of the transurethral resection biopsy to predict prostatic stromal invasion at RC was low [23].

Therefore, if we want to detect prostatic involvement or we find macroscopic tumor at this level, it seems advisable to perform a TUR including transurethral lateromontanal biopsies, assuming that it will not always be possible to detect stromal involvement. This difficulty to perform an accurate endoscopic staging of the prostatic urethra implies that, even though patients with prostatic urethra affected by CIS, papillary tumor and even by duct infiltration may respond to BCG, those are tumors with high progression risk and needing a strict follow-up with repeated biopsies of the prostatic urethra [24,25].

Herr et al. found relapse in prostatic urethra in 72 (39%) of 186 consecutive men with superficial bladder tumors treated with TUR and followed over 15 years. Twenty-seven (38%) cases had stromal invasion. The authors considered that they had silent tumor progression within the prostate that escaped detection during the follow-up [26]. They did not systematically use transurethral lateromontanal loop biopsies and no surveillance policy for the detection of tumor involvement of the prostate was applied. In our series, 6 of 43 patients without macroscopic tumor and negative cold cup biopsies had tumor in the RC specimen.

The question as yet unanswered is when to perform TUR of the prostate in the absence of macroscopic tumor to detect silent prostatic invasion. Previous tumor at the bladder neck, trigone, or prostatic urethra [27] and suspicion of TIS of the bladder [4] have been suggested as potential indications.

The importance of tumor involvement of the prostate lies in that it is a poor prognostic factor, especially in stromal invasion, with 5-year survival between 16–55% [26,28,29].

Solsona et al. observed stromal invasion in 15 (34%) out of 45 RCs performed in HRSBT refractory to TUR and intravesical therapy. Extravesical recurrences were responsible for an unexpectedly poor outcome and RC came to late in some cases. Solsona et al. consider that in primary HRSBT it is necessary to identify progression predictive factors in order to find the optimum timing for RC. No response to intravesical therapy
evaluated at 3 or 6 months seems to be an important factor for the indication of an early RC [18].

Even though our patients are a selected group, having the indication for a cystectomy, in our study we observe that the presence of a post-BCG superficial tumor in the prostatic urethra is a poor prognostic factor, and that it probably is also an important factor for the indication of an early RC.

5. Conclusions

In HRSBTs unresponsive to BCG, RC should be performed before progression. In patients with clinical and pathological nonmuscle invasive disease, RC provided excellent disease-free survival. One third of patients with HRSBT who underwent RC after BCG failure were understaged and had shorter survival. After BCG failure, tumor involvement of the prostatic urethra at endoscopic staging prior to RC was the only prognostic factor associated to understaging and a shorter survival. This is due to the fact that correct endoscopic staging of the prostatic urethra is difficult and in patients who have received BCG the prostatic urethra is a location in which a transitional tumor may progress silently. In those cases at risk of presenting with subclinical involvement, TUR biopsy of the prostatic urethra should be done.

References


Editorial Comment

P. Whelan, Leeds, UK
peter.whelan@leedsth.nhs.uk

Patients with high risk superficial bladder tumours who fail to respond or recur following BCG therapy are a relatively small, but nonetheless, highly significant and difficult group of patients to treat. BCG therapy has been imitated in the hope of eradicating disease and preserving normal bladder function and there is always the tension between attaining this goal and running the risk of being too late in offering radical ablative surgery (radical cystectomy).

This retrospective study from a well respected Urological Cancer Institute in Spain drives home this lesson, yet again emphasising in general terms the effectiveness in obtaining cure by radical cystectomy but warning of the precarious path that we tread in showing that a third of the patients who had BCG failure were upstaged after radical cystectomy and therefore their survival was shorter indicating that radical cystectomy had been performed later than optimally, and adds yet a further adverse prognostic factor, the only one in their factor analysis to prove consistent, that tumour in the prostatic urethra at endoscopic stage was the only factor associated with understating and a shorter survival.

It reinforces the persistent message that sampling from the urethra in high risk patients is essential, that radical treatment in the presence of aggressive, even if apparently superficial disease within the prostatic urethra, emphasises the need for early radical cystectomy.
Bladder Cancer

Adjuvant and Neoadjuvant Chemotherapy in Muscle Invasive Bladder Cancer: Literature Review

Dimitrios Pectasides, Melina Pectasides, Maria Nikolaou*

Second Department of Internal Medicine-Propaedeutic, Athens University Medical School, Attikon University Hospital, 8, Agias Lavras, Haidari, 124 61 Athens, Greece

Accepted 22 March 2005
Available online 7 April 2005

Abstract

Radical cystectomy is the standard treatment for patients with clinically localized muscle invasive bladder cancer, providing a 5-year survival rate of approximately 50%. Failure to cure is often due to the presence of occult metastases beyond the margins of local therapy, indicating a need for eradication of micrometastatic disease with systemic treatment, in order to improve survival. Combined chemotherapy regimens, such as methotrexate-vinblastine-cisplatin (CMV), methotrexate-vinblastine-cisplatin-doxorubicin (M-VAC) and gemcitabine-cisplatin (GC) have already demonstrated their effectiveness in patients with advanced or metastatic disease and have been considered as appropriate regimens in the peri-operative setting. Large randomized studies with a prolonged follow-up have been able to confirm a modest survival benefit with neoadjuvant therapy. A recent meta-analysis, including all previous reported randomized trials, concluded that neoadjuvant chemotherapy administration provides a significant survival benefit and can be administered without adverse outcomes resulting from delayed local therapy. Adjuvant chemotherapy trials, although promising, have failed to show statistically improved survival, mostly due to small sample sizes and absent or inconclusive data on overall survival. A multi-center randomized-controlled trial is currently ongoing, in order to elucidate the role of post-operative chemotherapy administration.

Keywords: Bladder cancer; Transitional cell carcinoma; Muscle invasive; Neoadjuvant therapy; Adjuvant therapy

1. Introduction

Bladder cancer is the fourth most commonly occurring cancer in men and the eighth in women, in United States and UK [1,2]. It is most common in the elderly with a peak incidence in the seventh decade. Transitional cell carcinoma (TIC) represents more than 90% of all bladder cancers, while squamous cell carcinoma and adenocarcinoma account for 5% and 2%, respectively [3]. About 20% of incident cases concern locally advanced, muscle invasive disease, although an additional 10–25% will occur in association with relapsed superficial bladder cancer [3,4].

Muscle-invasive bladder cancer may be treated with curative intent by either radical cystectomy or external beam radiotherapy. Despite local therapy with cystectomy and/or radical radiotherapy, the 5-year survival rate of patients with muscle invasive transitional cell carcinoma, is approximately 50% [5,6]. Recurrence is mostly related to tumor stage, grade and nodal status at the time of cystectomy. Nodal involvement is associated with a 5-year survival of 20–40%, while direct invasion into adjacent viscera reduces the rate to less than 10% [7]. Most patients relapse, within 2 years, in distant sites, with only one third of patients relapsing in the pelvis alone [8]. The presence of lymphatic or vascular invasion is associated with an increased risk of invasion and metastasis [9]. Other adverse prognostic factors include the absence of expression of blood group antigens on the tumor cell surface, DNA aneu-
ploidy, expression of epidermal growth factor receptor (EGFR), p53 mutations, absence of retinoblastoma (Rb) protein expression and absence of p21 expression [9].

Failure to cure is often due to the presence of occult metastases beyond the margins of local therapy, indicating a need for eradication of micrometastatic disease with systemic treatment in the peri-operative setting, as combination chemotherapy for metastatic disease has already proven its effectiveness.

Although several single agents have been successfully used in patients with advanced or metastatic disease, randomized clinical trials have favored combined chemotherapy regimens, such as methotrexate-vinblastine-cisplatin (CMV) and methotrexate-vinblastine-cisplatin plus doxorubicin (M-VAC) [10,11]. Recently, the combination of gemcitabine and cisplatin (GC) has demonstrated similar objective response and survival rates with less toxicity than M-VAC, in a large randomized trial and is also considered as a current therapeutic option [12].

This article will review the delivery of neoadjuvant and adjuvant systemic chemotherapy aimed at improving the outcome of muscle invasive TCC.

2. Neoadjuvant chemotherapy

Systemic chemotherapy combined with local modalities, has been studied extensively in the past two decades, in order to improve survival, or to preserve the bladder. The theoretical advantages of neo-adjuvant chemotherapy are the immediate treatment of micrometastatic disease, the ability to assess tumor response in vivo (this may permit the continuation of treatment to maximal response or discontinuation of ineffective therapy), more effective delivery of chemotherapy before surgical disturbance, lower toxicity due to better performance status and the possibility of bladder preservation in selected patients, as a result of tumor downstaging. The main disadvantages are the time delay to definitive local therapy and the possibility of exposing some patients to unnecessary cytotoxic therapy based on inaccurate clinical staging. Trials addressing this question must assess the efficacy of treatment in terms of long – term overall survival. Until recently there was no clear evidence for the use of neoadjuvant chemotherapy for potentially curable bladder cancer outwith the context of a clinical trial. Although several randomized trials with good patient accrual had been published, none had statistical power to demonstrate a statistically significant benefit in its own right.

Phase I and II trials of single agent chemotherapy have been promising, providing encouraging data on tumor downstaging and survival [13,14]. Randomized trials, though, failed to support statistical survival benefit from neoadjuvant cisplatin-based chemotherapy prior to radical cystectomy or radiotherapy, despite the prolongation of disease free interval [15–17]. These early reports were criticized because of the single agent chemotherapy regimen used, which is not currently accepted for advanced bladder cancer, and for insufficient number of patients enrolled.

The largest published single trial of neoadjuvant chemotherapy in bladder cancer, the MRC BA06/EORTC 30894 study [18], randomized 976 patients with locally advanced TCC (T2 G3, T3 or T4a) to receive either radical local therapy alone (cystectomy or full dose external beam radiotherapy), or three cycles of neoadjuvant CMV prior to local treatment, aiming to detect 10% difference in overall survival. After a median follow-up of 4 years, results showed an improved 3-year survival from 50.5% to 55.5% (p = 0.075) and an increased overall survival from 37.5 to 44 months, for the arm of neoadjuvant CMV. The trial though, failed to achieve the predefined level of 10% improvement in overall survival. However, updated results with a prolonged median follow-up of 7 years, finally revealed an overall survival benefit with a hazard ratio (HR) of 0.85 (95% confidence intervals 0.72–1.00) in favor of the neoadjuvant arm making this trial statistically significant in its own right (p = 0.05) [19].

Although CMV has never been compared to M-VAC, most clinicians support that M-VAC regimen appears to be superior in terms of efficacy. It might therefore be suggested that this trial utilized inadequate chemotherapy. Against this, the pathological complete response rate of 33% in 206 assessable patients is comparable to published results with the four-drug regimens [20,21]. The SWOG 8710 trial enrolled 317 patients with T2-T4a disease [22]. Patients were randomized either to undergo radical cystectomy alone or to receive three cycles of M-VAC followed by radical cystectomy. After a median follow-up of 8.4 years, median overall survival was 77 months for the combination therapy group compared to 46 months for those treated by cystectomy alone (p = 0.05 by one-sided t-test, p = 0.06 by two-sided stratified log-rank test). The hazard ratio for patients in the experimental arm was 0.74 (95% confidence intervals 0.55–0.99). In addition, pathological complete response rates were observed in 38% of patients having received neoadjuvant M-VAC chemotherapy, a result similar to that seen in the MRC/EORTC trial, versus 15% of patients in the
cystectomy arm \((p < 0.001)\). This trial has also demonstrated that most of the survival benefit was identified in patients who achieved surgical complete remission after neoadjuvant treatment.

Another study, similar to the SWOG one, is the Italian GUONE trial, which enrolled 206 patients [23]. Patients were randomized to either four cycles of M-VAC before cystectomy, or cystectomy alone. The sample size was calculated to detect 15% benefit in 3-year survival. The trial was closed early because failed to achieve any difference in survival. This is a small trial in which no difference in survival was observed (The 3-year survival was 62% for the M-VAC arm, versus 68% for the cystectomy alone arm). Another Italian trial substituted doxorubicin with epirubicin and studied the neoadjuvant M-VEC regimen plus cystectomy versus cystectomy alone [24]. Again, no difference in survival was observed.

In the first Nordic cystectomy trial (NCT1), 325 patients were enrolled [25]. The experimental arm included two cycles of cisplatin-doxorubicin, while all patients both in the experimental and in the control arm were planned to receive 40 Gy irradiation plus cystectomy. The trial reported a small difference in a subgroup analysis of patients with T3-T4 disease. In the second Nordic cystectomy trial (NCT2), 317 patients were randomized to receive either three cycles of cisplatin-methotrexate and leucovorin as rescue, prior to cystectomy, or cystectomy alone [26]. Despite the lack of statistical power to detect a difference in overall survival when presented separately, the combined analysis of the two Nordic studies, showed a hazard ratio of 0.80% (95% confidence intervals 0.64 – 0.99, \(p = 0.049\)) for overall survival in favor of neoadjuvant treatment [27]. The 5-year survival was 56% in the experimental group versus 48% in the control group.

Several other published randomized trials of neoadjuvant chemotherapy [28,29], have failed to show survival difference, mostly because in order to detect a 10% survival benefit of investigational chemotherapy arm over standard therapy, a randomized trial requires approximately 1000 patients [30] (Tables 1 and 2).

The first meta-analysis, included 2688 patients from 9 randomized published plus one unpublished trial [31]. The SWOG trial, although eligible, could not provide individual patient data in this meta-analysis. In five trials the planned local treatment was radical cystectomy, two used radical radiotherapy and one used preoperative radiotherapy and cystectomy. Two trials used a combination of one or more of these local treatments. All the trials used platinum-based chemotherapy, nine of which used cisplatin, either as a single agent, or in combination. The combined HR for trials using combination chemotherapy was 0.87 (95% confidence intervals 0.78–0.97, \(p = 0.016\)), equivalent to a 13% relative reduction in the risk of death, and an absolute benefit of 5% at 5 years, improving survival from 45% to 50%. The HR for trials with single agent chemotherapy is in favor of local treatment alone, although the result is not statistically significant (\(p = 0.264\)).

A more recent meta-analysis, including the SWOG trial, concluded in similar results. The combined HR for the total of trials was 0.90 (95% confidence intervals 0.82–0.99), in favor of the chemotherapy arm [32]. Although the overall benefit is modest, and the conclusions of trials which necessitate extensive patient selection may not be directly relevant to all patients,

<table>
<thead>
<tr>
<th>Trial</th>
<th>(n)</th>
<th>Med F/U (m)</th>
<th>CT arm</th>
<th>Standard arm</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Standard vs. neoadjuvant</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>CUETO [15]</td>
<td>122</td>
<td>78.2</td>
<td>CDDP</td>
<td>C</td>
<td>6.5-y 37.3% vs. 35.5% NS</td>
</tr>
<tr>
<td>WMURG [16]</td>
<td>159</td>
<td>16</td>
<td>CDDP</td>
<td>R/T</td>
<td>3-y 40% vs. 30% NS</td>
</tr>
<tr>
<td>ABCSG [17]</td>
<td>96</td>
<td>16</td>
<td>CDDP</td>
<td>R/T</td>
<td>3-y 41% vs. 38% NS</td>
</tr>
<tr>
<td>EORTC [18]</td>
<td>976</td>
<td>48</td>
<td>CMV</td>
<td>C or R/T</td>
<td>Median 37.5 vs. 44 mos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3-y 50% vs. 55.5%</td>
</tr>
<tr>
<td>EORTC [19]</td>
<td>976</td>
<td>78</td>
<td>CMV</td>
<td>C or R/T</td>
<td>0.85 (0.72–1.00)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(p = 0.05)</td>
</tr>
<tr>
<td>SWOG 8710 [22]</td>
<td>317</td>
<td>85.2</td>
<td>M-VAC</td>
<td>C</td>
<td>5-y 42% vs. 57%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Median 43.2 vs. 74.7 mos</td>
</tr>
<tr>
<td>GUONE [23]</td>
<td>206</td>
<td>NR</td>
<td>M-VAC</td>
<td>C</td>
<td>5-y 54% vs. 55% NS</td>
</tr>
<tr>
<td>GISTV [24]</td>
<td>171</td>
<td>37</td>
<td>M-VAC</td>
<td>C</td>
<td>NS</td>
</tr>
<tr>
<td>Nordic I [25]</td>
<td>325</td>
<td>60</td>
<td>CDDP-doxorubicin</td>
<td>R/T + C</td>
<td>5-y 51% vs. 58% NS</td>
</tr>
<tr>
<td>Nordic II [26]</td>
<td>316</td>
<td>40.8</td>
<td>CDDP-MTX</td>
<td>C</td>
<td>NS</td>
</tr>
<tr>
<td>DAVECA 8901 [28]</td>
<td>33</td>
<td>91.8</td>
<td>CDDP-MTX</td>
<td>C</td>
<td>5-y 46% vs. 64% NS</td>
</tr>
<tr>
<td>DAVECA 8902 [28]</td>
<td>120</td>
<td>77</td>
<td>CDDP-MTX</td>
<td>R/T</td>
<td>5-y 24% vs. 19% NS</td>
</tr>
<tr>
<td>RTOG 8903 [29]</td>
<td>126</td>
<td>60</td>
<td>CMV</td>
<td>R/T + CDDP</td>
<td>5-y 49% vs. 48% NS</td>
</tr>
</tbody>
</table>
this update analysis provides the first clear view of a significant benefit for chemotherapy in this setting and may represent a new standard of care for those patients with muscle–invasive TCC who are able to tolerate relatively aggressive chemotherapy. Future trials must now address the optimum chemotherapy regimen, determine whether the addition of new agents will add to the benefit of neoadjuvant chemotherapy and whether the benefit can also be transferred to patients who are unable to tolerate cisplatin–based combination chemotherapy. Large randomized studies with a prolonged follow-up have been able to confirm a modest survival benefit with neoadjuvant therapy. Moreover, the response to chemotherapy may be the most important predictor of survival. Data collected from 147 patients with muscle invasive TCC [33], showed that the 5-year survival was 75% in patients achieving tumor downstaging to pT0 or superficial disease with neoadjuvant therapy, versus only 20% in patients with residual muscle invasive disease (>pT2).

3. Neoadjuvant chemotherapy and bladder preservation

Although radical cystectomy remains the standard treatment option for muscle invasive bladder cancer and since orthotopic bladder substitution has become available, the chance of organ preservation should not be dismissed. The advantages of bladder preservation include less surgery, no need for urinary diversion and normal sexual life. Several phase II trials have supported the combined modality therapy with TUR-B, chemotherapy and irradiation, especially for patients with T3b and T4 disease [34,35].

The combination of neoadjuvant chemotherapy and radiotherapy is capable of producing 5-year survival rates of 42–63%, with organ preservation in approximately 40% of patients [29]. Prognostic factors for local curability are a small tumor, absence of hydronephrosis, papillary histology, a visible complete TUR-B and a complete response to induction chemotherapy. The evaluation of neo-adjuvant chemotherapy and bladder preservation was investigated in a series of 87 patients with T2-T4a TCC, who were treated with three cycles of M-VAC chemotherapy, followed by TUR-B (n = 42), or partial cystectomy (n = 13), or radical cystectomy (n = 32) [36]. In the first group, the 42 patients have attained a clinical complete response or a downstaging to superficial disease and they underwent a TUR-B. The 5-year survival for this group was 69%, while 57% have maintained an intact bladder. In another trial, with 111 surgical candidates who received neoadjuvant M-VAC, 10-year-survival of patients who underwent TUR-B or partial cystectomy is 74%, with 58% bearing an intact bladder [37].

Bladder preservation in patients based on the response to neoadjuvant chemotherapy is a feasible approach which must be confirmed in prospective randomized trials. The real value of bladder preservation need to be estimated in large randomized studies, as the goal of treatment remains the cure of patient with respect to the quality of life.

4. Adjuvant chemotherapy

For high risk patients with pT3-pT4 and/or pN+ M0 disease, 5 year survival after radical cystectomy is only 25–35% [3,38–40]. Post-operative chemotherapy has led to increased survival in patients with several malignancies. As a result, adjuvant chemotherapy has been used after cystectomy in high risk patients in an effort to delay recurrence and prolong survival.

The principal advantage of adjuvant setting is the immediate, without any delay performance of the definitive local treatment, especially for patients who do not respond to chemotherapy. Pathological evaluation of the cystectomy specimen offers the chance of accurate staging and detection of molecular prognostic markers. Moreover, the decreased morbidity of cystectomy because of orthotopic bladder substitution, is in favor of the immediate cystectomy and adjuvant chemotherapy.
Disadvantages of adjuvant chemotherapy include the delay of administering systemic chemotherapy, the lack of in vivo response assessment and the difficulty to administer chemotherapy after cystectomy. A number of randomized trials have assessed the role of adjuvant chemotherapy following either radiotherapy or cystectomy against an observation-controlled arm. The first comparative, nonrandomized trial was reported by Logothetis et al. [41], who divided patients into three groups, i.e. low risk controls, high risk controls (did not receive adjuvant chemotherapy because of refusal or medical reasons) and high risk patients treated with adjuvant chemotherapy. Follow-up of high-risk patients treated with five cycles of CISCA (cisplatin, cyclophosphamide, adriamycin) regimen after radical cystectomy demonstrated a significantly improved 2-year DFS (79% vs. 37%, \( p = 0.0012 \)). These high-risk patients who were defined by vascular invasion, extravesical involvement or direct invasion into adjacent prostate or vagina (pT3-pT4, pN+), had a similar DFS to that of untreated low risk patients (70% vs. 76%, \( p = 0.33 \)).

A single-center randomized trial of five cycles of M-VAC chemotherapy, given either as two neoadjuvant and three post-operative cycles, or five cycles of adjuvant therapy has recently been published [42]. 140 patients with T3b or T4a were enrolled. Significant difference in overall survival was not observed between the two arms. A disadvantage of this trial was that no observation-only arm was included.

A small number of randomized trials have evaluated the role of adjuvant chemotherapy following either cystectomy, or radiotherapy, against an observation-only control arm. The first randomized trial that showed at 5 years a benefit in DFS (51% vs. 34%) and survival (44% vs. 39%, n.s.) for the arm of adjuvant chemotherapy, was reported by Skinner et al. [43]. Ninety-one patients with pT3-pT4 or N+ were randomized to receive either the combination of cyclophosphamide, doxorubicin and cisplatin (CAP) after cystectomy, or cystectomy alone. The median survival for patients in the experimental group was 4.3 years versus 2.4 years in the control group. The trial marked as important prognostic factors the age, gender and lymph-node status, with the latter as the most important variable. However, after 7 years the DFS curves of the two patient groups crossed. This was somewhat unexpected, as cystectomy patients rarely relapse beyond 3 years after treatment. It has been speculated that the chemotherapy used in this study was too weak to cure the disease, but was strong enough to prolong DFS. The trial was criticized due to the small number of cases, the deviation from the predefined chemotherapy regimen and its statistical methodology (while chemotherapy appeared to prolong the median time to recurrence by 14 months, there was no residual advantage at 2 years).

In another trial of adjuvant therapy, 49 patients were finally randomized either to observation-only after cystectomy, or to adjuvant chemotherapy with 3 cycles of M-VAC or M-VEC regimen in patients with locally advanced bladder cancer (pT3-pT4, pN+) [44]. This trial was designed to demonstrate a 35% improvement of DFS from 25% to 35%. Twenty-six patients were randomized to the adjuvant treatment arm and 23 to cystectomy alone. Those patients who underwent cystectomy only had a very high-rate of progression (12 out of 13 node-positive patients), while only 3 out of 11 node-positive patients who received post-cystectomy chemotherapy were free of disease. In this study, patients randomized to observation after cystectomy, were not permitted to receive chemotherapy at any time (neither adjuvant, nor salvage). The study was prematurely closed because of a dramatic difference in progression free survival between the two arms (the 5-year progression free survival was 59% for the adjuvant group, versus 13% for the observation group). The authors later added non-randomized cases in order to increase the total number of cases, but this attempt reflected a serious error of statistical methodology and potential case selection bias. A later comparative analysis of 166 patients, including the initially treated 49 patients, found a significant difference (\( p = 0.0002 \)) in DFS for 89 patients treated with 3 cycles of adjuvant MVAC or MVEC, as compared to 86 patients who had only cystectomy. [45]. When stratifying patients, by the extend of lymph node involvement, it was found that adjuvant chemotherapy achieved the highest benefit in patients with pN+ disease. However, strong methodological problems of these studies may consider the conclusion as doubtful.

Another prospective, randomized trial which was reported by Freiha et al. investigated the benefit of CMV after radical cystectomy in 50 patients with pT3b-T4, N0/+, M0 [46]. Patients were treated with 4 cycles of CMV and those who did not have adjuvant therapy, received salvage chemotherapy after progression, thus decreasing the tumor-specific survival difference between the two arms. The study showed a statistically significant DFS benefit for the experimental arm (median 37 versus 12 months, \( p = 0.01 \), and concerning the overall survival benefit, a trend in favor of adjuvant therapy (median 63 versus 36 months, \( p = 0.32 \)). The lack of significant difference in overall survival was attributed to the unexpected ability to salvage patients with relapse on the observation arm.
This trial was also terminated prematurely before having included the full number of intended patients, due to an obvious progression-free survival, leaving the most important endpoint of an adjuvant trial (overall survival) unresolved.

Two studies out of 5 found no statistical difference between adjuvant treatment and cystectomy alone, but criticism addresses the inclusion of patients with organ-confined disease (pT2, pN0) [44,45]. Studer et al. [47] treated 37 of 77 patients with single-agent cisplatin post-cystectomy and no difference between the two groups was detected. More than 50% of patients had only organ-confined disease and patients who were diagnosed as node-positive disease, by imaging techniques, were excluded from the study. Bono et al. [48] randomized only pN0 patients to 4 cycles of adjuvant cisplatin and methotrexate post-cystectomy vs. cystectomy alone. They found a 10% (n.s.) advantage in DFS in favor of the adjuvant treatment after 18-month follow-up.

Although these trials appear to show a benefit in favor of adjuvant chemotherapy, they do not provide sufficient evidence to support its routine use in clinical practice for patients with locally advanced, particularly lymph-node-positive disease, due to small sample sizes and absent or inconclusive data on overall survival.

Therefore, EORTC and collaborating groups have developed a multi-center randomized-controlled trial (EORTC 30994) for pT3-pT4, and/or N+, M0 patients. Patients are randomized to either immediate chemotherapy following cystectomy, or delayed chemotherapy following relapse. M-VAC, dose-intense M-VAC with G-CSF support, or gemcitabine-cisplatin (GC) are considered acceptable regimens, providing that the same regimen is used for immediate and delayed chemotherapy within each institution. 1344 patients are required to detect the designed trial accrual, which is 20% relative increase in 5-year overall survival, at 80% power, using a two-sided log-rank test. A current adjuvant trial by the German Genito-Urinary Group was designed to demonstrate improved tolerability of 3 cycles of adjuvant cisplatin and methotrexate (CM) without loss of efficacy, when compared with 3 cycles of MVEC. The trial was recently closed (320 patients) including 120 patients with tumor-positive lymph-node disease. Preliminary results showed that the median survival was 25 months in patients receiving the intended-dose of either MVEC or CM, in contrast to 11 months after receiving either incomplete adjuvant chemotherapy or none at all. This was also observed in patients with node-positive disease [49]. It should be interesting to wait for the final results, concerning the two clear arms of the study. Another German study, that randomized 138 patients to receive MVEC as adjuvant chemotherapy or cystectomy alone was closed and the results are awaited. Another adjuvant study was conducted by the University of Chicago, in which 33 high-risk patients (pT3-pT4a, pN+) were treated with cisplatin and gemcitabine. The outcome data of this study were limited, since only 8 patients included in the study after 15 months [50].

Other current adjuvant phase III trials which include modern antineoplastic agents for locally advanced bladder cancer were performed by the Spanish Oncology Genito-Urinary Group (SOGUG) comparing 4 cycles of paclitaxel, cisplatin and gemcitabine post-cystectomy vs. cystectomy only and by the ECOG (Eastern Cooperative Oncology Group) that randomized 490 patients to 4 cycles of carboplatin and paclitaxel post-cystectomy vs. cystectomy only.

5. Novel cytotoxic agents

The recent introduction of gemcitabine and the taxanes as treatment options for bladder cancer is a promising development. Gemcitabine, a nucleoside antimetabolite that inhibits DNA synthesis, has shown single agent overall response (OR) rate of about 25%, with a complete response rate of 9% [52–55]. Disposing a synergistic action with cisplatin, the combination GC has already demonstrated similar objective response and survival rates with less toxicity than M-VAC, in patients with metastatic bladder cancer [12]. As it is considered an option for first line treatment, clinical randomized trials may use this combination in adjuvant and neoadjuvant settings. The currently ongoing EORTC trial (EORTC 30994), testing the benefit of adjuvant chemotherapy, has already included this combination as acceptable along with M-VAC (intensified, or standard doses).

The taxanes, as single agents, have yielded overall response rates of 7% to 56%, depending on whether the patients have received prior chemotherapy for metastatic disease, while in combination with cisplatin the OR rates are 61% for paclitaxel and 54% for docetaxel [55]. They have also been assessed in neoadjuvant regimens in combination with cisplatin, in phase II clinical trials. Three cycles of paclitaxel (175 mg/m²) and cisplatin (75 mg/m²) were administered before radiotherapy or radical surgery in 42 patients with locally advanced bladder cancer (T3a-T4a) [56]. Thirty-two out of 42 patients (76%) achieved a major response, while 20 patients (47.6%) remain free of disease at a median follow-up of three years. In another
phase II trial, 50 patients were treated with docetaxel (75 mg/m²) and cisplatin (75 mg/m²) for 3 cycles prior to cystectomy [57]. Chemotherapy was well tolerated. The 5-year survival and progression free survival were 51.92% and 52.47% respectively.

6. Conclusions

Muscle invasive bladder cancer is a chemosensitive disease and should be dealt with in a multimodality approach. Cisplatin-based combination chemotherapy has recently demonstrated to improve overall survival prior to definitive local treatment and may become the standard of care for patients who have undergone radical cystectomy. Results from the EORTC 30994 trial will probably clarify any survival benefit from post-operative chemotherapy.

Newer drugs such as gemcitabine and the taxanes, combined with cisplatin, may represent effective and less toxic regimens. The use of molecular markers, although investigational at present, may permit the detection of selected populations that could benefit from peri-operative therapy in the future.

References

More than 20 years after the first reports on systemic chemotherapy such as the M-VAC regimen, the role of systemic treatment for transitional cell carcinoma remains more or less undefined in spite of thousands of treated patients. In patients with metastatic disease, long term survival is exceptional, the treatment intention is therefore often described as “palliative”.

In the situation of patients undergoing cystectomy for muscle invasive or locally advanced disease, it was the matter of a long-lasting controversy, whether peri-

Editorial Comment
M. Stöckle, Homburg, Germany
michael.stoeckle@uniklinikum-saarland.de
operative chemotherapy can improve their doubtful prognosis by sterilizing potential micrometastases. The large meta-analysis of the neo-adjuvant trials gave a clear answer to this question: Eradication of micrometases is possible. However, the survival probability was only improved by 5%, which indicates that 19 of 20 treated patients have to accept treatment without efficacy: They would either be cured by cystectomy alone or they will die in spite of additional treatment. The question therefore arises, if this small 5% difference reflects the weakness of chemotherapy itself or the weakness of the neo-adjuvant trials that were summarized for the meta-analysis. And indeed, the trials as well as the concept of neo-adjuvant chemotherapy are characterized by two flaws that may have significantly contributed to this borderline result.

1. **Dilution** of “informative” patients (Namely those who would develop progression after cystectomy) by a high percentage of “non-informative” patients (Those who are cured by cystectomy alone): Almost all neo-adjuvant protocols called for patients with T2 or T3 disease. Patients with T2 disease are characterized by a cure rate of up to 80%. A significant improvement of such a favourable prognosis is extremely unlikely. Even the optimistic assumption that neo-adjuvant chemotherapy can prevent one out of three tumor-related deaths would only translate into a 6% survival advantage in a patient group with a progression risk as low as 20%. Including such patients will therefore probably “dilute” the potential benefit that might be observed in patients with a higher risk of progression, but will not add useful additional information. The inclusion of patients with T2 disease probably adds nothing but a significant increase of the number of patients necessary to detect a significant survival advantage and a decrease in survival difference.

2. Patients with *non-responding tumors* may not only not benefit from systemic pre-treatment, prognosis may even become worse by the delay of definitive treatment in favour of an ineffective pre-treatment. The 5% survival advantage must therefore be read as the summary effect of a prognostic improvement in patients with a tumor response minus the worsening in patients with non-responding tumors.

The majority of adjuvant trials focused on high risk patients based on the histopathological tumor stage, thus circumventing the dilution problem. Furthermore, adjuvant trials are not biased by the non-responder-problem, because adjuvant treatment does not lead to a delay of definitive treatment. For theoretical reasons, adjuvant trials should therefore be expected to reveal larger survival differences as compared to neo-adjuvant trials and it is regrettable that not more efforts were invested in large adjuvant trials during the late 1980s, when almost all international trial organisation planned for neo-adjuvant studies. The authors correctly state that all available adjuvant trials are characterized by small patient numbers, but on average, they concluded with a larger survival advantage as could be predicted by simple considerations.

How can these experiences be translated into the decision making process of the individual patient? The poorer his baseline prognosis, the higher is the probability that the patient will benefit from perioperative systemic treatment. When clear signs of lymph node involvement are seen on a preoperative CT or MRI, inductive chemotherapy should be seriously considered prior to cystectomy, because the prognosis of such a patient must be regarded as extremely poor with a pure surgical approach. When lymph node involvement is detected during cystectomy, adjuvant chemotherapy should also seriously be considered because of a progression risk as high as 80%.
Bladder Cancer

Urinary Levels of Soluble E-Cadherin in the Detection of Transitional Cell Carcinoma of the Urinary Bladder

Shahrokh F. Shariat, Kazumasa Matsumoto, Roberto Casella, Weiguo Jian, Seth P. Lerner

Department of Urology, University of Texas Southwestern Medical School; 5323 Harry Hines Blvd, Dallas, Texas 75390-9110, USA

Scott Department of Urology, Baylor College of Medicine, Houston, Texas, USA

Department of Urology, University of Basel, Basel, Switzerland

Accepted 16 February 2005

Available online 14 March 2005

Abstract

Objective: To test the hypothesis that elevated urinary levels of soluble E-cadherin (sE-cadherin) would aid in the detection of transitional cell carcinoma (TCC) of the urinary bladder.

Methods: We performed sE-cadherin staining of one murine (MBT2) and four human (RT4, 5637, T24, and TCCSUP) bladder cancer cell lines. sE-cadherin levels were also determined in voided urine of 188 consecutive subjects at risk for TCC recurrence, 31 patients with other uro-pathologic conditions, and 10 healthy subjects using a commercially-available ELISA kit. sE-cadherin was analyzed continuously and categorically on the basis of its median distribution.

Results: Moderately and poorly differentiated bladder cancer cell lines had decreased cellular E-cadherin expression, whereas RT4, a well differentiated cell line, had preserved expression. All cell lines had measurable sE-cadherin levels in their conditioned media. The area under the ROC curve of sE-cadherin for the detection of TCC was 0.719 (95% CI, 0.637–0.801; p < 0.001). Higher levels of sE-cadherin were associated with positive cytology results (p = 0.012) and muscle invasive tumor stage (p = 0.009). Urinary sE-cadherin was more sensitive, but less specific than urinary cytology for the detection of bladder TCC. In a multivariable logistic regression analysis, higher sE-cadherin and positive cytology were both associated with an increased risk of bladder TCC (p = 0.048 and p < 0.001, respectively). Combination of cytology and sE-cadherin allowed categorization of patients into three significantly different risk groups for bladder cancer. Adjustment of sE-cadherin for urinary creatinine levels did not affect any of the outcomes.

Conclusions: Urinary level of sE-cadherin may add information to cytology in the detection of bladder TCC.

#2005 Elsevier B.V. All rights reserved.

Keywords: Bladder cancer; Urine biomarker; Diagnosis; E-cadherin

1. Introduction

E-cadherin is a calcium-dependent cell-to-cell adhesion molecule expressed exclusively by epithelia [1,2]. Loss of or decreased expression of E-cadherin in bladder cancer tissue has been associated with advanced stage, higher grade, metastasis and a higher probability of cancer progression and death [3–7]. sE-cadherin is detected as a proteolytic cleavage product of the E-cadherin peptide from the cell surface.
sE-cadherin is found in the circulation of normal individuals but it is particularly elevated in patients with various malignancies [9–12] including transitional cell carcinoma (TCC) of the urinary bladder [13,14]. Banks and colleagues first reported the presence of sE-cadherin in urine [15]. In a follow-up study they showed that urinary levels of sE-cadherin were elevated in bladder cancer patients compared to healthy controls and patients with non-neoplastic urologic diseases [16].

We sought to confirm that urinary levels of sE-cadherin may aid in the detection of bladder TCC. In order to demonstrate that sE-cadherin is released by bladder cancer cells, we first evaluated the expression of E-cadherin in five established, stable bladder cancer cell lines, and measured the levels of sE-cadherin in their conditioned media. Then, we assessed the association of urinary levels of sE-cadherin with bladder cancer presence and characteristics in a case-control study of 229 consecutive subjects with past history of Ta, T1, and/or CIS TCC stages.

2. Materials and methods

2.1. Bladder cancer cell lines and immunohistochemistry

We measured sE-cadherin in the conditioned media of four human and one murine bladder cancer cell lines. RT4, S637, T24 and TCCSUP were obtained from American Type Culture Collection (Rockville, Maryland, USA) and the murine cell line MBT2 was a kind gift from Dr. Tim Ratliff (University of Iowa). All cell lines were maintained in the recommended growth medium. Cell blocks established for each cell line were fixed in buffered formalin at room temperature, and then dehydrated and paraffin embedded. Immunohistochemical staining and scoring methods have been described previously [3,4]. Briefly, the cell line slides were incubated with anti-E-cadherin monoclonal antibody (Transduction Labs) diluted 1:25 in blocking solution. Secondary antibody (Vector Labs) was applied at a dilution of 1:400 followed by avidine-biotin complex immunoperoxidase system using diaminobenzidine as the chromogen and methyl green and Alcian blue as the counterstain. The positive control was bladder tissue known to possess 100% preserved E-cadherin expression. The negative control was serial sections processed without incubation of the primary antibody. E-cadherin expression was classified as normal (90–100% positive cells with preserved cell border staining) and abnormal, which included negative (0–10% positive cells) and various degrees of heterogeneous expression (11–89% positive cells). All experiments were run in quintuplicate.

2.2. Patient population

All studies were performed after approval by a local Human Investigations Committee. Informed consent was obtained from each subject. The study comprised (1) 188 consecutive patients with history of bladder cancer presenting for surveillance cystoscopy; (2) 31 patients with other uro-pathologic conditions such as benign prostatic hyperplasia, urinary tract infection, urinary retention, incontinence, urolithiasis, and non-cancer related hematuria; and (3) 10 healthy subjects. We collected a voided urine sample for measurement of sE-cadherin and creatinine prior to cystoscopy in all patients. We also collected bladder washout samples during cystoscopy for cytology in a subset of 191 subjects (93 had bladder cancer at cystoscopy and 98 did not have bladder cancer at cystoscopy). There were 153 (67%) males and 76 (33%) females, and the median age was 71.0 years (range 21 to 94). Overall, 122 patients (53%) were found to have a bladder tumor. The 107 patients without bladder tumor belonged to three different categories: patients with past history of bladder cancer but without tumor evidence at cystoscopy (n = 66), patients with urological pathology other than bladder malignancy, and healthy volunteers.

2.3. sE-cadherin measurements

We used a commercially-available quantitative ELISA assay (Takara Shuzo, Japan) for measurement of sE-cadherin levels in urine of the study subjects and the conditioned media of bladder cancer cell lines. Each cell line conditioned media was collected two days after last passage with 90–100% cell confluence. Samples were centrifuged and stored at −80 °C. Before analysis, samples were slowly thawed and gently mixed. This assay is based on the capture of sE-cadherin using a solid-phase adsorbed monoclonal antibody followed by subsequent detection using a labeled second monoclonal antibody to E-cadherin. Every sample was run in duplicate and the mean calculated for data analysis. Differences between the two measurements were minimal, as shown by the mean intra-assay precision coefficient of variation (±standard deviation) of 7.3 ± 6.9%.

2.4. Pathologic examination and cytology grading

All histologic slides were reviewed without knowledge of clinical data. Bladder tumors were staged according to the 1997 TNM classification and assigned a grade according to the WHO classification. Cytological findings were grade 0 (no atypical cells), 1 to 2 (low-grade atypia), and 3 (high-grade atypia). Only high-grade atypia was considered positive.

2.5. Urinary creatinine measurements

We measured the levels of creatinine (Olympus America Inc., Melville, NY) in the voided urine from 170 of the original 229 patients. All samples were run in duplicate and the mean utilized for data analysis. Differences between the two measurements were minimal, as shown by the intra-assay precision coefficient of variation of only 5.8 ± 8.7%.

2.6. Statistical analysis

Spearman correlation coefficients were used to examine the correlation between sE-cadherin levels, creatinine levels, and patient age. sE-cadherin was analyzed either as continuous variable or categorically on the basis of its median distribution in the case and control subjects combined. The association between categorical data was tested using the Fisher’s exact test. Differences in continuous variables between dichotomous categories were tested using the Mann-Whitney U test. Discordances between two related dichotomous variables were tested using the non-parametric McNemar test. Non-parametric receiver operating characteristics (ROC) curves in which the value for sensitivity is plotted against false positive rate (1-specificity) were generated. Areas under the curves (AUC) were compared using non-parametric Mann Whitney U-statistics [17]. Univariable and multivariable logistic regression models were used to calculate odds ratios and 95% CI. Age had a skewed distribution and therefore was modeled with a logarith-
mical transformation. Tumor stage was stratified by Tis, Ta, and T1 versus T2 and above; tumor grade was stratified by grade 1 and 2 versus grade 3. Statistical significance in this study was set as $p < 0.05$ and all reported $p$ values were two-sided. All analyses were performed with SPSS statistical package (SPSS version 11.0 for Windows).

## 3. Results

### 3.1. sE-cadherin expression in bladder cancer cell lines and release in conditioned media

RT4, a well differentiated cell line, was the only cell line to exhibit preserved E-cadherin expression (Fig. 1A). TCCSUP exhibited heterogeneous E-cadherin staining (Fig. 1B). T24, 5637, and MBT2 did not express E-cadherin (Fig. 1C). All cell lines had measurable sE-cadherin levels in their conditioned media.

### 3.2. Association of urinary levels of sE-cadherin, cytology, and age with bladder cancer presence and characteristics

Association of urinary sE-cadherin, cytology, and age with cancer presence is shown in Table 1. sE-cadherin levels were higher in patients with bladder cancer than those in control subjects when analyzed as continuous variable and dichotomized variable split by the median ($p = 0.002$ and $p = 0.005$, respectively). Abnormal urinary cytology was associated with blad-

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>No. Subjects (%)</th>
<th>Case Subjects ($n = 122$)</th>
<th>Control Subjects ($n = 107$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>229</td>
<td>73.1 (40.2–94.2)</td>
<td>69.9 (21.0–86.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary creatinine (median, IQR; mg/dL)</td>
<td>170</td>
<td>66.0 (61.0)</td>
<td>65.1 (56.9)</td>
<td>0.691</td>
</tr>
<tr>
<td>Gender (No Pts, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>76 (33)</td>
<td>34 (28)</td>
<td>42 (39)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>153 (67)</td>
<td>88 (72)</td>
<td>65 (61)</td>
<td>0.091</td>
</tr>
<tr>
<td>Urinary cytology* (No Pts, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>60 (31)</td>
<td>50 (54)</td>
<td>10 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>131 (69)</td>
<td>43 (46)</td>
<td>88 (90)</td>
<td></td>
</tr>
<tr>
<td>Urinary sE-cadherin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous (median, IQR; ng/mL)</td>
<td>229</td>
<td>1606.9 (1826.9)</td>
<td>904.2 (1226.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>Dichotomous (No Pts, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below median</td>
<td>114 (50)</td>
<td>50 (41)</td>
<td>64 (60)</td>
<td>0.005</td>
</tr>
<tr>
<td>Above median</td>
<td>115 (50)</td>
<td>72 (59)</td>
<td>43 (40)</td>
<td></td>
</tr>
</tbody>
</table>

IQR = interquartile range.

* Wash urinary cytology was available in 191 patients only.

† Mann-Whitney $U$ test.

‡ Fisher’s exact test.
der cancer \((p < 0.001)\). Patients with bladder cancer were older than those without \((p < 0.001)\).

Association of urinary levels of sE-cadherin analyzed as continuous variable and dichotomous variable split by the median with patient characteristics is shown in Table 2. Males had significantly higher sE-cadherin levels than females. Patients with positive urinary cytology assay results had significantly higher sE-cadherin levels than those with negative assay results. Bladder cancer patients with muscle-invasive disease had significantly higher sE-cadherin levels than those with Tis, Ta, or T1 tumor stage. All five patients with carcinoma in situ had urinary sE-cadherin levels above the median (data not shown). There was no correlation between urinary sE-cadherin levels and patients’ age at time of specimen collection \((r = 0.173, p = 0.101)\). There was no difference in age between patients with sE-cadherin levels above versus those with sE-cadherin levels below the median level \((p = 0.195)\).

Age was higher in patients with positive cytology assay result than in those with negative assay result \((p = 0.023)\). Abnormal wash urinary cytology was associated with both invasive tumor stage, (positive in 25 of the 64 Ta or Tis patients versus 25 of the 29 \(\geq T1\) patients, \(p < 0.001\)) and higher tumor grade (positive in 27 of the 69 Grade 1 or 2 patients versus 23 of the 24 Grade 3 patients, \(p < 0.001\)). However, cytology was not associated with gender \((p = 0.736)\). Forty-three patients with bladder cancer had negative cytology. sE-cadherin levels were above the median in 31 (72%) of these patients.

### 3.3. Diagnostic performance of urinary sE-cadherin as continuous and dichotomous variable for prediction of bladder cancer presence

The ability of sE-cadherin levels to predict cystoscopic findings was analyzed using non-parametric ROC analyses. sE-cadherin was more accurate than guessing (null hypothesis; area under the null hypothesis = 0.5) for predicting the presence of bladder cancer in all patients \((p < 0.001; \text{Fig. 2})\) and in patients with negative cytology (AUC = 0.640, 95%CI, 0.568–0.712; \(p = 0.003\)).

In a multivariable logistic regression analysis (Table 3), higher sE-cadherin levels and positive cytology result were both significantly associated with an increased risk of bladder cancer presence after adjusting for the effect of age.

### 3.4. Diagnostic performance of urinary sE-cadherin and cytology as combined variable for prediction of bladder cancer presence

We combined the test results of urine cytology and sE-cadherin into two categories: both negative versus any positive. The sensitivity, specificity, PPV, NPV, and accuracy of the combined test results of cytology and sE-cadherin for the detection of bladder TCC are

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution of urinary levels of sE-cadherin across selected patient characteristics</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. Pts (%)</th>
<th>s-Ecadherin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Continuous variable</td>
</tr>
<tr>
<td></td>
<td>Median levels (IQR)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>76 (33)</td>
</tr>
<tr>
<td>Male</td>
<td>153 (67)</td>
</tr>
<tr>
<td>Cytology*</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Pathologic Stage†</td>
<td>Tis, Ta, or T1</td>
</tr>
<tr>
<td></td>
<td>T2 or higher stage</td>
</tr>
<tr>
<td>Pathologic Grade‡</td>
<td>1 or 2</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

IQR = interquartile range.
* Wash urinary cytology was available in 191 patients only.
† Pathologic stage and grade were available in the 122 patients who underwent surgery for bladder cancer.
‡ Fisher’s exact test.
§ Mann-Whitney U test.
shown in Table 4. The sensitivity of the combined cytology and sE-cadherin for the detection of bladder cancer was significantly higher than those of cytology or sE-cadherin alone (p-values < 0.001).

We then combined the test results of urine cytology and sE-cadherin split by the median into three categories: both negative versus one positive versus both positive. In multivariable logistic regression analyses (Table 3), the combined sE-cadherin and cytology variable was associated with an increased risk of bladder cancer when adjusted for the effect of age. Patients could be stratified into low, intermediate, and high risk for cancer based on the combined status of cytology and sE-cadherin.

3.5. Adjustment of sE-cadherin for urinary creatinine levels

The mean urinary level of creatinine level was 73.1 ± 46.4 mg/dL in all patients (median 65.2, range 9.8 to 236.1). Urinary sE-cadherin levels were weakly but significantly correlated with urinary creatinine levels (r = 0.315, p < 0.001). Median urinary creatinine levels in patients with sE-cadherin levels above the median were higher than those in patients with sE-cadherin below the median (75.8, IQR 60.3 versus 51.9, IQR 58.3; p < 0.001). There was no difference in urinary creatinine levels between patients with positive versus negative cytology assay result (p = 0.758).

sE-cadherin/Creatinine ratio was analyzed either as continuous variable or categorically on the basis of its median distribution in the case and control subjects combined. We found that the sE-cadherin/Creatinine ratio was higher in patients with bladder tumor compared to controls (p < 0.001). Patients with sE-cadherin/Creatinine ratio above the median were more likely to have bladder cancer than those with sE-cadherin/Creatinine ratio below the median (p < 0.001). There was no correlation between age at time specimen collection and urinary creatinine

Table 3
Multivariable logistic regression analyses of urinary levels of sE-cadherin, urinary cytology, and age for the prediction of transitional cell carcinoma of the bladder

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age*</td>
<td>3.628</td>
<td>0.539–24.425</td>
<td>0.185</td>
</tr>
<tr>
<td>sE-cadherin*</td>
<td>1.283</td>
<td>1.008–1.633</td>
<td>0.048</td>
</tr>
<tr>
<td>Cytology</td>
<td>8.312</td>
<td>3.523–19.613</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age*</td>
<td>2.998</td>
<td>0.487–18.458</td>
<td>0.236</td>
</tr>
<tr>
<td>sE-cadherin† and cytology combined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both negative</td>
<td>1.000</td>
<td>Referent</td>
<td>–</td>
</tr>
<tr>
<td>Either positive</td>
<td>2.812</td>
<td>1.396–5.664</td>
<td>0.004</td>
</tr>
<tr>
<td>Both positive</td>
<td>11.819</td>
<td>3.949–35.377</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Test for trend</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Age and sE-cadherin had a skewed distribution and therefore were modeled with a logarithmical transformation.
† sE-cadherin was analyzed categorically on the basis of its median distribution in the case and control subjects combined.

Table 4
Diagnostic performance of wash urinary cytology and urinary sE-cadherin for the detection of bladder cancer

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology*</td>
<td>53.8</td>
<td>89.8</td>
<td>83.3</td>
<td>67.2</td>
<td>72.3</td>
</tr>
<tr>
<td>sE-cadherin†</td>
<td>71.3</td>
<td>65.0</td>
<td>67.2</td>
<td>69.2</td>
<td>68.1</td>
</tr>
<tr>
<td>Combined cytology*/sE-cadherin†</td>
<td>77.4</td>
<td>56.1</td>
<td>66.5</td>
<td>72.4</td>
<td>62.6</td>
</tr>
</tbody>
</table>

* Wash urinary cytology was available in 191 patients only.
† sE-cadherin was analyzed categorically on the basis of its median distribution in the case and control subjects combined.
levels \((r = -0.138, p = 0.078)\). sE-cadherin/Creatinine ratios were higher in patients with positive urine cytology assay result than in those with negative assay result \((p = 0.009)\). sE-cadherin/Creatinine \((\text{AUC} = 0.646, 95\% \text{CI}, 0.575–0.717)\) was more accurate than guessing \((p = 0.016)\) but not different from sE-cadherin alone \((p = 0.982)\) for predicting the presence of bladder cancer.

In a multivariate logistic regression model, higher urinary sE-cadherin/Creatinine ratio and positive cytology were associated with the presence of bladder cancer \((p = 0.041\) and \(p < 0.001\), respectively\), when adjusted for the effects of age.

4. Discussion

The sequence of events in cancer progression is characterized in part by the ability of a tumor cell to overcome cell-cell adhesion and to invade the surrounding tissue. E-cadherin is the primary mediator of calcium-dependent cell-cell adhesion in epithelial tissues [18,19]. Loss of E-cadherin mediated cell adhesion coincides with the transition from well-differentiated adenoma to invasive carcinoma supporting its role as a rate-limiting step in the progression to invasive disease [20]. In addition re-establishment of the functional cadherin complex in tumor cell lines resulted in suppression of invasiveness [21,22]. We found that moderately and poorly differentiated bladder cancer cell lines had decreased or absent cellular E-cadherin expression, whereas RT4, a well differentiated cell line, had preserved E-cadherin expression. However, sE-cadherin was measurable in the conditioned media of all bladder cancer cell lines.

We confirmed that urinary levels of sE-cadherin are significantly higher in bladder cancer patients compared to control subjects [15,16]. In addition, we found that higher urinary levels of sE-cadherin were associated with positive urinary cytology assay results and muscle invasive bladder cancer stage. Moreover, sE-cadherin was more sensitive but less specific than cytology for the detection of recurrent bladder cancer. Most importantly, we found that urinary sE-cadherin was an independent predictor of the presence of bladder cancer in a large set of consecutive patients. Since there is no biologically or statistically validated cut point for urinary sE-cadherin, we performed all analyses using sE-cadherin either as continuous variable or as categorical variable stratified on the basis of its median distribution in the case and control subjects combined. We found no differences in the statistical significance of any of the outcomes between using sE-cadherin as continuous or categorical variable. We found that the combination of cytology and sE-cadherin stratified by the median allowed a more optimal categorization of patients who are likely (at high risk) or unlikely (at low risk) to have bladder cancer. However, while increasing the sensitivity for detection of bladder cancer, the combination of sE-cadherin with cytology reduced the specificity and resulted in an increased number of false positive results. Finally, we adjusted sE-cadherin for the effect of urine concentration by dividing urine levels of sE-cadherin by urine levels of creatinine. We found that adjustment for creatinine levels did not affect any of the statistically significant of any of the outcomes.

Higher urinary levels of sE-cadherin were associated with presence of bladder TCC after adjustment for the effects of urine cytology and patient age. Seventy two percent of the 43 bladder cancer patients who had a negative cytology had urinary sE-cadherin levels above the median. Although urinary cytology has excellent sensitivity and specificity in bladder cancer patients with high grade lesions, it has low sensitivity for the detection of intermediate and low grade tumors. In our study, an expert cytopathologist examined all washout cytologies and considered only high grade disease as positive. This taken together with the independent predictive value of sE-cadherin for bladder cancer suggests that the additional evaluation of urine sE-cadherin may help improve bladder cancer detection over cytology alone.

Interestingly, we found that all our five patients with CIS had urine sE-cadherin levels above the median. CIS is associated with the loss of intercellular cohesion causing cells or clusters of cells to detach from the surface epithelium, as reflected by the high incidence of positive urine cytology in patients with CIS of the bladder. In addition, E-cadherin plays an essential role in maintaining cell adhesion in epithelium [23]. We have previously shown that tissue expression of E-cadherin is decreased or absent in 32% of patients with CIS [3]. While sE-cadherin is considered to be the degradation product of intact E-cadherin from the cell surface generated by proteolytic movement, [8] the small sample size of patients with CIS in our study limits conclusions on a possible association between urinary sE-cadherin and CIS.

We found that urinary sE-cadherin levels were higher in males compared to female subjects. To our knowledge, there are no studies showing any association between gender and local or circulating E-cadherin levels. In addition, E-cadherin expression has not been reported to be regulated by sex hormones. In absence of a clear explanation for this...
association, it could be that E-cadherin expression is related to the male preponderance of patients who had cancer or this association may simply be unique to this particular patient population.

Various sources may contribute to the total level of sE-cadherin found in the urine of patients with bladder TCC. In addition to direct release from cells in the urinary epithelium, the urine levels of sE-cadherin may result from blood levels filtered through the kidney. We previously found that plasma levels of sE-cadherin were significantly higher in bladder cancer patients than in healthy controls [13]. In addition, within bladder cancer patients undergoing radical cystectomy, preoperative plasma sE-cadherin levels were independently associated with an increased risk of metastases to lymph nodes and of disease progression. However, Griffiths et al. did not find an association between decreased tissue expression of E-cadherin and elevated serum levels of sE-cadherin [14]. In addition, the glomerular capillary wall functions as a filter that allows the passage of small molecules but almost completely restricts the passage of molecules larger than albumin. Molecular radii less than about 2 nm are filtered as freely as inulin (5 kD, molecular radius 1.4 nm), whereas molecules larger than 5 nm are normally excluded from filtration. Since sE-cadherin has a molecular weight of 80 kD and sE-cadherin in urine is not fragmented, it is unlikely that urinary sE-cadherin is filtered by the kidney. This together with the findings that bladder urothelium expresses E-cadherin and that these levels are associated with poor outcome in patients with TCC suggests that the sE-cadherin in urine originates from and reflects the biology of the urinary epithelium [3–7]. A better understanding of the biological mechanisms underlying the altered levels of circulating, urinary, and tissue levels of sE-cadherin in patients with bladder TCC would potentially lead to more effective clinical management as well as provide new target pathways for therapy in these patients.

We confirmed the strong independent value of urinary cytology for bladder cancer detection and staging. The high sensitivity of cytology in our study is due to the fact that all urine samples were evaluated by a single expert cytopathologist [24]. In addition, we used bladder washout specimens, which have been shown to yield a higher sensitivity than voided urine specimens. In contrast to the high sensitivity, the specificity of cytology in our study was lower than that reported previously. All ten patients who had a presumed false positive urinary cytology had a past history of bladder cancer. Five of the ten patients underwent multiple random biopsies of the bladder without evidence of cancer and remained tumor-free at follow-up. These patients remain at high risk for TCC and must be aggressively followed, and upper tract tumors and prostate urethra CIS must be ruled out. Recent data from the Southwest Oncology Group trial of maintenance BCG demonstrates that cytology may convert to normal up to six months or longer after a single induction course of BCG [25]. Two of the five patients that were not biopsied later developed bladder cancer. The first patient developed a Ta grade 3 TCC two years later, and the second patient developed a Ta grade 1 TCC only seven months after inclusion in the study. Interestingly, his urine was positive for both cytology and sE-cadherin supporting the potential role of these urine markers for predicting the subsequent occurrence of bladder cancer. Possibly, this patient may also represent a “false negative” of cystoscopy. Although considered as the gold standard for diagnosis, cystoscopy has a false negative rate up to 20%, due either to operator error or to small areas of carcinoma in situ, which may be difficult to detect [26].

Acknowledgment

SFS is supported by the Austrian Program for Advanced Research and Technology

References


Kidney Cancer

Prognostic Factors and Survival after Pulmonary Resection of Metastatic Renal Cell Carcinoma

Hans-Stefan Hofmann*, Heinz Neef, Katharina Krohe, Petko Andreev, Rolf-Edgar Silber

Department of Cardio-Thoracic Surgery, Martin-Luther-University Halle-Wittenberg, Ernst-Grube-Strasse 40, 06097 Halle, Germany

Accepted 2 March 2005
Available online 20 March 2005

Abstract

Objective: Pulmonary metastasectomy as well as immunotherapy have reproducible, albeit limited efficacy in advanced renal cell carcinoma (RCC). We examined whether metastasectomy improved overall survival compared with results of immunotherapy.

Methods: Between 1975 and 2003, 64 patients (41 men, 23 women) underwent pulmonary resection of metastatic RCC. Only patients who met the criteria for potentially curative operation, that means, control of primary tumor, ability to resect metastatic disease and no other extrapulmonary metastases, were included.

Results: The overall 5-year survival was 33.4% (median survival: 39.2 months). A significant longer survival was observed using multivariate analysis in patients with complete pulmonary resection (R0), with a 5-year survival of 39.9% and a median survival of 46.6 months in correlation to patients with incomplete resection (5-year survival 0%, median survival 13.3 months). In multivariate analysis patients with synchronous metastases had a significant worse prognosis in correlation to patients with metachronous metastases. The 5-year survival of curative resected patients with metachronous metastases was 43.7% versus 0% for synchronous metastases, respectively. In patients with solitary metastasis and R0 resection, we observed a 5-year survival of 49%, whereas the rate was 23% in patients with more than a single metastasis. When establishing prognostic groups as suggested by the International Registry based on the risk factors disease-free interval, number of metastasis and complete resection the group with the best prognosis showed a 5-year survival of 52% (median survival 75.2 months).

Conclusion: Metastasectomy nowadays is the best treatment option in cases with technical resectable metastases with as much as possible good prognostic factors (metachronous metastases with long DFI, number up to 6 metastases).

© 2005 Elsevier B.V. All rights reserved.

Keywords: Lung metastasis; Renal cell carcinoma; Survival; Prognostic factors

1. Introduction

Renal cell carcinoma (RCC) accounts for about 2% of all cancers, with a world-wide annual increase of 1.5–5.9% due to enhanced detection of tumors by expanded use of imaging techniques. A total of 25–30% of patients with RCC have overt metastases at initial presentation [1,2]. About one third of patients with tumor of the kidney at diagnosis will develop metastatic disease during the following time. Thus, nearly 50–60% of all patients with RCC will eventually present with metastatic disease that requires individual treatment decision. Patients with untreated metastatic disease have a 5-year survival of 0% to 18% [3].

The first resection of a pulmonary metastasis in a patient with RCC was performed by Barney and Churchill in 1939 [4]. Since then, surgery remains the only effective treatment for patients with isolated pulmonary metastases. The published 5-year survival rates after pulmonary metastasectomy of renal origin...
range from 36–54%. Good prognostic factors for pulmonary resection are solitary metastasis and long disease free interval.

While RCC responds poorly to single-agent chemotherapy or hormonal therapy, immunotherapy with subcutaneous recombinant interleukin-2 (IL-2) alone or in combination with recombinant interferon–alpha (IFN-α) yielded significant therapeutic efficacy in RCC.

In the era of emerging effective systemic therapy (usually immunotherapy) the role and indications of surgery for RCC must be scrutinized and eventually again defined. This retrospective analysis of patients subjected to aggressive surgical management of lung metastasis after radical tumornephrectomy reinforces the need to find indications/contraindications for complete eradication of metastatic disease or conservative treatment with immunotherapy.

2. Patients and methods

64 patients (41 men, 23 women) with lung metastasis of RCC were operated on for curative purposes in our department between September 1975 and September 2003 (Table 1). The indications for the operation as well the operation itself were performed by the same surgeon. In all patients the primary RCC was treated by radical nephrectomy. Six patients had lung metastases at the same time as RCC diagnosis (synchronous metastasizing). The mean disease free interval between primary resection of the kidney and pulmonary metastasectomy for patients with metachronous metastases was 40.9 months. Pulmonary metastases were bilateral in 22 patients and unilateral in 42 patients. Ten sternotomies, 10 staged bilateral and 44 unilateral thoracotomies were performed. In four patients with bilateral metastases the planed second lateral thoracotomy was not carried out because of incomplete resection (R1/R2) after the first lateral thoracotomy. Wedge resection was performed in 48 patients, lobectomy in 7 and pneumonectomy in two patients. Because of miliary metastasizing seven patients had only biopsy. Finally, the resection was complete (R0) in 84% (n = 54).

After metastasectomy, a microscopic residual tumor remained in two patients (R1) and a macroscopic residual tumor in 8 patients (R2). 14% (9/64) of the patients received adjuvant therapy as chemo- or immunotherapy. Those patients however did receive different schemes so no grouping for survival analysis depending on adjuvant treatment was possible.

All patients were post-examined in our outpatient department. Survival was calculated from the time of metastasectomy. For analysis of follow-up data, life table curves were calculated with Kaplan-Meier methods, and survival distributions were compared by use of log-rank statistics. The Cox proportional hazards model was applied for multivariate analysis using the SPSS software program (SPSS Inc., Chiacago, U.S.).

3. Results

There were no operative deaths. Follow-up of all patients ranged from 60 days to 13 years (median 23.2 month). Detectable recurrent disease occurred in 31 (57%) of the 54 patients with RO resection. Local recurrence in the lung occurred in eight and distant metastases in only one organ in 11 patients (kidney n = 4, brain = 3, liver n = 2, bone n = 1, thyroid n = 1), 12 patients suffered from multiple metastases. Of these, 25 patients died and 9 patients died without knowledge of recurrent disease during the follow-up. Overall estimated long term survival was 33% and 13% at 5 and 10 years, respectively. The median survival of all patients was 30 months.

4. Influence of prognostic factors

4.1. Synchronous versus metachronous metastases

Of the 54 patients who underwent complete pulmonary resection (R = 0), 5 patients had synchronous pulmonary metastases at the time of RCC diagnosis. The other 49 patients developed the metachronous metastases during follow-up after nephrectomy. The 5-year survival of the patients with metachronous metastases was 43.7% (median survival 56.7 months). No patient with synchronous metastases survived the fourth year after thoracotomy (median survival 15.3 months). There was a significant difference between these two groups (p = 0.033).

4.2. Disease free interval

The occurrence of metastasis can also be expressed as the time from treatment of the primary tumor to the development of pulmonary metastases – disease free

---

Table 1
Clinical and pathological characteristics of patients and their pulmonary metastases

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>64</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>60.0 ± 8.8</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>41/23</td>
</tr>
<tr>
<td>Disease free interval (month)</td>
<td>40.9 ± 40.6</td>
</tr>
<tr>
<td>Synchronous/metachronous</td>
<td>6/58</td>
</tr>
<tr>
<td>Uni-/bilateral</td>
<td>41/23</td>
</tr>
<tr>
<td>Access</td>
<td></td>
</tr>
<tr>
<td>Sternotomy</td>
<td>10 16%</td>
</tr>
<tr>
<td>Thoracotomy (unilateral)</td>
<td>44 68%</td>
</tr>
<tr>
<td>Thoracotomy (bilateral)</td>
<td>10 16%</td>
</tr>
<tr>
<td>Surgical Procedure</td>
<td></td>
</tr>
<tr>
<td>PE/diagnostic thoracotomy</td>
<td>7 (11%)</td>
</tr>
<tr>
<td>Atypical resection</td>
<td>48 (75%)</td>
</tr>
<tr>
<td>Lobectomy</td>
<td>7 (11%)</td>
</tr>
<tr>
<td>Pneumonectomy</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Residual tumor situation</td>
<td></td>
</tr>
<tr>
<td>R0</td>
<td>54 (84%)</td>
</tr>
<tr>
<td>R1</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>R2</td>
<td>8 (13%)</td>
</tr>
</tbody>
</table>
interval (DFI). Thirty-one patients with R0 resection had a DFI of less than three years with a 5-year survival of 32.6% (median survival 36.9 months). This was not significantly lower (p = 0.17) than the 5 year-survival of 49.8% (median survival 59 months) in the 23 patients with R0 resection, but with a longer disease free interval.

4.3. Localization of metastases

The majority of patients (37 of 54) in this study had unilateral pulmonary metastases. In patients with complete resection, there was no difference in postthoracotomy survival between patients who had unilateral metastases (5-year survival 40.3%) versus patients with bilateral localization of the metastasis (40.4%; p = 0.93).

4.4. Number of metastases

The mean number of nodules resected was 2.7 ± 2.9 (range 1 to 19). The survival of the 22 patients with solitary metastases was 54.7% at 5 years (median survival 71.6 months). Five-year survival rate for the 32 patients with multiple metastases was 29.8% (median survival 32.7 months). This difference in survival was not significant (p = 0.07). However, the classification in solitary metastasis, 2–6 metastases and more than six metastases showed with 5-year survival rates of 54.7%, 32.0% and 0% significant differences (p = 0.02).

4.5. Resectability

The overall survival of the 54 patients who underwent complete resection (R0) of the pulmonary metastases was 39.9% with a median survival of 46.5 months. None of the patients with incomplete resection (R1 and R2) survived more than four years. Three-year survival rate of the patients with incomplete resection was 22%, the median survival 13.3 months. These differences were significant (p = 0.0006).

4.6. Multivariate analysis

Relative risks of death and multivariate analysis were calculated on all 64 patients. When considered separately, metachronous versus synchronous, number of metastases (solitary, 2–6 and >6 metastases) and resectability were significant prognostic variables; localization (uni- vs. bilateral) and age (not shown) were not significant. The relative risks of death for each variable are adjusted for all variables of interest: age, synchronous vs. metachronous, number of metastases (solitary vs. multiple), localization and resectability (Table 2). The best prognosis was observed for metachronous metastases, single metastases and curative resectability. Patients with incomplete resections had clearly the worst prognosis.

4.7. Staging of the international registry of lung metastases

The International registry of lung metastases performed a prognostic grouping that used three parameters of prognostic significance: resectability, DFI and number of metastases. We staged our patients in accordance to these four prognostic groups: Group I: resectable, no risk factors (DFI ≥ 36 months and single metastasis); Group II: resectable, one risk factor (DFI < 36 months or multiple metastases); Group III: resectable, two risk factors (DFI < 36 months and multiple metastases) and Group IV unresectable (Fig. 1). The 5-year survival rates were 52.5% (median survival 75.2 months), 48.2% (59.0 months), 21.5% (35.0 months) and 0% (13.3 months) for group I, II, III and IV, respectively. The differences between the groups were highly significant (p = 0.0003).

5. Discussion

Barney and Churchill in 1939 were the first to demonstrate the value of resecting a pulmonary metastases in a patient with RCC [4]. Since than metastasectomy of lung metastases from RCC represents the best treatment in selected cases, especially in patients with limited RCC and good performance status. Recent advances in immunochemotherapy have led to response rates as high as 20% with concomitant improvements in cancer specific survival. The present study was conducted to describe criteria for selecting patients with isolated pulmonary metastases. The aim was to identify patients who would benefit from metastasectomy or who should be treated with immunochemotherapy.

---

**Table 2**

Multivariate Cox regression analysis of potential risk factors for pulmonary metastasectomy in patients with advanced RCC

<table>
<thead>
<tr>
<th>Location</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60 years</td>
<td>0.06</td>
<td>0.5–1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metachronous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synchronous</td>
<td>7.8</td>
<td>1.5–11.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>1.2</td>
<td>0.72–3.4</td>
<td>0.25</td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solitary</td>
<td>5.4</td>
<td>0.16–0.83</td>
<td>0.02</td>
</tr>
<tr>
<td>Multiple</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1/R2</td>
<td>11.1</td>
<td>0.1–0.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Overall 5-year survival in our series was 33% and 39.9% for complete resection, respectively. Similar results for surgery were observed in the literature, with 5-year survival rates ranging from 36 to 54% for complete resection. The most important and multivariate significant prognostic factor influencing survival is resectability. Patients with incomplete resection had a 3-year survival rate of 22% and a median survival of 13.3 months a 11.1-fold evaluated relative risk in correlation to patients with complete resection. Median survival with untreated metastatic RCC is 10 months [1]. Atzpodien has reported overall 5-year survival of 16% with a median survival of 20 months for 425 patients with metastatic RCC treated with subcutaneous IL-2/INF-alpha2A therapy [5]. Patients with metastatic RCC limited to the lung fared significantly better than patients who had evidence of disease in multiple organs [6]. Thus, preoperative diagnostic assessment must analyze the curative resectability of the metastases, because patients with residual tumor tissue or bulky disease did not benefit from operation. This confirms data from other studies, who also detected significant worse survival for patients with incomplete resection of metastases [7,8].

The second multivariate significant risk factor is the time between treatment of primary RCC and occurrence of pulmonary metastases, but only in the criteria of synchronous versus metachronous metastases. Patients with synchronous metastases have a very short survival after metastasectomy and the relative risk in correlation to patients with metachronous metastases is 7.8 higher. Synchronous metastasizing is also a significant risk factor in patients treated only with combined immunotherapy [9]. May et al. reported a relative risk of 1.81 for patients with synchronous different organ metastases in RCC in correlation to metachronous occurrence. The median survival after nephrectomy of node-negative RCC and immunotherapy for patients with only synchronous lung metastases is 31 months. This is nearly twice as long as our median survival (15.3 months). Nevertheless, that the results of May et al. only include RCC patients with a very good prognostic factor – tumor negative lymph nodes at the time of diagnosis, this outcome implicates, that patients with synchronous metastases may profit from immunochemotherapy in correlation to surgery. In the group of completely resected patients the DFI, which had a cut-off of 36 months, is not a significant prognostic factor. Recent reports [8], 10) appear to support our results, in other metastasectomy studies DFI is a prognostic parameter and varied largely between one to four years [11–13].

Univariate analysis of our data shows that the number of resected metastases is relevant for survival of complete resected patients. The best prognosis have patients with solitary metastasis, therefore many reports [10,11] as well as the International Registry of Lung Metastases [14] limit their staging to patients with solitary versus multiple metastases. However, the results of this study is the finding that worse prognosis is combined with more than 6 metastases. We only found one immunotherapy study that investigate the correlation of the number of lung metastases in RCC patients and survival [6]. Han et al. identified 9 of 120 patients with lung metastases who had only a solitary lesion in the lung. These 9 patients had a 5-year survival rate of 67%, which was significant in correlation to patients with two or more metastases. The overall response rate for immunotherapy after nephrectomy in the study of Han et al. was very high (44%) for patients with lung metastases, which may be connected with the fact that only favorable risk groups (primary tumor without node infiltration, lung metastases) were recruited. Most study groups report more modest response rates for immunotherapy with IL-2, IFN-α and 5-FU [15].

The role of metastasectomy in combination with immunotherapy as neoadjuvant or adjuvant therapy is discussed controversially. Kim and Louie reviewed 399 patients with metastatic RCC who received IL-2 with or without lymphokine-activated killer cell immunotherapy [16]. Eleven patients underwent surgical resection of the residual tumor in the lung, kidney, retroperitoneum, or pelvis, and were rendered surgically free of disease after immunotherapy and remained alive without recurrence after a median follow up of 21 months. Progrebniak et al. reported
about 23 patients who underwent resection of pulmonary metastases of RCC of whom 18 had previously received interleukin immunotherapies [17]. Patients who underwent complete resection of the metastatic disease had a mean survival of 49 months. Walther et al. describe their experiences of adjuvant immunotherapy after pulmonary metastasectomy. All 93 patients received palliative tumornephrectomy. 32% an additional resection of the lung metastases [18]. But only 40% of the surgically treated patients could receive immunotherapy because of fast tumor progression. Summarizing the present data of combined therapy from the early 90’s no conclusions can be drawn and newer studies are lacking.

6. Conclusions

In RCC the results of lung metastatic surgery are increasingly recognized because of the obvious failure of systematic therapeutic approaches. On the basis of our data we can recommend pulmonary metastasectomy in RCC patients who have technical resectable metastases with as much as possible positive prognostic factors (metachronous metastases with long DFI, solitary up to 6 metastases) and a good functional status. In patients with unresectable metastases or several bad prognostic factors (synchronous metastases or short DFI, more than 6 metastases) immunotherapy should be the preferred treatment.

References


Editorial Comment

Allan J. Pantuck, Los Angeles, CA, USA
apantuck@mednet.ucla.edu

Approximately 30% to 50% of patients with RCC will eventually develop metastatic disease. Patients with metastatic RCC generally face a poor prognosis, however subsets of patients with advanced disease display a heterogeneous prognosis and outcome with some patients experiencing prolonged survival. Immunotherapy can be expected to produce objective responses in only 10 to 20% of metastatic RCC patients, of which only 5–10% will be complete responders. The role of surgery in metastatic RCC is becoming clearer, and the optimal management of many patients will involve a combined approach with surgery and systemic immunotherapy. Clinical situations in which surgery is potentially appropriate for patients with metastatic RCC include: excision of solitary or multiple metastases, excision of locally recurrent disease, resection of a residual mass after systemic therapy, and palliation. Despite the recent interest in algorithms to predict RCC recurrence and survival, the literature provides little guidance to the clinician faced with the management of these patients,
and selection criteria are not well defined. Hofmann et al. [1] help fill this gap in the literature.

Previously, several series have suggested a potential benefit for complete surgical resection of all tumor burden, including removal of both the primary renal mass as well as metastatic deposits in carefully selected patients with minimal volume metastatic disease. Surgical removal of solitary metastasis was widely accepted as potentially effective by the mid-1970s after several groups reported five-year overall survival rates of 29% to 35% [2–4]. It is possible that these rates may in fact be better or worse depending on other clinical and/or pathologic factors such as performance status, site of metastasis, interval after nephrectomy, and whether the metastasis is completely resected.

Hofmann et al. contribute significantly to the literature by describing the outcome of 64 patients undergoing pulmonary resection of metastatic RCC, making it the largest such series to date. It should be emphasized that it took nearly 30 years to accumulate this experience and noted that these 64 patients represent a carefully selected group. Only patients who had disease that was judged to be potentially curative by surgical means and no evidence of extrapulmonary metastases were treated with this aggressive approach.

In this population 5-year survival was 33.4%, and median survival was 39.2 months. More importantly, they were able to further stratify the patients into prognostic groups whose 5 year survival ranged from 0% to 52%. Factors which predicted for a better survival included ability to perform a complete resection, disease free interval greater than 36 months, and limited number of metastases. Overall, the data supports the notion that metastasectomy is a viable treatment option for carefully selected patients, and that this approach can by tailored to individual patients using standard eligibility criteria.

References

Laparoscopy

Morbidity of Laparoscopic Extraperitoneal versus Transperitoneal Radical Prostatectomy versus Open Retropubic Radical Prostatectomy

M. Remzi*, H.C. Klingler, M.V. Tinzl, Y.K. Fong, M. Lodde, B. Kiss, M. Marberger

Department of Urology, University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria

Accepted 18 March 2005

Available online 12 April 2005

Abstract

Objective: Morbidity and postoperative pain after extraperitoneal (E-LRPE) and transperitoneal (T-LRPE) laparoscopic radical prostatectomy was compared to open extraperitoneal radical prostatectomy (O-RPE).

Material and methods: Between January 2002 and October 2003, we evaluated 41 E-LRPE, 39 T-LRPE and 41 O-RPE prospectively. All operations were performed as standard procedures by the same group of surgeons and perioperative results and complications were evaluated. Pain management was performed with tramadol 50–100 mg on demand, and no other form of anaesthesia was given. Postoperative pain was assessed daily in all patients quantifying analgesic requirement and evaluation of Visual Analogue Scale (VAS). All patients had at least a 12 month follow-up.

Results: Mean age, prostate volume, PSA and Gleason score were comparable between all three groups (p > 0.05). Mean blood loss was lower with laparoscopy (189 ± 140 and 290 ± 254 ml), as compared to 385 ± 410 ml for O-RPE (p = 0.002). However, mean operating times were significantly longer in L-TRPE (279 ± 70 min) as compared to E-LRPE (217 ± 51 min) and O-RPE (195 ± 72 min) (p < 0.001), but E-LRPE and O-RPE showed no statistical difference (p = 0.1143). Average VAS score on the 1st and 5th postoperative day for E-LRPE versus T-LRPE versus O-RPE was 4.9 ± 1.0 versus 7.8 ± 1.5 versus 5.8 ± 1.9 and 1.6 ± 0.9 versus 2.3 ± 1.2 versus 2.3 ± 0.9 respectively, which was significant lower (p = 0.02) between E-LRPE versus T-LRPE (p < 0.001) and O-RPE (p = 0.008), but equal (p = 0.655) between T-LRPE and O-RPE since postoperative day 3. Mean tramadol analgesic consumption within the first postoperative week was 290 versus 490 versus 300 mg respectively, which was statistical different between E-LRPE and T-LRPE (p < 0.001), O-RPE and T-LRPE (p < 0.001), but not between E-LRPE and O-RPE (p = 0.550). Statistical analysis revealed a strong correlation of urinary leakage with increased postoperative pain (p = 0.029) in all groups, especially for T-LRPE (p = 0.007). Likewise, increased operating times (>240 min) were associated with increased post-operative pain (p = 0.049). Full continence defined as no pads at one year was achieved in 36/41 (88%, E-LRPE) versus 33/39 (85%, T-LRPE) versus 33/41 (81%, O-RPE), respectively (p = 0.2).

Conclusion: E-LRPE resulted in a significant subjective (VAS Score, p < 0.001) and objective (analgetic consumption, p < 0.001) pain reduction compared to T-LRPE, but only in VAS Score compared to O-RPE (p = 0.008). Analgetic consumption during first postoperative week was equal in E-LRPE (290 mg) and O-RPE (300 mg) (p = 0.550). Shorter operating times, lower urinary leakage rates, lower stricture rates and lower blood loss in E-LRPE compared to T-LRPE are mainly explained due to the long learning curve in LRPE, which we did not overcome yet, and not due to the approach (extraperitoneal versus transperitoneal).

© 2005 Elsevier B.V. All rights reserved.

Keywords: Prostate cancer; Radical prostatectomy; Laparoscopy; Morbidity
1. Introduction

Guillonneau and Vallancien reported their experience with over 1000 transperitoneal laparoscopic radical prostatectomy (T-LRPE) and many centres could repeat their promising results and data obtained from these studies demonstrated competitive results in respect to oncological findings [1–3], continence [3,4] and potency rates [4].

We started laparoscopic radical prostatectomy in April 2000, using the transperitoneal laparoscopic radical prostatectomy (T-LRPE) technique described by Guillonneau [1]. We overcame the so-called learning curve with about 30 initial cases. However, it was striking to us, that patients undergoing T-LRPE still complained of marked postoperative pain which did not seem to be less than in patients undergoing open extraperitoneal radical prostatectomy (O-RPE) at the same institution within this period. Similar results were shown by Fornara et al. [5], who reported that the surgical trauma measured by acute-phase-C-reactive protein, serum amyloid A, interleukin 6 and interleukin 10 was equal between open radical prostatectomy and laparoscopic radical prostatectomy.

Already in 1997 Raboy et al. [6] published 2 cases of extraperitoneal laparoscopic radical prostatectomy (E-LRPE), but it was Bollens et al. [7,8] who clearly demonstrated the technical feasibility and competitiveness of this approach, particularly in respect to perioperative morbidity. Their findings were supported by other studies [9,10]. We therefore changed our standard T-LRPE surgical approach to the E-LRPE technique in an attempt to reduce peri-operative morbidity and pain. Objective of this prospective study was to determine the results, morbidity and postoperative pain of E-LRPE and T-LRPE, as compared with O-RPE.

2. Patients and methods

We evaluated 121 consecutive patients undergoing radical surgical treatment for adenocarcinoma of the prostate between January 2002 and October 2003. The aim of this study was to compare the morbidity of LRPE versus O-RPE. 41 of the patients had E-LRPE, 39 T-LRPE and 41 were treated by O-RPE. Decision to perform either laparoscopic or open RPE was based on the preferences of the performing surgeons or the referring institution. After overcoming the initial learning curve for laparoscopic RPE case 34 to 73 was included as T-RPE and the next 41 were performed as the first E-LRPE. All laparoscopic procedures were performed by an experienced laparoscopic surgeon (HCK), who performed more than 300 major laparoscopic procedures like tumornephrectomy, tumornephroureterectomy, pyeloplasty and others.

Pertinent patient characteristics were shown in Table 1, demonstrating a comparable patient cohort in each group. All operations were performed as standard procedures and evaluated for results and complications. All patients had histological confirmed adenocarcinoma of the prostate and were clinically ≤T2. Organ confined organ involvement was determined by unsuspicious digital rectal examination, low serum total PSA levels (<10 ng/mL), Gleason score < 7 and negative bone-scan imaging. At PSA levels > 10 ng/mL and/or Gleason score > 7 CT scans or MRI were routinely performed (n = 12).

2.1. Preoperative preparation

After introduction of general anaesthesia with mechanical ventilation, a naso-gastric tube and an indwelling 20 F Foley catheter were inserted. An external warming system (Bair Hugger®; USA) was draped around the patient and an intravenous broad-spectrum antibiotic was administered intraoperatively and continued for further 5 postoperative days.

2.2. Laparoscopic techniques

In all laparoscopic operations a voice-controlled robotic arm (AESOP®; Computer Motion, USA) was used for camera guidance. For laparoscopic cutting and dissection a harmonic scalpel (Ultracision, Ethicon, USA) and bipolar forceps (Ethicon, USA) were used. Carbon dioxide gas insufflation was kept at a maximum intra-abdominal pressure of 12–14 mmHg. The patients were

Table 1
Patient, operative and pain characteristics (means values ± SD)

<table>
<thead>
<tr>
<th>Patients position (head down)</th>
<th>E-LRPE (n = 41)</th>
<th>T-LRPE (n = 39)</th>
<th>O-RPE (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59 ± 12</td>
<td>61 ± 11</td>
<td>60 ± 14</td>
</tr>
<tr>
<td>Prostate volume (ml)</td>
<td>32 ± 14</td>
<td>37 ± 16</td>
<td>44 ± 18</td>
</tr>
<tr>
<td>Total PSA (ng/mL)</td>
<td>8.1 ± 6.1</td>
<td>5.5 ± 3.7</td>
<td>6.9 ± 4.4</td>
</tr>
<tr>
<td>Gleason score</td>
<td>5.5 ± 1.3</td>
<td>5.1 ± 1.2</td>
<td>4.7 ± 1.5</td>
</tr>
<tr>
<td>Operating times (min)</td>
<td>217 ± 51⁷</td>
<td>279 ± 70</td>
<td>195 ± 72⁷</td>
</tr>
<tr>
<td>Hospitalisation time (days)</td>
<td>7 ± 2b</td>
<td>7 ± 2</td>
<td>10 ± 4⁷</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>189 ± 140⁸,⁹</td>
<td>290 ± 254</td>
<td>385 ± 410</td>
</tr>
<tr>
<td>VAS score post OP day 1</td>
<td>4.9 ± 1.0⁷,⁹</td>
<td>7.8 ± 1.5</td>
<td>5.8 ± 1.9⁹</td>
</tr>
<tr>
<td>VAS score post OP day 5</td>
<td>1.6 ± 0.9⁸</td>
<td>2.3 ± 1.2</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>Tramadol consumption (mg)</td>
<td>290 ± 60⁹</td>
<td>490 ± 120</td>
<td>300 ± 90⁹</td>
</tr>
</tbody>
</table>

All other showed no statistical difference (p > 0.05). Statistical significant difference between:
8 E-LRPE and T-LRPE.
9 E-LRPE and O-RPE.
10 T-LRPE and O-RPE.
placed in a slightly over-stretched supine position with either a 10° (E-LRPE) or 30° head down (T-LRPE) Trendelenburg position to facilitate rectal access and dissection.

For extraperitoneal access a 3 cm semi-lunar umbilical incision was used, the extraperitoneal cavity is created primarily digitally and thereafter with the aid of a 12 mm balloon system (AutoSuture – Tyco, Norwalk, USA) (10). At the umbilical site a 12 mm blunt-tipped balloon-port system (AutoSuture – Tyco, Norwalk, USA) served as the optical access.

In the transperitoneal group the pneumo-peritoneum was generated via a Veress needle, the first 12 mm port (Auto Suture – Tycho, Norwalk, USA) was inserted blindly thereafter.

In both laparoscopic groups we used two additional 12 mm and two 5 mm ports (AutoSuture – Tycho, USA) in a semilunar position – placed under direct vision, as described elsewhere [4].

2.2.1. Operative techniques

All laparoscopic patients, but two in the T-LRPE group had a staging lymphadenectomy of the obturator fossa. In O-RPE only 71% had a staging lymphadenectomy (PSA > 10 ng/mL, Gleason Score 6, more than 30% of biopsy cores positive). The specimens were sent for frozen section to exclude lymph node involvement. No nodal involvement was reported. The extraperitoneal laparoscopic radical prostatectomy was performed in a descending technique as described by Bollens [7,8] and the transperitoneal radical prostatectomy was performed using a standard Montsouris technique [1]. Dissection of the bladder neck was performed with a combination of harmonic scalpel and bipolar forceps. In cases where a nerve sparing approach was considered (n = 32) we used solely endo-clips (Hem-o-loc system, Weck, USA) for ligation of vessels and control of the prostatic pedicle. In all other cases bipolar dissection was also used. The dorsal venous complex was ligated with Vicryl 0 sutures (Ethicon Johnson & Johnson, Vienna, Austria) and the proximal portion of the complex was divided using the harmonic scalpel. The proximal urethral stump was solely dissected with endoscissors, without any electrocautery.

Retrieval of the specimen was achieved in T-RPE through enlargement of one 12 mm port in the right abdomen, converting it into a 3 cm muscle splitting incision. In E-LRPE the subumbilical incision was used for this purpose. All specimens were entrapped in a cell-tight organ bag (Rüsch, Kernen, Germany) and thereafter the specimens were sent for frozen section to control for negative resection margins.

In all patients a waterbath anastomosis was performed with the aid of a 24 F metal Guyon bougie and two running Vicryl 2-0 sutures. An 18-F Foley catheter (Rüsch, Kernen, Germany) was inserted for postoperative bladder drainage and a 21-F silicone drainage tube was placed via one port. All other 12 mm ports were closed with Vicryl 0 endo-sutures. A voiding cystogram was performed at the 6th postoperative day. Provided no urinary leakage was documented the catheter was removed.

2.3. Open surgical access and techniques

Open extraperitoneal ascending radical prostatectomy was performed via a standard midline incision in the lower abdomen, including staging lymphadenectomy of the obturator fossa [11]. A Mercedes-star type bladder neck reconstruction was performed using Dexon 2-0 (Tyco Healthcare, Norwalk, USA) sutures and in addition Dexon 4-0 (Tyco Healthcare, Norwalk, USA) sutures were used for mucosal eversion. For the anastomosis seven Maxon 3-0 (Sherwood Medical, St. Louis, USA) single sutures were used. An 18-F Foley catheter was inserted for the post-operative bladder drainage and another two 21-F silicone drainage tubes for wound drainage. A cystogram was performed on the 7th postoperative day and the catheter was removed, if there was no urinary extravasation.

2.4. Postoperative care

All patients started ambulation within 24 hours after surgery and fluids were given both orally and intravenously. On the second post-operative day wound drains were removed, laxatives were given, and regular alimentation started. For post-operative pain relief 50–100 mg tramadol was given either intravenously or orally upon demand. No other form of anaesthesia was given.

Data collected for this study included patient demographics, operation time, intraoperative blood loss, complication rate, Visual Analogue Symptom Scale (VAS) score (scale 0–10) (12) which was evaluated daily from the pre-operative day to the 6–8th post-operative day, consumption of analgesics and hospitalisation time. The daily collected VAS-Scores were named VAS0 for pre-operative, VAS1 to VAS9 for the 1st to 9th post-operative day respectively. Patients were further evaluated at scheduled follow-up visits at 1, 6 and 12 months post-operatively which included a detailed medical history with incontinence evaluation and pad-count, serum PSA levels, urinalysis, Valsalva stress test and postvoid residual volume measurement. Follow-up in all patients achieved at least 12 months, mean follow up was 14.9 months. Full continence was defined as no pad needed.

2.4.1. Statistical analysis

For all variables mean, standard deviation and range were given. To determine if distributions across factor levels were centred at the same location a non-parametric Wilcoxon rank score was used or Kruskal-Wallis if there were more than 2 groups. A Shapiro-Wilk W test was performed to evaluate the distribution of the variables. With a p-value less than 0.05 the distribution was not normal. For comparison of nominal variables Fisher’s exact test was performed. The computed F-Ratio that evaluates the effectiveness of the model, the function of the regression analysis, as well as the root square value and the root mean square error were given. A p-value ≤ 0.05 was considered to be statistically significant.

3. Results

Pertinent patient characteristics and intraoperative results are given in Table 1. No difference was seen between E-LRPE, T-LRPE, and O-RPE regarding age, prostate volume, total PSA and biopsy Gleason Score (p > 0.05). Mean operating times were significantly longer in the L-TRPE (279 ± 70 min) group as compared to E-LRPE (217 ± 51 min) and O-RPE (195 ± 72 min) (p < 0.001), but E-LRPE and O-RPE showed no statistical difference (p = 0.1143).

3.1. Operative morbidity

Mean blood loss was lower with both laparoscopic approaches E-LRPE (189 ± 140 ml, p = 0.0049) and T-LRPE (290 ± 254 ml, p = 0.021) in comparison to O-RPE (385 ± 410 ml). Two rectal injuries (O-RPE and T-LRPE) occurred and were recognised and repaired intraoperatively, but the case with T-LRPE was converted to open surgery.
3.1. Postoperative morbidity

One post-operative ileus occurred with T-LRPE but the patient recovered well with conservative treatment. Prolonged urinary leakage was associated with a decreased early continence rate in all groups (p = 0.020). Early full continence (no pad) rate one month post-operatively was highest in the laparoscopic groups with 11/41 (26.8%); E-LRPE) and 10/39 (25.6%; T-LRPE) versus 8/41 (19.5%) for O-RPE (p = 0.02). Similarly, full continence at 12 months was achieved in 36/41 (87.8%) versus 33/39 (84.6%) versus 33/41 (80.3%) respectively (p = 0.02).

The incidence of anastomotic stricture was lowest in E-LRPE (1/41 = 2.4%) as compared to T-LRPE (2/39 = 5.1%) and O-RPE (4/41 = 9.7%) (p > 0.05). Two strictures after laparoscopic procedure were dilated under flexible cystoscopic guidance. One laparoscopic and three strictures in the open group were endoscopically incised. Hospitalisation time was in both laparoscopic groups 7 ± 2 days, which was significantly lower than in the open group 10 ± 4 days (p < 0.001).

3.1.2. Pain (Table 3)

Average VAS score on the 1st and 5th postoperative day was 4.9 ± 1.0 (E-LRPE) versus 7.8 ± 1.5 (T-LRPE) versus 5.8 ± 1.9 (O-RPE) and 1.6 ± 0.9 versus 2.3 ± 1.2 versus 2.3 ± 0.9, respectively (Fig. 1). VAS Scores were significant lower in E-LRPE versus T-LRPE (p < 0.001) and O-RPE (p = 0.008) from day 1 to 5, and in O-RPE versus T-LRPE on day 1 to 2 (p = 0.007). From day 3 on no statistical differences were seen between the T-RPE and the O-RPE group regarding VAS-scores (p > 0.05) (Fig. 1). Mean VAS score after catheter removal on 6th (laparoscopy) or 8th post-operative day (open surgery) demonstrated a slightly increase in pain score. This however, was only significant in T-LRPE (p = 0.04).

Mean analgesic consumption within the first post-operative week was 290 ± 60 (E-LRPE) versus 490 ± 120 (T-LRPE) versus 300 ± 90 mg (O-RPE) respectively. Statistical analysis favoured extraperitoneal and open techniques over transperitoneal laparoscopic surgery (p < 0.001). No difference in analgesic consumption within the first postoperative week was seen between E-LRPE and O-RPE (p = 0.555). In addition, analysis revealed a strong correlation of prolonged urinary leakage with increased postoperative pain (p = 0.029) in all groups, however it was more prominent with T-LRPE (0.007). Likewise, increased operating times (more than 240 min) were associated with increased post-operative pain (p = 0.049).

3.1.3. Histopathological features

Staging lymphadenectomy was negative in all patients. Histopathological results at final histology were similar in all groups (Table 2) with localized prostate cancer (pT2) in 65.8%, 61.5% and 63.4% in E-LRPE, T-LRPE, and O-RPE, respectively (p > 0.05). Positive margins were found in 19.5% (n = 8), 25.6% (n = 10) and 19.5% (n = 8) for E-LRPE, T-LRPE, and O-RPE, respectively (p > 0.05).

4. Discussion

A variety of studies have confirmed the feasibility and equal oncological efficacy of LRPE for treatment of organ confined prostate cancer as compared to O-RPE and the results obtained in this study are in agreement to those reported previously [1–3,8–10,13–21]. Nowadays, one of the most important questions is therefore focussed on morbidity. In the recent literature it is not clear, if E-LRPE can reduce morbidity, especially pain compared to T-LRPE. Nevertheless, as many open surgeons question a marked benefit of the laparoscopic approach compared to O-RPE this study was conducted to clarify this issue. A match-pair analysis by the Heilbronn team [13] using the ascending T-LRPE and E-LRPE showed no benefit of E-LRPE over T-LRPE regarding analgesics dosage (21.9 ± 15.4 versus 26.3 ± 15.8, p = 0.111). Another study by Fornara et al. comparing morbidity of E-LRPE and O-RPE showed no significant benefit of the laparoscopic approach [5].

In our study mean blood loss was significantly lower with laparoscopy (189 versus 290 ml), as compared with 385 ml for O-RPE (p < 0.05), particularly in our E-LRPE group (p = 0.0049) with no need for transfusion. This clearly results from better visualisation, in particular of the dorsal venous complex, and the
increase in either the intra-abdominal or extra-peritoneal pressure generated within the working space.

Similarly, mean hospitalisation time was lower in the laparoscopic groups, as compared with open surgery \( (p < 0.0001) \). Hospitalisation time was longer particularly when compared to series from USA, where a mean hospital stay of 1.2 days was reported [14]. This difference is based on a different insurance coverage policy in Austria, where earlier patient dismissal is not promoted as the system is based on complete patient recovering rather than hospitalisation costs per day. Thus there was the same hospitalisation time for all in agreement with findings by other European centres [15].

Mean operating times were statistical longer in the T-LRPE (279 min) group compared to the E-LRPE (217 min, \( p < 0.001 \)) and to O-RPE (195 min, \( p < 0.001 \)). No statistical difference was seen between E-LRPE and O-RPE \( (p = 0.114) \). This however, reflects gaining experience and a still ongoing learning curve even in the E-LRPE group [16]. With the extraperitoneal approach, operating time is now similar to open surgery but the operating time in open surgery has increased with the routine use of bipolar forceps and loop magnification at nerve sparing surgery. But it has also taken into consideration that in E-LRPE 100% had a staging lymphadenectomy, but only 71% in the O-RPE group. These results underline the findings of Rassweiler et al. [16], who compared the first 219 with the second 219 LRPE and found a significant reduction in operating times from 288 min to 218 min, which was only slightly higher compared to most O-RPE (range 135–263 min). Cathelineau et al. from Institute Montsouris compared the first 100 E-LRPE with the last 100 T-RPE out of 400 LRPE performed in the year 2002 [17]. They found a significant reduction in operating times in the E-LRPE group \( (p = 0.003) \) from 10 min (163 min versus 173 min).

Prolonged anastomotic urinary leakage was more frequent for O-RPE (14.6%, \( n = 6 \)) and T-LRPE (13.5%, \( n = 5 \)) as compared to E-LRPE (7.3%, \( n = 3 \)). Because of the low number of patients this was not statistical difference \( (p > 0.05) \). The lower leakage rate in the extraperitoneal laparoscopic group may be caused by an improved suturing technique using continuous versus single suture, better visualisation in a more bloodless field and by an obviously improved drainage of the anastomotic region, thus avoiding local inflammation and irritation of the healing process in case of significant early urine leakage. However, it seems to be obvious that this also results, because of a still ongoing learning curve, which reflects also the results by Rassweiler et al. [16]. Presumably as a result of this incidence of anastomotic stricture rate was lowest in ELRPE (2.4%, \( n = 1 \)) as compared to TLRPE (5.1%, \( n = 2 \)) and O-RPE (9.7%, \( n = 4 \), \( p > 0.05 \)).

It was an interesting finding, that urinary leakage was associated with increased post-operative pain \( (p = 0.029) \) and decreased early continence rates \( (p = 0.020) \). This finding was more prominent in the T-LRPE group \( (p = 0.007) \). Apparently urinary leakage causes significant discomfort for the patient and drainage at this site may be inadequate likewise. Patients

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Histopathological results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E-LRPE ( (n = 41) )</td>
</tr>
<tr>
<td>Histology:</td>
<td></td>
</tr>
<tr>
<td>( pT2 ):</td>
<td>27 (65.8%)</td>
</tr>
<tr>
<td>( pT3 ):</td>
<td>14 (34.2%)</td>
</tr>
<tr>
<td>( pT4 ):</td>
<td>0%</td>
</tr>
<tr>
<td>pN+:</td>
<td>0</td>
</tr>
<tr>
<td>Postive margins (final histology)</td>
<td>8 (19.5%)</td>
</tr>
<tr>
<td>Gleason score</td>
<td>5.5 ± 1.9</td>
</tr>
<tr>
<td>Surgical access</td>
<td>5 ports – semilunar</td>
</tr>
<tr>
<td>Lymphadenectomy</td>
<td>41 (100%)</td>
</tr>
<tr>
<td>Nerve sparing</td>
<td>23 (56%)</td>
</tr>
<tr>
<td>Anastomosis</td>
<td>Running suture Vicryl 2-0</td>
</tr>
<tr>
<td>Catheter removal (mean days)</td>
<td>6.1</td>
</tr>
<tr>
<td>range</td>
<td>4–24</td>
</tr>
<tr>
<td>Prolonged anastomotic leakage</td>
<td>3 (7.3%)</td>
</tr>
<tr>
<td>Anastomatic stricture</td>
<td>1 (2.4%)</td>
</tr>
<tr>
<td>Rectal injury</td>
<td>0</td>
</tr>
<tr>
<td>Postoperativ ileus</td>
<td>0</td>
</tr>
<tr>
<td>Haemorrage</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histopathological results</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-LRPE ( (n = 41) )</td>
</tr>
<tr>
<td>Histology:</td>
</tr>
<tr>
<td>( pT2 ):</td>
</tr>
<tr>
<td>( pT3 ):</td>
</tr>
<tr>
<td>( pT4 ):</td>
</tr>
<tr>
<td>pN+:</td>
</tr>
<tr>
<td>Postive margins (final histology)</td>
</tr>
<tr>
<td>Gleason score</td>
</tr>
<tr>
<td>Surgical access</td>
</tr>
<tr>
<td>Lymphadenectomy</td>
</tr>
<tr>
<td>Nerve sparing</td>
</tr>
<tr>
<td>Anastomosis</td>
</tr>
<tr>
<td>Catheter removal (mean days)</td>
</tr>
<tr>
<td>range</td>
</tr>
<tr>
<td>Prolonged anastomotic leakage</td>
</tr>
<tr>
<td>Anastomatic stricture</td>
</tr>
<tr>
<td>Rectal injury</td>
</tr>
<tr>
<td>Postoperativ ileus</td>
</tr>
<tr>
<td>Haemorrage</td>
</tr>
</tbody>
</table>
with minimal urinary leakage after catheter removal may experience significant pain (Fig. 1), as demonstrated by the significant \( p = 0.04 \) pain peak on postoperative day 7–9 for T-LRPE. The only case of postoperative ileus occurred with T-LRPE and was associated with significant urinary leakage after catheter removal.

Artibani et al. [18] however, demonstrated in his study, that he could not find a significant difference favouring either group of laparoscopic techniques. This however, is not in agreement with findings in our study. However, the study from Creteil, France underlines our results, which described a significant benefit of the E-LRPE compared to T-LRPE even dose (6 versus 12.8 mg), and duration (0.5 versus 0.9 days) of morphine administration was not significant different, due to the low number of patients [10]. Besides to the fact that, that even after overcoming the initial learning curve of L-TRPE, patients did not seem to benefit significantly from laparoscopic surgery in respect to postoperative pain and morbidity as opposed to patients undergoing renal ablative surgery, in which we could demonstrate definite benefit with a laparoscopic approach [12]. Postoperative pain, as assessed by VAS score analysis revealed significant benefits with the extraperitoneal techniques \( p < 0.05 \) Table 3, though E-LRPE scored better than O-RPE in this respect the difference in analgetic consumption was not statistically significant \( p = 0.550 \). This might be due to the fact that postoperative pain was treated by tramadol 50–100 mg on demand. The subjective parameter of VAS scores were 4.9 versus 5.8 and 7.8 on first postoperative pain, which resulted in our study also in the reduction of analgesic consumption within the first postoperative week of 290, 300 and 490 mg for E-RPE, O-RPE and T-RPE, respectively. Subjectively, as a measure of VAS Score, patient had significant less pain with E-LRPE, compared to both T-LRPE and O-RPE \( p < 0.001 \). However this resulted not in higher pain consumption during the first week between E-LRPE and O-RPE \( p = 0.555 \). This might be due to the fact that tramadol was given in 50 mg steps on demand and this was sufficient also for VAS Score difference of 0.9 (5.8 O-RPE – 4.9 E-LRPE), but not for 2.9 (7.8 T-LRPE – 4.9 E-LRPE) or 2 (7.8 T-LRPE – 5.8 O-RPE). Likewise, mean analgesic consumption within the first postoperative week was lower in the extraperitoneal groups. Statistical analysis revealed a strong correlation of urinary leakage with increased postoperative pain in all groups, being most obvious with T-LRPE \( p = 0.001 \).

Likewise, increased operating time was associated with increased post-operative pain \( p = 0.011 \). This may be partially explained by findings by Meininger et al. [19]. In their study they could demonstrate increased heart rate and changes in acid-base balances during the prolonged pneumo-peritoneum. Whether this may directly influence post-operative pain remains unclear since these changes were reported to be minor only.

### Table 3

<table>
<thead>
<tr>
<th>Factor</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating times (&gt;240 min)</td>
<td>0.049</td>
</tr>
<tr>
<td>Urinary leakage</td>
<td>0.029</td>
</tr>
<tr>
<td>T-LRPE</td>
<td>0.007</td>
</tr>
<tr>
<td>Catheter removal in T-LRPE (day 7–9)</td>
<td>0.04</td>
</tr>
<tr>
<td>VAS Scores:</td>
<td></td>
</tr>
<tr>
<td>E-LRPE vs. T-LRPE</td>
<td>(&lt; 0.0001 )</td>
</tr>
<tr>
<td>E-LRPE vs. O-RPE</td>
<td>0.008</td>
</tr>
<tr>
<td>O-RPE vs. T-LRPE (day 1–2)</td>
<td>0.007</td>
</tr>
<tr>
<td>O-RPE vs. T-LRPE (day 3–8)</td>
<td>0.655</td>
</tr>
<tr>
<td>Analgetic consumption:</td>
<td></td>
</tr>
<tr>
<td>E-LRPE vs. T-LRPE</td>
<td>(&lt; 0.001 )</td>
</tr>
<tr>
<td>E-LRPE vs. O-RPE</td>
<td>0.550</td>
</tr>
<tr>
<td>O-RPE vs. T-LRPE (day 1–8)</td>
<td>(&lt; 0.001 )</td>
</tr>
</tbody>
</table>

5. Conclusion

In our study the introduction if E-LRPE resulted in a significant subjective (VAS Score, \( p < 0.001 \)) and objective (analgetic consumption, \( p < 0.001 \)) pain reduction compared to T-LRPE, but only in VAS Score compared to O-RPE \( p = 0.008 \). Analgetic consumption during first postoperative week was equal in E-LRPE (290 mg) and O-RPE (300 mg) \( p = 0.550 \). Shorter operating times, lower urinary leakage rates, lower stricture rates and lower blood loss in E-LRPE compared to T-LRPE are mainly explained due to the long learning curve in LRPE, which we did not overcome yet, and not due to the approach (extraperitoneal versus transperitoneal). However, E-LRPE has significantly less post-operative pain as compared to T-LRPE. Oncological results, morbidity and postoperative complication rates compare favourably with open surgery, but continence rates improved by laparoscopic radical surgery. Patients undergoing radical prostatectomy may benefit most from an extraperitoneal laparoscopic approach, which is the operation of choice today at our institution.

### References


**Editorial Comment**

Jens Rassweiler, *Heilbronn, Germany*

jens.rassweiler@slk-kliniken.de

The authors should be congratulated for their effort comparing three different approaches of radical prostatectomy. However, the results of this study have to be considered carefully. The measurable morbidity of all three techniques does not differ very much according to the minor access trauma caused by an extraperitoneal midline incision versus a trans- or extraperitoneal 5 port technique including a 3 cm periumbilical incision (Ref. [5]). Therefore, other factors become more important, such as the quality of dissection and urethrovesical anastomosis. This depends mainly on the experience of the surgeon with the respective technique. In this study, like in other comparative series (Ref. [5,18]), the level of surgeons’ experience differed significantly: Whereas, open radical prostatectomy has been performed by the respective surgeons at least more than 500 times (i.e M.M.), the laparoscopic series included case 34 to 73 (transperitoneal) and 74 to 115 (extraperitoneal) of the laparoscopic surgeon (H.C.K.). This bias regarding the level of experience is evident. Our own experience including more than 1,200 transperitoneal laparoscopic radical prostatectomies did not reveal any significant advantages for the extraperitoneal approach (Ref. [13]).

However, there are certain clear indications for this approach, such as previous abdominal surgery, gross obesity and requirement of simultaneous inguinal hernia repair. Moreover, there is no doubt, that in the rare case of urine leak or hematoma, the extraperitoneal approach is advantageous, but it is also responsible for a higher rate of lymphoceles. With the use of the single-knot technique described by van Velthoven, the problem of traction during the vesico-urethral anastomosis has become less important. Based on this, we strongly support the parallel training of both techniques at centers of expertise.

Concerning the direct comparison of the different operative techniques, one should be very careful with the results of studies based on significant differences regarding the level of surgeons’ expertise.
Laparoscopy

Retroperitoneoscopic Donor Nephrectomy: A Retrospective, Non-Randomized Comparison of Early Complications, Donor and Recipient Outcome with the Standard Open Approach

Alexander Bachmann\textsuperscript{a,d,*}, Thomas Wolff\textsuperscript{c}, Robin Ruszat\textsuperscript{a}, Olivier Giannini\textsuperscript{b}, Michael Dickenmann\textsuperscript{b}, Lorenz Gürke\textsuperscript{c}, Jürg Steiger\textsuperscript{b}, Thomas C. Gasser\textsuperscript{a}, Christian G. Stief\textsuperscript{d}, Tullio Sulser\textsuperscript{a}

\textsuperscript{a}Department of Urology, Basel University Hospital, Spitalstr. 21, CH-4031 Basel, Switzerland
\textsuperscript{b}Division of Transplantation Immunology and Nephrology, University Hospital Basel, Switzerland
\textsuperscript{c}Department of Surgery, University Hospital Basel, Switzerland
\textsuperscript{d}Department of Urology, Ludwig Maximilian University of Munich, Germany

Accepted 3 March 2005
Available online 20 March 2005

Abstract

Objectives: We retrospectively performed a comparative analysis of retroperitoneoscopic and open donor nephrectomy in terms of donor complications, as well as recipient complications and functional graft outcome.

Methods: A total of 134 donor nephrectomies including 69 open (ODN) and 65 retroperitoneoscopic (RDN) nephrectomies was analyzed retrospectively. Both groups were comparable in terms of age, body mass index (BMI), operating time (OPT), warm ischemia time (WIT) and blood loss.

Results: There were no statistically significant differences with respect to recipient outcome, mean values for age, BMI, OPT and cold ischemia time (CIT). The overall donor complication rate did not differ. Early functional graft follow-up showed significant differences in 24 h-urine output between the two groups ($p < 0.001$), but serum creatinine was comparable after 7, 30, 180 and 365 days. The early rejection rate in the recipients was similar in the two groups.

Conclusion: Retroperitoneoscopic donor nephrectomy (RDN) provides comparable perioperative features, such as operating time, warm ischemia time (WIT) and overall complication rate to the open donor nephrectomy (ODN). Additionally, it has no negative impact on recipients’ operating time, graft ischemia and early graft function.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Retroperitoneoscopy; Donor nephrectomy; Comparison of techniques; Recipient outcome; Laparoscopy

1. Introduction

Living donor nephrectomy is unique in that it affects a healthy individual rather than a sick person. This makes it a very demanding and sophisticated surgical procedure. The safety and efficiency of the surgical technique are of utmost concern for the donor and the recipient. Therefore, the surgical technique recommended must entail the lowest possible morbidity.
without compromising the functional outcome of the graft.

Since the early 1990s, transperitoneal laparoscopic techniques have been successfully adapted for various open urologic procedures, including laparoscopic living donor nephrectomy which was first described in 1995 [1].

Only few centers have reported a large number of kidney donations performed with the retroperitoneoscopic approach [2–5]. The most frequent arguments against the retroperitoneoscopic approach are the difficulty in establishing the topography, the smaller working space and a probably steeper learning curve compared to the transperitoneal approach.

In this retrospective study, we analyzed the periproductive outcome and early complication rate of donors and recipients after retroperitoneoscopic donor nephrectomy (RDN) as compared to standard open donor nephrectomy (ODN).

2. Materials and methods

From November 1997 to March 2004, 69 ODN and 65 RDN were performed at the Basel University Hospital. Since November 2001, retroperitoneoscopy has become our favored approach for living donor nephrectomy after we had used a standard open approach for donor nephrectomy for more than ten years. Right-sided donor nephrectomy was performed in 45 donors (34%). Indications for right-sided nephrectomy are listed in Table 1. All potential donors were routinely evaluated according to a donation protocol. Their suitability was discussed in detail by the transplantation team comprising nephrologists, urologists, visceral and vascular surgeons, transplantation coordinators, immunological laboratories and psychosomatics experts. Preoperatively, a conventional or a contrast enhanced magnet resonance angiography was performed to evaluate the vascular anatomy in all donors.

All perioperative data including operating time (OPT), warm ischemia time (WIT) and complication rate of donors and recipients were prospectively collected in the RDN group and compared to the ODN group within a period of 30 days were analyzed for this study. Intraoperative complications were immediately documented in the patient’s chart by the surgeon. Postoperative complications were documented by the ward nurse or the ward resident. Complications after discharge were documented by an outpatient resident. Major complications were defined as complications that significantly detract from donor well-being, graft function or recipient well-being, including conversion, transfusion, re-operation or surgical graft damage.

A standard open extraperitoneal approach through a subcostal flank incision without rib resection was used in ODN. Our technique for RDN has recently been published in detail [6]. With the donor in a slightly overextended flank position, a 1–2 cm skin incision just below the tip of the twelfth rib is made and a small initial retroperitoneal space is created by index finger dissection. After insertion of a balloon dissector, the retroperitoneal space is bluntly dissected with infusion of approximately 800–1200 ml sterile 0.9% saline solution into the dissection balloon. We prefer to use water instead of air, because the volume of infused water correlates exactly with extraperitoneal volume created by the following blunt balloon dissection. After removal of the balloon-dissector, a pneumoperitoneum is established with an intraabdominal pressure of 12–15 mmHg and the peritoneal reflection is bluntly mobilized antero-medially from the undersurface of the anterior abdominal wall with the tip of the camera in order to get a larger working space and to be able to insert the additional trocars safely under vision. Intraabdominal pressures during nephrectomy above 15 mmHg are avoided. Finally, three more trocars (2 × 12 mm, 1 × 5 mm) are inserted in a typically diamond position. Gerota’s fascia is incised laterally and the hilum is exposed. Dissection of the renal vessels is performed first after the kidney has been freed from the covering fatty tissue. The ureter is carefully dissected and clipped with two absorbable 12 mm clips. Only harvesting of the kidney is performed with hand-assistance. For this purpose, the lower trocar access is enlarged up to 7–9 cm by a muscle split incision and the surgeon’s hand is inserted directly into the retroperitoneum. The incision diameter is large enough to ensure a safe, quick and careful removal of the kidney. Pre-( and postoperative) administration of diuretics was abandoned after February 2003. However for intra vessel volume expansion saline infusion is increased immediately prior to transection in order to improve early onset of renal graft function. The kidney is raised and the renal vessels are optimally exposed for transection that is performed using a TA*-30–2.5 (AutoSuture®) disposable stapler on both artery and vein. Subsequently, the kidney is stored on cold storage solution (Viaspan®) until a clear venous effluvium is visible. The kidney is put in a sterile plastic bag and taken forthwith to the next operation room, where the implantation is performed immediately.

Operating time (OPT) was defined as the period between skin incision and skin closure. We defined warm ischemia time (WIT) as the time from closure of the renal artery to the time when clear outflow of the cold irrigation solution (Viaspan®) in the renal vein was detected.

As part of a study protocol, recipients transplanted from February 1998 to December 2000 were randomized 1:1 to either triple therapy with CsA/MMF/Pred or FK/AZA/Pred. As part of a second study protocol, patients transplanted from January 2001 to October 2003 were randomized 1:1 to either SRL/MMF/Pred or CsA/MMF/Pred. From October 2002 all patients additionally received two doses of anti-interleukin-2 receptor antibody basiliximab (day 0 and day 4). All patients with clinical suspected acute rejection (creatinine increase more than 25% from baseline, weight gain, and
elevated blood pressure) were biopsied within 72 hours. Between January 2001 and February 2004, as a part of a study, we performed a renal protocol biopsy at the end of month 3 and 6 post transplantation.

All data expressed as mean ± standard deviation or available numbers of cases, if appropriate. Statistical analysis was performed with the SPSS 11.5 (SPSS Inc., Chicago, IL) statistical software package. The Mann–Whitney U test was used to compare the two categories of independent non-parametric numerical data. The chi-square test was employed to compare categorical data. A p value of less than 0.05 was considered to be statistically significant.

3. Results

From November 1997 to March 2004, we retrospectively analyzed 134 medical records after 69 ODN operations and 65 RDN operations. Detailed donor characteristics are presented in Table 2. With RDN, two (3%) conversions were necessary because of a renal artery disruption (n = 1) and because of two very short renal veins (<1 cm) on the right side, which opened directly into the vena cava. In both cases, the kidney was harvested and subsequently transplanted successfully without persistent impairments for the donors or recipients.

Both groups were comparable in terms of age, body mass index (BMI), OPT, WIT and blood loss. Donors after RDN were discharged from the hospital significantly earlier (p < 0.001) than after ODN. In RDN donors, serum creatinine was significantly higher during the first five postoperative days when compared to the ODN group (Fig. 1).

Overall, major, minor, intraoperative and postoperative donor complication rates did not differ significantly between the two groups. Donor complications are listed subsequently in Table 3.

General demographic recipient data and functional follow-up is presented in Table 4. Differences of mean values for age, OPT and cold ischemia time (CIT) were not statistically significant. Early functional follow-up showed significant differences in 24 h-urine output between the two groups (p < 0.001), but this parameter was comparable after 7, 30, 180 and finally 365 days (Table 4). Corresponding to the higher serum creatinine values in the RDN group, grafts after RDN showed a greater delay in onset of function than after ODN. However, from the 7th postoperative day there was no statistically significant difference between the two groups.

Overall recipient complication rate and total early rejection rate within 30 days were similar within the two groups. There was no elevation of the rate of surgical complications due to technical problems during endoscopic kidney delivery in the RDN group. However, a renal artery kinking led to a primary nonfunctioning of the graft in one recipient after RDN. This recipient was re-operated. Moreover, a venous saphena patch was necessary in two right-sided donations because of very short right renal veins (<2 cm). Longer functional follow-up of both grafts was uneventful, although early glomerular function was delayed in these grafts. The recipients’ major complication rate was comparable in the two groups (Table 4). After RDN, we observed a significantly delayed onset of early function (p = 0.025), but this did not entail repercussions for longer-term follow-up (Table 4).

4. Discussion

During the last fifty years, the standard open extraperitoneal or transperitoneal access has proved to be a safe approach for donor nephrectomy and is associated with a low complication rate [6]. However, flank incision entails significant postoperative problems including prolonged postoperative pain, scarring, permanent flank bulging, pleural irritation or subcostal
nerve injury [7]. Therefore, some surgeons were happy to carry out minimal flank incision for living donor nephrectomy in order to minimize skin incision [8]. Usually, the main argument for choosing this technique is to avoid the well-known learning curve associated with laparoscopic donor nephrectomy. Drawbacks of the minimal flank incision technique are the limited overview of the anatomical structures and the confined working space which can make it very difficult to handle intraoperative problems. Since Ratner et al. first performed a laparoscopic living donor nephrectomy in 1995 this procedure has become a widely accepted alternative for living donation [1]. Data is also available suggesting that it can increase the rate of kidney donations [9]. The laparoscopic access is technically challenging and is usually associated with longer OPT and WIT. However, the conventional laparoscopic technique leads to less perioperative pain, earlier mobilization and return to normal activities when compared to the open approach [10–12]. Besides these advantages of minimally-invasive techniques, graft integrity must be maintained and adequate lengths of ureter and renal vasculature must be available.

Our preliminary experience with RDN in comparison to the ODN has recently been published [13]. The retroperitoneoscopic approach combines the blunt and quick endoscopic creation of the anatomical retroperitoneal space and obviates lateral-colic peritoneal dissection or the adhesiolysis entailed in the conventional laparoscopic approach. OPT comparable to those for the ODN have not been published for the conventional laparoscopic approach up to now. Hand-assistance would appear to be an appropriate tool for improving safety and shortening OPT [4,14,15]. However, hand-assistance does not necessarily speed up OPT with the retroperitoneoscopic approach. In contrast to the conventional laparoscopic approach, hilum preparation is performed first during retroperitoneoscopy. That guarantees fixation of the kidney by anterior connective tissue with the peritoneum with enough room for vessel dissection. Therefore, hand-assistance itself only entails limited advantages during this step. However, hand-assistance makes the harvesting process safe and quick during retroperitoneoscopic donor nephrectomy. Additionally, the artery that has to be transected is easily reached for the transection process because of the dorsal

Table 3
Perioperative donor complications within 30 days after open (OLDN) and retroperitoneoscopic living donor nephrectomy (RLDN; n = 134)

<table>
<thead>
<tr>
<th></th>
<th>OLDN</th>
<th>RLDN</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>69</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Intraoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conversion to the open approach</td>
<td>1</td>
<td>0</td>
<td>0.198</td>
</tr>
<tr>
<td>Pleural laceration with necessity of drainage</td>
<td>0</td>
<td>1†</td>
<td></td>
</tr>
<tr>
<td>Bleeding with blood transfusion</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ureter injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1/69 (1.5%)</td>
<td>4/65 (6.2%)</td>
<td>0.198</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe wound pain</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hematoma requiring transfusion</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chyloretroperitoneum with chylothorax requiring re-operation†</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Paralysis, nausea, vomiting</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Wound infection/dehiscence/large hematoma (no transfusion)</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Transient fever within 24 h postoperative</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Pleural emphysema/effusion</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cardiac ischemia</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Asthmatic distress†</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Urinary problems (retention, prostatitis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16/69 (23.2%)</td>
<td>13/65 (20%)</td>
<td>0.839</td>
</tr>
<tr>
<td>Major complication†</td>
<td>2/69 (2.9%)</td>
<td>7/65 (10.7%)</td>
<td>0.090</td>
</tr>
<tr>
<td>Minor complication</td>
<td>15/69 (22.1%)</td>
<td>10/65 (15.4%)</td>
<td>0.382</td>
</tr>
<tr>
<td>Overall complications</td>
<td>17/69 (24.6%)</td>
<td>17/65 (26.1%)</td>
<td>0.846</td>
</tr>
</tbody>
</table>

Data presented as frequencies.
† This case is one of the cases that had to be converted.
† Fisher’s exact test (2-sided).
access to the vessels. This contrasts with the conventional laparoscopic approach. Therefore, we observed statistically comparable WIT for both approaches in our series (Table 2). It should be noted that a WIT of 2–4 minutes has never been demonstrated to affect delayed graft function or acute tubular necrosis. To our knowledge, no study confirming that hand-assisted retroperitoneoscopic donor nephrectomy is superior to fully retroperitoneoscopic donor nephrectomy in terms of OPT, WIT and complication rate has been published. However, the advantage of the hand-assisted retroperitoneal approach compared to the standard open or conventional laparoscopic approach has been demonstrated [3,14,15].

Besides optimized graft function, donor safety is of utmost concern. It is therefore mandatory to offer the donor a surgical technique that is safe and subject to minimal complications. Laparoscopic donor nephrectomy is a complex procedure and should be performed only by surgeons skilled in laparoscopy to maximize safety and assure efficient complication management. As a result of our series, the overall complication rates in donors and recipients were statistically similar in the two groups (Tables 3 and 4). The present study thus confirms that RDN has a low rate of major and minor complications that is comparable with that of the standard open and laparoscopic technique [6,10,11,14,16,17]. We found that the greater complexity of the retroperitoneoscopic procedure led to a slight tendency to more intraoperative problems during RDN (Table 3). However, differences in the rate of intraoperative and postoperative complications did not reach significant levels.

The absence of intraoperative and postoperative visceral complications is an advantage of endoscopic extraperitoneal surgery. Most of the donors typically recommenced oral food intake and were fully mobilized without any delay. RDN donors experienced less postoperative pain, irrespective of the type of perioperative regional anesthesia [18]. Depending on how these were defined, the rate of major complications in our series was in the range of published data for open (1–6%) or laparoscopic techniques (1–6.3%) [10,13,14,18,19]. The pleural and pulmonary complications observed after RDN (6.3%) have to be seen in conjunction with the close proximity of the first trocar to the 12th rib. Pleural complications are not confined to retroperitoneoscopy, as 5.6% pneumomediastinum and atelectasis have been reported with open donor nephrectomy, too [19]. Because of our experience, we try to place the first 12 mm trocar more distally to the tip of the 12th rib in order to avoid these complications in RDN. Our reported complication rate represents the whole learning curve of

### Table 4
Recipient outcome after standard open (ODN) and retroperitoneoscopic (RDN) donor nephrectomy

<table>
<thead>
<tr>
<th></th>
<th>ODN</th>
<th>RDN</th>
<th>p^j</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perioperative data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. recipients</td>
<td>69</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 (19–69)</td>
<td>47 (17–72)</td>
<td>0.897</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 (15–33)</td>
<td>23 (19–32)</td>
<td>0.116</td>
</tr>
<tr>
<td>Gender female/male</td>
<td>45/24</td>
<td>44/21</td>
<td></td>
</tr>
<tr>
<td>Operating time (min)</td>
<td>138 (75–270)</td>
<td>150 (90–240)</td>
<td>0.051</td>
</tr>
<tr>
<td>CIT (min)</td>
<td>58 (29–120)</td>
<td>67 (40–127)</td>
<td>0.055</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>17 (6–46)</td>
<td>12 (6–39)</td>
<td>0.001</td>
</tr>
<tr>
<td>Functional follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Creatinine (μmol/l) preoperative</td>
<td>710 (451–1138)</td>
<td>791 (391–1561)</td>
<td>0.095</td>
</tr>
<tr>
<td>Urine output, −24 h (l)</td>
<td>11 (2–22)</td>
<td>7 (2–22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine fall (%, −24 h)</td>
<td>43 (40–86)</td>
<td>41 (8–82)</td>
<td>0.275</td>
</tr>
<tr>
<td>S-Creatinine-day 7 postoperative</td>
<td>153 (66–752)</td>
<td>173 (67–549)</td>
<td>0.255</td>
</tr>
<tr>
<td>S-Creatinine-day 30 postoperative</td>
<td>134 (78–313)</td>
<td>146 (62–542)</td>
<td>0.363</td>
</tr>
<tr>
<td>S-Creatinine-day 180 postoperative</td>
<td>155 (85–298)</td>
<td>166 (79–461)</td>
<td>0.586</td>
</tr>
<tr>
<td>S-Creatinine-day 365 postoperative</td>
<td>159 (77–430)</td>
<td>148 (62–570)</td>
<td>0.312</td>
</tr>
<tr>
<td>Complications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rejection rate overall within 30 days (based on renal biopsy)</td>
<td>30 (43.5%)</td>
<td>20 (30.8%)</td>
<td>0.110</td>
</tr>
<tr>
<td>Interstitial</td>
<td>23</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Vasculare</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Major complication^1</td>
<td>15 (21.7%)</td>
<td>10 (15.4%)</td>
<td>0.382</td>
</tr>
<tr>
<td>Delayed non function</td>
<td>0</td>
<td>5 (7.6%)</td>
<td></td>
</tr>
<tr>
<td>Primary non function</td>
<td>0</td>
<td>1 (artery kinking, reoperation)</td>
<td></td>
</tr>
</tbody>
</table>

S-creatinin, serum creatinin.

^1 Mann–Whitney U test.

^1 Related to surgery, re-operation required or vascular revision (angiography with PTA) or bleeding needing transfusions (>3 units) or consequences on graft function.
two laparoscopic experienced surgeons. The retroperitoneoscopic approach has become the favoured approach for renal surgery since years at our institution. Even after having experiences with all kind of renal surgery including tumor enucleation and partial nephrectomy, pyeloplasty, adrenalectomy and laparoscopic radical prostatectomy for years, we have adopted donor nephrectomy to the retroperitoneoscopic approach. This is important to mention, because donor nephrectomy remain a high-risk operation with potential to harm donor and the graft and learning curve for donor nephrectomy should and has to be zero [20].

Carbon dioxide absorption is an issue familiar from conventional laparoscopic surgery. In order to prevent this, RDN was performed in a gasless technique [4]. The authors emphasized that this technique seems to suffer from the disadvantages that more wound pain is caused by the retractor and that the working space achieved is smaller than with a retropneumoperitoneum. Similar to the problem of carbon dioxide absorption, donors after RDN experienced significantly higher serum creatinine retention than donors after ODN within the first postoperative days in our series. Correspondingly, graft function in recipients after RDN shows a tendency to delayed onset of renal function as measured by 24 hours urine output. However, this effect was not significant in the subsequent postoperative days (Table 4). We observed no statistically significant difference between the groups for decrease of creatinine within 24 hours postoperatively or in the serum creatinine at 7, 30, 180 and 365 days (Table 4). However, we observed a tendency of delayed creatinine decrease (graft function) within the first weeks in the RDN group. Graft function within a follow-up of one year shows comparable results in the two groups. It still remains speculative whether the delayed onset of renal function after RDN (Fig. 1) in donors and grafts is associated with the CO₂-based respiratory acidosis as a result of pneumoperitoneum during RDN. In addition, the higher abdominal pressure that diminishes renal blood flow can also cause renal failure. Additionally, it has been shown by experimental studies, that the increased intra-abdominal pressure during laparoscopic nephrectomy significantly reduces renal blood flow, especially diastolic perfusion [21]. Thus, intra-abdominal pressures of 20 mmHg lead to a decrease of glomerular filtration rate by approximately 25% of normal [22, 23]. Although we used a strictly retroperitoneal access, the problems associated with carbon dioxide gas resorption encountered in the conventional laparoscopic approach also seem to be present during retroperitoneoscopy. Others have shown that retroperitoneal carbon dioxide insufflation causes more carbon dioxide absorption than intraperitoneal insufflation [24]. However, the issue of retroperitoneal carbon dioxide absorption during retroperitoneoscopy remain unsolved, as prospective clinical studies have shown no greater carbon dioxide absorption compared to transperitoneal laparoscopy [25].

The number of acute rejection episodes within the first 30 days was comparable in the two techniques. We are aware that the early rejection rate we observed was considerably higher than others have reported. However, this has to be seen in the context of our biopsy policy, that might leading to a higher rejection detection rate. One-year results of serum creatinine, which are a strong predictor for long-term graft outcome, were not negatively influenced by the choice of the harvesting technique [26, 27].

5. Conclusion

The retroperitoneoscopic approach is a safe, quick and practicable access route for living donor nephrectomy. The perioperative features of retroperitoneoscopic living donor nephrectomy such as operating time, warm ischemia time and overall complication rate are comparable to those of open donor nephrectomy. It has no negative impact on recipients’ operating time, graft ischemia and graft function.

References

Due to the dorsal approach to the kidney and the peritoneal dissection, the renal artery is easily reached. This approach has some advantages: it obviates laterocolic manipulation, the renal artery is easily reached due to the dorsal approach to the kidney and the procedure is entirely extraperitoneal, reducing the risks of intraoperative visceral lesions.

Bachmann et al. reported one of the longer series in the literature on retroperitoneoscopic nephrectomy for renal transplant with two important details: It is the first reference in which an endoscopic approach reflects a comparable operative time to the classical open approach with a similar first warm ischemia time. Secondly, the long-term functional outcome of the grafts harvested by retroperitoneoscopy is similar to those obtained by open techniques.

Anyway, from a practical point of view, considering the shortage of cadaveric kidneys for transplantation, living organ donation should be potentiated. The less invasive character of laparoscopic or retroperitoneoscopic LDN is an important factor in order to increase the number of kidneys for transplantation, especially in young recipients.
Laparoscopy

Urological Retroperitoneoscopic Surgery for Patients with Prior Intra-Abdominal Surgery


Department of Urology, Graduate school of medical sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima City, Hiroshima, Japan

Accepted 22 February 2005
Available online 16 March 2005

Abstract

Objective: To determine whether previous intra-abdominal surgery is associated with surgical outcome in patients undergoing urological retroperitoneoscopic surgery.

Patients and methods: One hundred seventeen cases of urological retroperitoneoscopic surgery, including 78 cases of retroperitoneoscopic radical nephrectomy (RN) for localized renal tumor and 39 cases of retroperitoneoscope-assisted radical nephroureterectomy (RNU) for upper urinary tract cancer, were evaluated. Thirty (38.5%) of the 78 patients who underwent RN and 13 (33.3%) of the 39 patients who underwent RNU had a history of intra-abdominal surgery. The patients were divided into two groups: those who had undergone prior intra-abdominal surgery (OP+) and those who had not (OP−). Patients’ backgrounds, degree of surgical invasiveness, and period of convalescence were compared between the OP+ and OP− groups.

Results: There was no significant difference between the OP+ and OP− groups in terms of background, surgical invasiveness or convalescence, except for age in the patients who had undergone RN. Complications in the studied cases were unrelated to any history of intra-abdominal surgery.

Conclusion: Previous intra-abdominal surgery is not associated with a negative outcome of urological retroperitoneoscopic surgery in patients with localized renal tumors and those with upper urinary tract cancer.

Keywords: Laparoscopy; Nephrectomy; Prior surgery

1. Objective

Clayman was the first to report the use of laparoscopic nephrectomy for a benign renal tumor in 1991 [1]. Since then, laparoscopic radical nephrectomy for malignant renal tumors has been investigated closely [2] and is now accepted as a standard treatment [3].

Intra-abdominal surgical procedures usually induce scarring and bowel adhesions to the abdominal wall. A history of intra-abdominal surgery has been reported to be a contraindication for transperitoneal laparoscopy, necessitating the use of open surgery [4]. Recently, the indications for surgical treatment in patients at risk have expanded widely. In patients who have undergone extensive prior intra-abdominal surgery, it would be expected that retroperitoneal access might avoid peritoneal adhesion and potential complications. However, there has been no thorough evaluation of the relative safety and efficacy of urological retroperitoneoscopic surgery in patients with a history of intra-abdominal surgery.

The aim of this study was to determine whether previous intra-abdominal surgery is associated with surgical outcome in patients undergoing urological retroperitoneoscopic surgery.
2. Patients and methods

Between 1 January, 1999, and 1 December, 2004, 117 cases of urological retroperitoneoscopic surgery, including 78 cases of retroperitoneoscopic radical nephrectomy (RN) for localized renal tumor and 39 cases of retroperitoneoscope-assisted radical nephroureterectomy (RNU) for upper urinary tract cancer, were evaluated. All transperitoneal laparoscopic procedures conducted during the same period were excluded.

Thirty of the 78 patients (38.5%) who underwent RN and 13 of the 39 patients (33.3%) who underwent RNU had undergone previous intra-abdominal surgery. The characteristics of the patients are summarized in Table 1. There was no statistically significant difference in background between patients with a history of intra-abdominal surgery and those without, except for age in the RN group. Data for prior intra-abdominal surgery and the side of retroperitoneoscopic surgery in patients who underwent RN and RNU are summarized in Table 2.

The patients were divided into two groups: those who had undergone prior intra-abdominal surgery and those who had not. The following factors were then compared: patient age, gender, side on which the tumor was located, body mass index, clinical stage, insufflation time during the endoscopic procedure, estimated blood loss, time until the start of oral intake of rice gruel, time to ambulation and postoperative hospital stay.

2.1. Surgical procedure

Under general anesthesia, the patient was placed in the kidney position. The first port of 12 mm was created by open laparotomy in the midaxillary line at the level of the umbilicus, and a retroperitoneal space outside the renal fascia was created using balloon dissection as described previously [5,6]. Two other ports of 10 mm were then placed 7 cm dorsal and anterior to the first port at the same horizontal level under laparoscopic vision. During the procedure, pneumoretroperitoneal pressure was maintained at 8 mmHg with carbon dioxide insufflation. After the quadratus lumborum fascia had been identified, the lateroconal fascia and posterior renal fascia were opened longitudinally. Blunt dissection was performed between the layers covering the perirenal fat and the renal fascia upwards and medially while the kidney was lifted anteriorly with an endoretractor (Autosuture, CT, USA). The lymphatic channels around the renal pedicle were then dissected to identify the renal artery and vein located at the renal hilum. First the renal artery was isolated, clipped and dissected. The renal vein was then mobilized and dissected with a vascular stapler (EndoGIA; Autosuture). The medial layer of the posterior fascia, which continues to the anterior renal fascia, was dissected from the

Table 1
The characteristics of all patients

<table>
<thead>
<tr>
<th></th>
<th>RN with prior surgery (n = 30)</th>
<th>RN without prior surgery (n = 48)</th>
<th>p value</th>
<th>RNU with prior surgery (n = 13)</th>
<th>RNU without prior surgery (n = 26)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean agea (range)</td>
<td>65.0 (42–86)</td>
<td>57.2 (32–82)</td>
<td>0.006</td>
<td>69.9 (54–86)</td>
<td>71.3 (47–89)</td>
<td>0.670</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>22:8</td>
<td>32:16</td>
<td>0.535</td>
<td>9:4</td>
<td>22:4</td>
<td>0.262</td>
</tr>
<tr>
<td>Tumor side (right:left)</td>
<td>10:20</td>
<td>17:31</td>
<td>0.851</td>
<td>2:11</td>
<td>12:14</td>
<td>0.059</td>
</tr>
<tr>
<td>Mean BMIB (range)</td>
<td>22.8 (17.8–29.3)</td>
<td>23.4 (16.8–33.1)</td>
<td>0.423</td>
<td>22.4 (17.1–27.9)</td>
<td>23.4 (15.1–30.1)</td>
<td>0.392</td>
</tr>
<tr>
<td>Clinical stagec</td>
<td>T1a: 22</td>
<td>T1a: 30</td>
<td>0.398</td>
<td>T1: 5</td>
<td>T1: 10</td>
<td>0.144</td>
</tr>
<tr>
<td></td>
<td>T1b: 8</td>
<td>T1b: 16</td>
<td></td>
<td>T2: 7</td>
<td>T2: 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2: 0</td>
<td>T2: 2</td>
<td></td>
<td>T3: 1</td>
<td>T3: 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T4: 0</td>
<td>T4: 1</td>
<td></td>
</tr>
</tbody>
</table>

a Years old.

b BMI: Body Mass Index.

c TNM classification.

Table 2
Prior intra-abdominal surgery and the side of retroperitoneoscopic surgery

<table>
<thead>
<tr>
<th>Prior intra-abdominal surgery</th>
<th>RN No. of pts. (Right:Left)</th>
<th>RNU No. of pts. (Right:Left)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrectomy</td>
<td>4 (3:1)</td>
<td>2 (0:2)</td>
</tr>
<tr>
<td>Surgical resection for colon cancer</td>
<td>3 (1:2)</td>
<td>2 (1:1)</td>
</tr>
<tr>
<td>Cholecystectomy</td>
<td>1 (0:1)</td>
<td>1 (0:1)</td>
</tr>
<tr>
<td>Hepatectomy</td>
<td>1 (0:1)</td>
<td>1 (0:1)</td>
</tr>
<tr>
<td>Pancreatectomy</td>
<td>1 (0:1)</td>
<td>1 (0:1)</td>
</tr>
<tr>
<td>Abdominal injury (traffic, iatrogenic)</td>
<td>1 (1:0)</td>
<td>1 (0:1)</td>
</tr>
<tr>
<td>Appendectomy</td>
<td>8 (2:6)</td>
<td>2 (0:2)</td>
</tr>
<tr>
<td>Continuous ambulatory peritoneal dialysis</td>
<td>4 (0:4)</td>
<td>0 (0:0)</td>
</tr>
<tr>
<td>Total hysterectomy</td>
<td>4 (3:1)</td>
<td>0 (0:0)</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>1 (0:1)</td>
<td>1 (0:1)</td>
</tr>
<tr>
<td>Radical nephrectomy (contralateral)</td>
<td>1 (0:1)</td>
<td>0 (0:0)</td>
</tr>
<tr>
<td>Total cystectomy with ileal urinary diversion</td>
<td>1 (0:1)</td>
<td>2 (0:2)</td>
</tr>
<tr>
<td></td>
<td>30 (10:20)</td>
<td>13 (2:11)</td>
</tr>
</tbody>
</table>
anterior portion around the perirenal fat. If adrenalectomy was not planned, the upper pole and medial aspect of the renal fascia were dissected from the fat tissue including the adrenal gland. After the fat tissue had been divided, the ureter was clipped.

For RN, the ureter was dissected and then the specimen was removed intact by creating a skin incision that joined the two ports and dividing the underlying muscle.

For RNU, nephrectomy was carried out in the same way as for RN; after RN a 7–10-cm oblique iliac incision was created to dissect the lower part of the ureter in an open surgical manner and the total specimen was removed with the cuff of the bladder.

2.2. Statistical analysis

Statistical analysis was performed with the unpaired t test and the chi-squared test for all successful cases. Differences at $p < 0.05$ were considered statistically significant.

3. Results

Table 3 shows that there were no significant differences in the degree of surgical invasiveness or period of convalescence between patients with a history of intra-abdominal surgery and those without such a history.

There were three open conversions (2.6%) among the 117 cases. One of these, in a patient with a history of appendectomy in the RN group, was because of difficulty in the control of bleeding from a lumbar vein injury. The other two open conversions were done because of advanced-stage upper urinary tract cancer with tight adhesions that could not be removed completely. Intraoperative complications included minor injuries to vessels in six patients, although no blood transfusion was required. Postoperative complications included hematoma in one patient, bleeding from the wound in three and wound infection in one. The patient with hematoma had been receiving hemodialysis for chronic renal failure, and required a second operation for removal of the hematoma due to bleeding at the port site on postoperative day 1. None of these complications were related to a history of intra-abdominal surgery.

Three of 117 studied patients had obvious anatomical abnormality between the perirenal tissue and renal fascia, and in the hilar area and medial border of the kidney. These three patients respectively had a history of pancreatitis, ipsilateral adrenalectomy and surgical resection for ascending colon cancer that was confirmed by retroperitoneoscopy to have disseminated to the retroperitoneum. Despite firm adhesions and disappearance of the anatomical layers between the perirenal fat and renal fascia, dissection of those layers was performed without any complications. No other dissection difficulties during the surgical procedure were encountered in any of the other patients because the anatomical layers remained intact.

4. Discussion

Although open intra-abdominal surgery induces scarring and adhesion of the bowel to the abdominal wall, the results of urological retroperitoneoscopy in patients with a history of intra-abdominal surgery showed that the rates of both open conversion and complications were low and that postoperative recovery was good in patients with localized renal tumors and those with upper urinary tract cancer. These results were very similar to those for a cohort who had not undergone prior intra-abdominal surgery.

The presence of ipsilateral widespread and dense retroperitoneal inflammatory fibrosis is thought to...
contraindicate a retroperitoneoscopic procedure, whereas limited fibrosis caused by previous nephrostomy or percutaneous renal surgery is not [6]. Apart from exploration of the retroperitoneal area, previous studies have not sufficiently addressed the influence of previous intra-abdominal surgery in the retroperitoneal space through the perirenal fascia. Retroperitoneoscopy during pneumoretroperitoneum can clearly demonstrate the anatomy of the perirenal fascial structure [5]. However, from an anatomical viewpoint, there is still some controversy about communication between the inside and the outside of the perirenal space. It is thought that the perirenal space is closed superiorly and that its layers are fused weakly or blend with the iliac fascia inferiorly, and thus there can be no actual or potential communication between the right and left perirenal spaces across the midline [7,8]. A network of interlobular septa acts as a barrier or pathway to the free spread of disease from the perirenal space to the central retroperitoneum or vice versa.

Conversely, infiltrating soft tissue or accumulating retroperitoneal fluid may travel into or out of the perirenal space via the perirenal bridging septa and renal fascia [9]. The normal anatomy and pathways of disease spread, such as the variety of specific pathologic conditions that may involve the renal fascia and perirenal space, including pancreatitis, retroperitoneal hematoma, urinoma and metastatic disease, have been discussed previously [9]. Although we recognized abnormality of the anatomical layers adjacent to the renal fascia in three patients with a history of pancreatitis, ipsilateral adrenalectomy, and retroperitoneal dissemination of ascending colon cancer, respectively, no dissection difficulties were encountered during retroperitoneoscopic surgery in any of the other patients who had undergone previous intra-abdominal surgery. These findings indicated that the anatomical layers in the retroperitoneum had remained intact and that no postoperative scarring had occurred within the perirenal space through the renal fascia, except under specific pathological conditions after intra-abdominal surgery.

To investigate the risk of intra-abdominal adhesions when using a transperitoneal approach, a retrospective cohort study showed no difference in identifiable preoperative risk factors [10,11]. However, in marked contrast, one study that re-examined 25,764 laparoscopic procedures identified that being female and having previously undergone abdominal laparoscopic were major risk factors for complications during surgery [12]. To avoid complications during the transperitoneal approach, the original retroperitoneal access to the peritoneum and subsequent trocar placement was carried out to provide access to the peritoneal cavity in eight patients at high risk of intra-abdominal scarring [13]. As an upper midline scar or a scar in the upper quadrant on the same side of the abdomen increases the risk of transperitoneal access complications, the location of the scar has an impact on the access complication rate [14].

It is well established that both the retroperitoneal and transperitoneal approaches have distinct advantages and disadvantages with regard to urological laparoscopic surgery [15,16]. In practical terms, the selection of one approach over the other depends on an individual surgeon’s experience and training [17]. Although the lack of clear anatomical landmarks and the narrow working space are drawbacks to a retroperitoneoscopic procedure, a 3D navigator system can be used to avoid operative risk and possible complications as appropriate [18]. Also, use of the balloon dissection technique can make retroperitoneal access a reasonably safe, efficient and reliable, minimally invasive procedure even for patients with a history of intra-abdominal surgery [5,6]. Use of a retroperitoneoscopic procedure, along with a transperitoneal procedure, is warranted for the treatment of urological malignancy because it allows complete en bloc dissection, including the layer of perirenal fat tissue covered by the renal fascia [19]. Our present results, combined with those of previous studies, suggest that urological retroperitoneoscopic surgery can be the first option for patients with localized renal tumors or upper urinary tract cancer who have undergone previous intra-abdominal surgery, without any significant increase in morbidity or negative outcome. Prior abdominal surgery is not a contraindication for urological retroperitoneoscopic surgery.

References


Comparison of Darifenacin and Oxybutynin in Patients with Overactive Bladder: Assessment of Ambulatory Urodynamics and Impact on Salivary Flow

C.R. Chapple, P. Abrams

Department of Urology, Royal Hallamshire Hospital, Glossop Road, Sheffield, S10 2JF, UK
Bristol Urological Institute, Southmead Hospital, Westbury on Trym, Bristol, BS10 5NB, UK

Accepted 20 April 2005
Available online 17 May 2005

Abstract
Objectives: To evaluate the effects of darifenacin, an M3 selective receptor antagonist, compared with oxybutynin, on ambulatory urodynamics, salivary flow, heart rate and visual nearpoint in patients with overactive bladder (OAB).

Methods: A double-blind, randomized, crossover study \( n = 65 \) with three treatment cohorts: darifenacin immediate release (IR) 2.5 mg three times a day (t.i.d.) or oxybutynin 2.5 mg t.i.d.; darifenacin controlled release (CR) 15 mg once daily (q.d.) or oxybutynin 5 mg q.d.; darifenacin CR 30 mg q.d. or oxybutynin 5 mg t.i.d. Within cohorts, patients received 7 days’ treatment with each agent separated by 14 days’ washout.

Results: All active treatments improved urodynamic parameters. Both darifenacin CR doses had significantly less effect on salivary flow than oxybutynin. Effects on urodynamic parameters, heart rate and visual nearpoint were comparable.

Conclusion: Ambulatory urodynamics appears to be an innovative and potentially useful investigative tool in the evaluation of the efficacy of new therapeutic agents. Darifenacin CR is an efficacious therapy for OAB with comparable effects on urodynamic parameters but producing significantly less dry mouth than oxybutynin.

Keywords: Darifenacin; Heart rate; Oxybutynin; Overactive bladder; Salivary flow; Urodynamics

1. Introduction

The clinical diagnosis of overactive bladder (OAB) is based on symptoms of urinary urgency, with or without urge incontinence, usually with elevated micturition frequency and nocturia, in the absence of pathological or metabolic factors that would explain these symptoms [1]. OAB is usually associated with detrusor overactivity, a urodynamic observation characterized by involuntary detrusor contractions during bladder filling, whether idiopathic or neurogenic in origin. Assessment of symptoms alone may be insufficient to accurately diagnose OAB [2], so urodynamic assessments may provide a complementary, objective measure of disease severity and treatment effect.

Pharmacological treatment for OAB is commonly with muscarinic receptor antagonists to reduce the frequency and amplitude of detrusor contractions and relieve the symptoms of OAB [3]. Although both M2 and M3 receptors can be found in the human detrusor (3:1 ratio), the post-junctional M3 receptor is primarily responsible for mediating detrusor contraction during bladder emptying [4]. Since muscarinic receptors are located in many other tissues throughout the body (including the salivary glands, gastrointestinal tract, heart, central nervous system and eye), indiscriminate blockade of the different muscarinic receptor subtypes (M1–M5) may produce a number of adverse effects. These include dry mouth, constipation and less
common effects such as tachycardia, cognitive dysfunction and impaired visual accommodation [3].

Darifenacin is a new treatment for OAB that has shown a high affinity and selectivity in vitro for M3 receptors compared with the other muscarinic receptor subtypes [5]. In contrast, oxybutynin shows high affinity for both the M1 and M3 receptor subtypes [5]. While darifenacin and oxybutynin would be expected to yield comparable efficacy through inhibition of M3 mediated signalling in the bladder, darifenacin may improve tolerability and safety compared with oxybutynin for M1-mediated adverse effects.

This study aimed to assess the differential effects of darifenacin immediate release (IR), darifenacin controlled release (CR) and oxybutynin IR in patients with OAB. Their effects were assessed on salivary flow, heart rate, heart-rate variability, visual nearpoint and parameters derived from 6-hour ambulatory urodynamic measurements of detrusor pressure.

2. Patients and methods

2.1. Study design

This was a randomized, double-blind, two-way crossover study in three cohorts of patients with OAB. Pre-study screening assessments included medical history, physical examination (including 12-lead electrocardiogram [ECG]) and clinical laboratory tests. Within each cohort, patients were randomly assigned using computer-generated random permuted blocks to the first treatment for 7 days, followed by a washout period of ≥14 days, then the second treatment for 7 days. Cohort 1 received darifenacin IR 2.5 mg three times daily (t.i.d.) or oxybutynin 2.5 mg t.i.d.; cohort 2 received darifenacin CR 15 mg once daily (q.d.) or oxybutynin 5 mg t.i.d.; and cohort 3 received darifenacin CR 30 mg q.d. or oxybutynin 5 mg t.i.d. Double blinding was maintained in cohorts 2 and 3 using a double-dummy technique. Compliance was determined from unused drug at study end.

Male and female patients aged 18–75 years were included if they showed cystometric evidence of detrusor overactivity within the previous 6 months, either idiopathic or neurogenic (secondary to a neurological lesion present for ≥12 months), with ≥2 associated symptoms (average of ≥7 micturitions/day, ≥7 episodes of urgency/week, ≥1 urge incontinence episode/week necessitating change of clothing or pads). Exclusion criteria included: previous bladder surgery for detrusor overactivity; prostatectomy in the last 6 months; bladder stones; treatment with diuretics, antimuscarinics, tricyclic antidepressants or digoxin within the previous 2 weeks; and stress and mixed incontinence, unless detrusor overactivity was the principal urodynamic observation and the patient was experiencing <1 stress incontinence leak/week. Patients were also excluded if they were pregnant, breastfeeding or not using adequate contraception, if they intended to start or to modify an existing bladder training programme, if their alcohol intake exceeded normal recommended limits, or if they had any condition in which anticholinergics would be contraindicated (e.g. untreated or narrow angle glaucoma, bladder outlet obstruction). Over-the-counter and prescribed medications were not permitted for 2 weeks prior to and during the study without agreement from the sponsor, except for paracetamol (acetaminophen), which could be taken up to 48 hours before the study. The study was conducted in accordance with the Declaration of Helsinki and was approved by an independent ethics committee. Written, informed consent was obtained from all subjects.

2.2. Pharmacodynamic assessments

Patients attended the clinic on days 1 and 7 of each treatment. Ambulatory urodynamics, salivary flow, visual nearpoint and heart rate were measured at baseline (prior to the first treatment), and at the end (day 7) of each treatment period. Patients recorded the time and nature of OAB symptoms (urgency, urinary leaks, voids and pain) during non-visit days in a paper diary.

A continuous ambulatory ECG (Holter) recording was undertaken, coinciding with the time of ambulatory urodynamic recording (see below). The recordings were analyzed centrally (Cardio Data Systems, NJ) and used to derive average heart rate, PNN50 (percent of normal-to-normal intervals that are more than 50 msec different from the normal-to-normal interval immediately preceding them) and St George’s Index [6]. Heart rate and variations in heart rate were calculated for the middle 5 minutes of each time period using a validated system.

Ambulatory bladder pressure measurements were taken with catheter tip solid-state transducers (Galeta) catheters which were carefully taped in position. The position of the catheters was carefully assessed if pressure measurements appeared inconsistent at any stage (pressure measurements were visible on the ambulatory units and were checked at regular intervals). All patients were given routine antibiotic medication to minimize the risk of urinary tract infection as a result of the bladder catheter, avoiding the use of antibiotics with smooth muscle activity (e.g. erythromycin). Transducer calibration and atmospheric pressure adjustments were made prior to each recording and new batteries were used on each study day to ensure the reliability of ambulatory data collection from the bladder transducer. Measurements were taken over a 6-hour period at baseline and starting 2 hours before the final dose on day 7 of each treatment period, i.e. from the time of trough drug levels to beyond the time of peak drug concentration, and data immediately downloaded. A predetermined sampling frequency of between 0.5–4 Hz was used. Caffeine-containing drinks, alcohol and unaccustomed exercise were not permitted during the ambulatory monitoring periods. Rectal pressure (representing intra-abdominal pressure) was subtracted from intravesical pressure to give detrusor pressure. Derived parameters were: duration of detrusor overactivity, activity index (area under the duration of activity effect–time curve, AUEC), and the number of phasic detrusor contractions. Responder rates (≥25% and >30% improvement from baseline) were determined from these parameters.

Salivary flow, another primary endpoint, was measured at baseline and at 2, 3 and 0.5 hours after, and 1, 2, 3 and 4 hours after, the final dose of each treatment. All meals and snacks were to be started at least 20 minutes after, and completed at least 20 minutes before, the salivary flow tests. A hard candy (Fox’s glacier mint) was pre-weighed to the nearest 0.1 g in a container. After swallowing all saliva, subjects sucked on the candy for 1 minute without swallowing and then discharged the candy with all saliva into the container for re-weighing. Salivary flow rate (ml/min) was calculated as the difference in weight between candy + container and candy remains + saliva + container.

Visual nearpoint was measured at baseline, pre-dose, and 2 and 4 hours after the final dose on day 7 of each treatment period using a
standard instrument, the RAF nearpoint rule. The rule was positioned over the subject’s nose, resting on the cheekbones. The subject slid the text block away from their nose until the top line of text came into focus. The distance was read off the scale and the mean nearpoint of the three measurements was calculated.

2.3. Tolerability and safety

Tolerability and safety were based on observed or volunteered adverse events, serious adverse events and discontinuations, clinical laboratory tests (haematology, biochemistry, urinalysis) and physical examinations.

2.4. Statistical analysis

Based on a previous study reported in part elsewhere [7], a sample size of 14 patients per cohort was considered sufficient to detect a difference of 60% in maximum inhibition of salivary flow with a probability of 0.80 when testing at the 5% level (two-sided). At least 14 patients were required to complete treatment in cohort 1 and between 14 and 30 patients in cohorts 2 and 3. For each cohort, urodynamic variables, maximum inhibition of salivary flow and other variables were subjected to an analysis of variance appropriate for the study design with terms for sequence, subject, treatment and treatment period. Each cohort was analyzed separately, and the treatment difference estimated using patient data relevant to that cohort only. Where appropriate and as indicated, means were adjusted with respect to sequence, subject and period. Differences between treatment means, standard errors and 95% confidence intervals (CIs) were calculated. For activity index, the ratio of anti-logged treatment means and the corresponding 95% CI was also calculated.

3. Results

3.1. Patient characteristics

Of 103 patients screened, 65 were randomized and received treatment and 59 completed the study (Table 1). Six patients discontinued treatment, three because of treatment-related adverse events, two with adverse events not related to treatment, and one withdrew consent. Five of the discontinuations occurred during the first treatment period and one during the second treatment period. Baseline demographics and medical history were comparable between treatment arms within each cohort (Table 2). There were more males than females (67.7% male; 32.3% female), age range was 21–75 years, and most patients (93.8%) were diagnosed as having idiopathic detrusor overactivity (IDO), with few (6.2%) having neurogenic detrusor overactivity (NDO). The use of concomitant medications was comparable between treatments within each cohort, consisting mainly of analgesics, anti-inflammatory drugs and antibiotics.

3.2. Urodynamic parameters

All urodynamic pressure parameters showed a substantial and statistically significant decrease from baseline after 7 days’ therapy with each treatment, with the exception of the number of phasic detrusor contractions during oxybutynin 5 mg t.i.d. therapy in cohort 3 (Table 3). However, the results after 7 days’ treatment showed large variability within the different treatment cohorts, and no significant differences between treatments were observed for any dose of darifenacin versus oxybutynin; the mean differences between treatments in cohorts 1, 2 and 3 (darifenacin–oxybutynin) were 77.5, 34.6 and −80.0 seconds for duration of activity, 198, 682 and 172 cmH2O.s for activity index AUEC, and 4.6, 2.9 and −7.4 for the number of phasic detrusor contractions, respectively.

There were also no meaningful differences between treatments in responder rates, i.e. patients achieving 25% or 30% improvement from baseline, for any of the ambulatory urodynamic parameters. At the 25% level, responder rates for duration of urodynamic activity were 71% and 92% for darifenacin IR 2.5 mg t.i.d. and oxybutynin 2.5 mg t.i.d., respectively, 70% and 83% for darifenacin CR 15 mg q.d. and oxybutynin 5 mg t.i.d., respectively, and 76% and 67% for darifenacin CR 30 mg q.d. and oxybutynin 5 mg t.i.d., respectively. At the 30% level, the corresponding numbers of responders were almost identical to those at the 25% level. Responder rates for activity index and number of peaks were generally comparable or higher than those observed for duration of activity.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient flow through the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort 1 (randomized, n = 16)</td>
<td>Cohort 2 (randomized, n = 24)</td>
</tr>
<tr>
<td>Darifenacin</td>
<td>Oxybutynin</td>
</tr>
<tr>
<td>Treatment period 1</td>
<td></td>
</tr>
<tr>
<td>Discontinued</td>
<td></td>
</tr>
<tr>
<td>n = 1</td>
<td>n = 1</td>
</tr>
<tr>
<td>Crossover</td>
<td></td>
</tr>
<tr>
<td>n = 0</td>
<td>n = 0</td>
</tr>
<tr>
<td>Treatment period 2</td>
<td></td>
</tr>
<tr>
<td>Discontinued</td>
<td></td>
</tr>
<tr>
<td>n = 0</td>
<td>n = 0</td>
</tr>
<tr>
<td>n = 1</td>
<td>n = 1</td>
</tr>
<tr>
<td>n = 2</td>
<td>n = 2</td>
</tr>
<tr>
<td>n = 13</td>
<td>n = 13</td>
</tr>
<tr>
<td>n = 12</td>
<td>n = 12</td>
</tr>
</tbody>
</table>
3.3. Salivary flow

Baseline salivary flow rates were a mean 1.89 ml/min in the whole study population, with no significant differences between groups. Measurement of salivary flow rate over the 6-h monitoring period on day 7 showed that reduction in salivary flow was significantly less with darifenacin CR 15 mg q.d. (cohort 2) and 30 mg q.d. (cohort 3) than with oxybutynin 5 mg t.i.d., while it was comparable for darifenacin IR 2.5 mg t.i.d. and oxybutynin 2.5 mg t.i.d. This was shown by significantly greater mean AUEC values for salivary flow with darifenacin CR 15 mg (p < 0.001) and 30 mg q.d. (p < 0.05) than with oxybutynin 5 mg q.d. (Fig. 1a). Similarly, the mean maximum decrease in salivary flow from baseline to treatment day 7 (observed at 2–4 hours post-dose) was significantly greater with oxybutynin 5 mg t.i.d. (−1.55 ml/min) than with darifenacin CR 15 mg q.d. (−0.98 ml/min; between treatment difference 0.56 ml/min; p < 0.01) and numerically greater with oxybutynin 5 mg t.i.d. (−1.30 ml/min) than with darifenacin CR 30 mg q.d. (−1.06 ml/min; between treatment difference 0.25 ml/min) (Fig. 1b).

3.4. Heart rate

Although data were not available for all patients at both timepoints, overall there were no meaningful differences in mean heart rate for darifenacin and oxybutynin within each cohort at baseline and on day 7 (Table 4). PNN50 and St George’s Index values suggested greater heart-rate variability after oxybutynin treatment compared with darifenacin in cohorts 1 and 2, though there was no difference between treatments in cohort 3 (Table 4).

Table 2
Baseline characteristics of patients randomized to treatment

<table>
<thead>
<tr>
<th>First treatment</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Darifenacin</td>
<td>Oxybutynin</td>
<td>Darifenacin</td>
</tr>
<tr>
<td>2.5 mg t.i.d.</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 12)</td>
</tr>
<tr>
<td>Females/males</td>
<td>4/4</td>
<td>3/5</td>
<td>4/8</td>
</tr>
<tr>
<td>Mean age (range; years)</td>
<td>53 (21–75)</td>
<td>49 (26–69)</td>
<td>50 (32–72)</td>
</tr>
<tr>
<td>Mean weight (range; kg)</td>
<td>73 (51–105)</td>
<td>74 (57–95)</td>
<td>85 (51–111)</td>
</tr>
<tr>
<td>Race, black/white</td>
<td>2/6</td>
<td>0/8</td>
<td>2/10</td>
</tr>
<tr>
<td>Primary diagnosis, NDO/IDO</td>
<td>0/8</td>
<td>0/8</td>
<td>2/10</td>
</tr>
</tbody>
</table>

NDO: neurogenic detrusor overactivity; IDO: idiopathic detrusor overactivity.

Table 3
Ambulatory urodynamic pressure measurements assessed over a 6-hour monitoring period

<table>
<thead>
<tr>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Darifenacin</td>
<td>Oxybutynin</td>
</tr>
<tr>
<td>2.5 mg t.i.d.</td>
<td>(n = 14)</td>
<td>(n = 13)</td>
</tr>
<tr>
<td>Duration of activity, adjusted means (s)</td>
<td>1063.4</td>
<td>1126.6</td>
</tr>
<tr>
<td>Day 7</td>
<td>227.8</td>
<td>150.3</td>
</tr>
<tr>
<td>LSM difference</td>
<td>−551.4</td>
<td>−618.25</td>
</tr>
<tr>
<td>p-Value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Activity index, log transformed geometric means (AUEC, cmH2O-s)</td>
<td>16576</td>
<td>18103</td>
</tr>
<tr>
<td>Day 7</td>
<td>1707</td>
<td>861</td>
</tr>
<tr>
<td>LSM difference</td>
<td>−17913</td>
<td>−23216</td>
</tr>
<tr>
<td>p-Value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of phasic detrusor contractions, adjusted means</td>
<td>59.6</td>
<td>63.4</td>
</tr>
<tr>
<td>Day 7</td>
<td>12.7</td>
<td>8.1</td>
</tr>
<tr>
<td>LSM difference</td>
<td>−27.3</td>
<td>−31.4</td>
</tr>
<tr>
<td>p-Value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

LSM difference = least squares mean difference from overall baseline value measured prior to any study treatment. p-Values are calculated for change from baseline by cohort using an ANOVA model correcting for sequence, subject within sequence, period and treatment.
3.5. Visual nearpoint

For visual nearpoint, there were no significant differences for any dose of darifenacin versus oxybutynin. Adjusted mean visual nearpoints were 24.0 and 24.9 cm for darifenacin IR 2.5 mg t.i.d. and oxybutynin 2.5 mg t.i.d., 23.2 and 24.0 cm for darifenacin CR 15 mg q.d. and oxybutynin 5 mg t.i.d., and 27.6 and 25.1 cm for darifenacin CR 30 mg q.d. and oxybutynin 5 mg t.i.d., respectively.

3.6. General tolerability and safety

The majority of adverse events were those commonly associated with antimuscarinic therapy, namely dry mouth and constipation, all of which were considered to be treatment related and most of which were mild or moderate in severity (Table 5). Within each of the three cohorts, dry mouth was consistently reported more frequently in oxybutynin-treated patients than in darifenacin-treated patients. No patient reported severe dry mouth during darifenacin IR 2.5 mg t.i.d. treatment, compared with reporting of severe dry mouth by three patients during oxybutynin 2.5 mg t.i.d. treatment; two patients reported severe dry mouth during darifenacin CR 15 mg q.d. treatment compared with seven patients during oxybutynin 5 mg t.i.d. treatment; and nine patients reported severe dry mouth during darifenacin CR 30 mg q.d. treatment compared with six patients taking oxybutynin 5 mg t.i.d. treatment. Three of the six discontinuations were due to adverse events that were all considered to be treatment related: severe dry mouth, blurred vision and sore throat during oxybutynin 5 mg t.i.d. treatment; severe dry mouth during darifenacin CR 15 mg q.d. treatment; and dry mouth, sore throat and confusion (all severities unknown) during darifenacin CR 30 mg q.d. treatment. Two patients discontinued with adverse events not related to treatment: vomiting and diarrhoea due to gastroenteritis, and pain due to the urodynamic investigations. Median changes in laboratory test values from baseline to last observation showed no evidence of a relationship between test variable and study treatment. No discontinuations due to laboratory abnormalities were reported.

Table 4
Heart rate (arithmetic mean) at baseline and treatment day 7

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Darifenacin 2.5 mg t.i.d.</td>
<td>Darifenacin 15 mg q.d.</td>
<td>Darifenacin 30 mg q.d.</td>
</tr>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 22)</td>
<td>(n = 12)</td>
</tr>
<tr>
<td></td>
<td>Oxybutynin 2.5 mg t.i.d.</td>
<td>Oxybutynin 5 mg t.i.d.</td>
<td>Oxybutynin 5 mg t.i.d.</td>
</tr>
<tr>
<td></td>
<td>(n = 13)</td>
<td>(n = 23)</td>
<td>(n = 14)</td>
</tr>
<tr>
<td>Average heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort baseline</td>
<td>74.3 (n = 11)</td>
<td>83.3 (n = 21)</td>
<td>80.8 (n = 12)</td>
</tr>
<tr>
<td>Day 7</td>
<td>75.7</td>
<td>84.0</td>
<td>72.5</td>
</tr>
<tr>
<td>PNN50 (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort baseline</td>
<td>17.2 (n = 11)</td>
<td>12.5 (n = 21)</td>
<td>10.1 (n = 12)</td>
</tr>
<tr>
<td>Day 7</td>
<td>12.2</td>
<td>9.3</td>
<td>9.8</td>
</tr>
<tr>
<td>St George’s index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort baseline</td>
<td>13.2 (n = 11)</td>
<td>12.8 (n = 21)</td>
<td>12.3 (n = 12)</td>
</tr>
<tr>
<td>Day 7</td>
<td>12.6</td>
<td>11.5</td>
<td>14.9</td>
</tr>
</tbody>
</table>

Fig. 1. (a) Adjusted arithmetic mean AUEC for salivary flow over a 6-hour monitoring period (−2 to 4 hour post-dose) on treatment day 7; (b) adjusted arithmetic mean maximum decrease in salivary flow from baseline to treatment day 7.
4. Discussion

The objectives of this study were to evaluate the effects of darifenacin IR, darifenacin CR and oxybutynin IR on ambulatory urodynamic parameters, salivary flow, visual nearpoint, heart rate and heart-rate variability in patients with OAB. In comparison with other available antimuscarinic agents, darifenacin is a more selective antagonist at the M3 receptor subtype that is primarily responsible for parasympathetic detrusor contraction. Indiscriminate blockade of the other subtypes may contribute to the broad spectrum of adverse effects associated with traditional antimuscarinic agents. Although other factors are also involved, the association between specific receptor subtypes and adverse events may contribute to the broad spectrum of adverse effects associated with traditional antimuscarinic agents. Although other factors are also involved, the association between specific receptor subtypes and adverse events may contribute to the broad spectrum of adverse effects associated with traditional antimuscarinic agents. Although other factors are also involved, the association between specific receptor subtypes and adverse events may contribute to the broad spectrum of adverse effects associated with traditional antimuscarinic agents.

Indiscriminate blockade of the other subtypes may contribute to the broad spectrum of adverse effects associated with traditional antimuscarinic agents. Although other factors are also involved, the association between specific receptor subtypes and adverse events may contribute to the broad spectrum of adverse effects associated with traditional antimuscarinic agents. Although other factors are also involved, the association between specific receptor subtypes and adverse events may contribute to the broad spectrum of adverse effects associated with traditional antimuscarinic agents. Although other factors are also involved, the association between specific receptor subtypes and adverse events may contribute to the broad spectrum of adverse effects associated with traditional antimuscarinic agents. Although other factors are also involved, the association between specific receptor subtypes and adverse events may contribute to the broad spectrum of adverse effects associated with traditional antimuscarinic agents.

Darifenacin could lead to an efficacious treatment for OAB with an adverse event profile on cognition and cardiac function that compares favourably with less selective antimuscarinic agents [8]. While other studies have specifically explored the potential cognitive and cardiac effects of darifenacin [9,10], this study provided a broader exploratory comparison of the pharmacodynamic effects of two antimuscarinic agents differing in selectivity profiles.

A particularly important feature of this study was the use of ambulatory measurement techniques, which demonstrated that both darifenacin and oxybutynin produced statistically and clinically significant, comparable improvements in urodynamic parameters. Ambulatory urodynamic monitoring is an alternative way of recording detrusor function that does not rely on conventional ‘static’ cystometric measures and is gaining in acceptance as a clinical research tool [7,11–13]. Ambulatory urodynamic monitoring techniques carry the advantage of using a more physiological state of natural bladder filling with urine, being less prone to subjective bias, allowing assessment over longer periods of time while patients carry out normal activities, and causing less embarrassment to patients [7]. In contrast to conventional (video) cystometry, ambulatory urodynamics have provided objective evidence of clinically important detrusor overactivity in the majority of women with symptoms suggestive of OAB [14]. The authors also reported a positive correlation between symptoms and ambulatory urodynamics, implying that a greater reliance may be placed on the symptomatic diagnosis of bladder overactivity [14]. In the present study, over a longer 6-hour assessment period, ambulatory techniques were able to detect statistically significant effects of treatment on the duration of activity, activity index, and the number of phasic detrusor contractions. The effects of darifenacin were comparable to those of oxybutynin, placing this novel agent firmly amongst the most effective treatments for OAB. These positive clinical findings, together with those reported elsewhere [7,11,13,14], suggest that ambulatory urody-

### Table 5

<table>
<thead>
<tr>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darifenacin</td>
<td>Oxybutynin</td>
<td>Darifenacin</td>
</tr>
<tr>
<td>2.5 mg t.i.d.</td>
<td>2.5 mg t.i.d.</td>
<td>15 mg q.d.</td>
</tr>
<tr>
<td>(n = 15)</td>
<td>(n = 15)</td>
<td>(n = 24)</td>
</tr>
</tbody>
</table>

| Patients with adverse events (treatment related) | 5 (4) | 8 (8) | 16 (14) | 19 (19) | 22 (22) | 24 (24) |
| Discontinued due to adverse events (treatment related) | 0 (0) | 1 (0) | 1 (1) | 0 (0) | 1 (1) | 2 (1) |

<table>
<thead>
<tr>
<th>Patients with all-causality adverse events (≥2 for any treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry mouth</td>
</tr>
<tr>
<td>Constipation</td>
</tr>
<tr>
<td>Dyspepsia</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Abnormal vision</td>
</tr>
<tr>
<td>Somnolence</td>
</tr>
<tr>
<td>Asthenia</td>
</tr>
<tr>
<td>Pharyngitis</td>
</tr>
<tr>
<td>Dysphagia</td>
</tr>
<tr>
<td>Pruritus</td>
</tr>
<tr>
<td>Dry eyes</td>
</tr>
<tr>
<td>Urinary tract disorder</td>
</tr>
<tr>
<td>Confusion</td>
</tr>
<tr>
<td>Epistaxis</td>
</tr>
<tr>
<td>Dysuria</td>
</tr>
</tbody>
</table>
dynamic monitoring is an effective tool for assessing drugs in clinical development and evaluating their potential effects on bladder filling function.

In contrast to the comparable efficacy for OAB demonstrated with darifenacin and oxybutynin, salivary flow showed a significantly smaller reduction with darifenacin CR 15 and 30 mg q.d. than with oxybutynin IR 5 mg t.i.d. administration, indicating less dry mouth with the CR formulation of darifenacin compared with oxybutynin IR. Similarly, the incidence of dry mouth reported as an adverse event was lower during darifenacin IR 2.5 mg t.i.d. and darifenacin CR 15 mg q.d. treatment than during oxybutynin IR treatment, though patient numbers were too small to undertake statistical analysis of treatment differences for tolerability.

The rationale for this difference between darifenacin and oxybutynin remains speculative given that M1 and M3 receptor subtypes are both present in the salivary glands; however, the importance of evaluating any novel antimuscarinic therapy on salivary flow is clear as dry mouth is one of the most common adverse effects of antimuscarinic treatment in patients with OAB, and is a frequent reason for discontinuation [15]. Whilst, darifenacin has a higher affinity for M3 receptors than for other muscarinic receptor subtypes, oxybutynin has similar affinity for M1 and M3 receptor subtypes [5]. There may therefore be an overall reduction in muscarinic receptor occupancy in the salivary glands with darifenacin compared with oxybutynin, since darifenacin targets only one of the two muscarinic receptor subtypes involved in salivary secretion [16]. In addition, the distinct distributions of the M1 and M3 receptor subtypes in the salivary glands may affect the ‘nature’ of dry mouth as well as the magnitude of the effect [16]. M3 receptors alone are involved in the control of low-viscosity secretions by serous cells (predominantly affecting saliva volume), while M3 and M1 receptors are both involved in the control of high-viscosity secretions by mucous cells (predominantly affecting lubrication) [16]. Darifenacin’s reduced effect on high-viscosity (mucous) secretion may, therefore, play a greater role in the sensation and severity of dry mouth felt by patients, since although saliva volume is reduced, lubrication is maintained [16]. In support of this hypothesis, although pilocarpine-stimulated salivation is attenuated in M3-knockout mice, the oral cavity remains lubricated and the mice are free of complications of human xerostomia such as mucosal injury and dental caries [17]. Furthermore, results from another study indicate that pilocarpine-induced salivation is completely abrogated in M1/M3 receptor double-knockout mice, whereas maximal salivary secretion can still be elicited by high doses of pilocarpine in both M3-knockout and M1-knockout mice [18].

The absence of any significant effect of darifenacin and oxybutynin on heart rate and heart-rate variability, particularly between cohorts, is consistent with their receptor selectivity profiles since cardiac effects of antimuscarinic agents are likely to involve M2 receptor antagonism [8]. Cardiac measurements were in line with recognized measurements of heart-rate variability [6]. Although ocular effects may be related to both M3 and M5 receptor antagonism and, therefore, it may be predicted that visual adverse events may be seen with both agents, the eye is protected by a blood–eye barrier [19]; any accommodation effects are therefore dependent on the amount of active drug that crosses this barrier and is available to bind to muscarinic receptors in the ciliary muscle.

In summary, at doses with similar urodynamic efficacy, controlled-release darifenacin (15 mg and 30 mg q.d.) had significantly less impact on salivary flow than immediate-release oxybutynin (5 mg t.i.d.), consistent with the receptor-binding profiles of these two antimuscarinic agents. Darifenacin was well tolerated and had no significant impact on visual nearpoint or heart rate.

Acknowledgment

Investigators: Prof P Abrams, Bristol Urological Institute, Bristol, UK; Mr CR Chapple, Royal Hallamshire Hospital, Sheffield, UK; Prof. AR Mundy, Institute of Urology, The Middlesex Hospital, London, UK; Prof. DE Neal, Medical School, University of Newcastle, Newcastle-upon-Tyne, UK.

This study was funded by Pfizer Inc. Preparation of the manuscript was supported by an educational grant from Novartis Pharma AG. Editorial and project management services were provided by Thomson ACUMED®.

References


Female Urology—Incontinence

Comparison of Symptom Severity and Treatment Response in Patients with Incontinent and Continent Overactive Bladder

Martin C. Michel*a,*, Jean J.M.C.H. de la Rosetteb, Maria Piroa, Tim Schneiderc

aDepartment of Pharmacology and Pharmacotherapy, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands
bDepartment of Urology, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands
cDepartment of Urology, University of Duisburg-Essen, Essen, Germany

Accepted 24 November 2004
Available online 15 December 2004

Abstract

Purpose: Two thirds of patients with overactive bladder (OAB) are continent, but our knowledge on the treatment of the syndrome is largely based on studies with incontinent patients. Therefore, we have explored baseline symptoms and treatment responses to tolterodine in continent relative to incontinent OAB patients.

Materials and Methods: Data from an open-label, observational study involving 3824 patients with OAB symptoms were analyzed for baseline symptoms and alterations thereof upon a 9 months treatment with 4 mg q.d. tolterodine ER.

Results: Baseline symptoms (number of urgency episodes, total urination frequency, daytime frequency, nocturia, and three scales of OAB severity) were similar in 1147 continent and 2571 incontinent patients, the latter having 4.8 ± 3.7 incontinence episodes per 24 h. Tolterodine ER-induced reduction of OAB symptoms was very similar in both groups of patients.

Conclusions: The severity of OAB in continent patients can be similar to that in incontinent ones. Symptom improvement upon treatment with tolterodine ER is very similar in both groups. We propose that continent patients may similarly deserve treatment with a muscarinic receptor antagonist as incontinent ones.

#2004 Elsevier B.V. All rights reserved.

Keywords: Overactive bladder; Urgency; Frequency; Incontinence; Tolterodine

1. Introduction

The International Continence Society has defined the syndrome of overactive bladder (OAB) as “urgency, with or without incontinence, usually accompanied by frequency and nocturia” [1]. Thus, according to this definition urgency is a mandatory part of the syndrome, frequency and nocturia occur very frequently, but incontinence is facultative. Epidemiological surveys in Europe and the US have shown that OAB is present in approximately 16% of the general population aged 40 and older but only approximately one third of all OAB patients is incontinent [2,3]. Whether continent OAB patients reflect a less severe and perhaps earlier form of OAB or a distinct group of patients is unknown.

Muscarinic receptor antagonists are the mainstay of medical treatment for OAB. Studies on the efficacy of muscarinic receptor antagonists in OAB patients have used either incontinence or, more recently, frequency as primary outcome variables to determine their efficacy [4]. Although urgency is the defining symptom of OAB, studies demonstrating that muscarinic receptor antagonists can effectively reduce urgency relative to placebo were published only within the last year [5–8], but even these studies were largely based on
incontinent patients. To the best of our knowledge, no study ever has determined the efficacy of muscarinic receptor antagonists in continent OAB patients.

In the analysis of open-label post-marketing surveillance data on the use of tolterodine (immediate release formulation) by office-based urologists, we have previously found that 28% of OAB patients receiving this muscarinic receptor antagonist were continent [9]. Despite the lack of any controlled data on the use of muscarinic receptor antagonists in continent OAB patients, we found a similar percentage of continent OAB patients in a more recent analysis of an even larger, long-term post-marketing surveillance study into the safety and efficacy of the extended release (ER) formulation of tolterodine. Therefore, we have used the latter database to address two questions: Do OAB symptoms such as urgency, frequency or nocturia differ between continent and incontinent patients? Does the efficacy and safety of tolterodine ER differ between these two subgroups of patients? By looking at both quantitative and qualitative indicators of OAB, we have additionally attempted to compare their value. Our data suggest that continent OAB does not necessarily reflect a less severe form of the syndrome and that muscarinic receptor antagonists may have similar efficacy in the treatment of continent as of incontinent OAB patients.

2. Patients and methods

We have performed a post-hoc analysis of the results of a large-scale, open-label, observational study which had been performed as part of the post-marketing surveillance of tolterodine ER in Germany. Thus, no specific inclusion or exclusion criteria were required. Rather the participating board-certified, office-based urologists (n = 492) or general practitioners and other physicians (n = 498) were asked to document their observations in a standardized questionnaire for patients with OAB who received tolterodine ER prescriptions according to the physician’s medical judgment for a planned observation time of 9 months. Between November 2001 and June 2003 the study recruited 3824 patients with a mean age 64.8 ± 13.3 years, of which 75.8% were female. The patients were investigated during 5 visits, i.e. before and after 1, 3, 6 and 9 months of treatment with 4 mg tolterodine ER q.d. (mean observation time 8.9 ± 2.2 months with no difference between continent and incontinent patients).

Prior to treatment, a patient history including concomitant diseases and previous medications was obtained from each patient. Based on patient history, OAB had been present for 47 ± 51 months, but 51.2% of patients had not received any prior treatment for this condition. Concomitant diseases of the cardiovascular, metabolic, nervous and gastrointestinal system were present in 44.5%, 21.7%, 11.9% and 6.7% of patients (multiple nominations possible).

With regard to urological symptoms, urgency, total urination frequency, daytime frequency, nocturia, incontinence and pad use were determined as quantitative parameters at each visit; in line with the methodology of our previous studies of a similar type [9,10], specific instructions how these symptoms should be quantified had not been given. Additionally, three scales of OAB symptoms were assessed. One of them was a validated 3-point urgency scale, which had been used in a previous study on the effects of tolterodine [7]. A second scale asked the patient to rate the bother of the OAB symptoms on a validated 6-point scale (“my condition causes me no, few very minor, few minor, moderate, severe, many severe problems”) which also had been used in a previous study with tolterodine [8]. Since it remains unclear whether rating on either of the two scales is linear, we did not calculate mean values but rather show frequency distribution graphs. Finally, patients were asked how much their bladder problems limited their daily life activities on a visual analog scale of 0 to 10 (none to very much). For the purpose of this analysis, a patient was defined as continent if not reporting incontinence prior to treatment.

Approximately 11% of patients prematurely discontinued the study (see safety data). If at least one symptom assessment had been performed after initiation of tolterodine treatment, their data were included in the analyses on a “last observation carried forward” basis. Data compilation was performed by Anfomed (Erlangen, Germany), a contract research organization. Where alterations relative to baseline values are given, only those patients were included for whom data were available before and during treatment for the parameter in question. Data are presented as means ± standard deviation. Descriptive comparison of statistical differences between continent and incontinent patients was performed by the Prism program (Graphpad Software, San Diego, CA, USA), and a p < 0.05 was considered statistically significant.

3. Results

3.1. Baseline data

The incontinent patients (2571 of 3824, i.e. 69% of all patients) reported an average of almost 5 incontinence episodes per day (Table 1). By average the continent patients (n = 1147, i.e. 31% of all patients) were about 5 years younger and had a 1 year shorter history of their symptoms; they were somewhat less

<table>
<thead>
<tr>
<th>Incontinent patients</th>
<th>Continent patients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66.3 ± 12.6</td>
<td>61.4 ± 14.1</td>
</tr>
<tr>
<td>% female</td>
<td>81.7%</td>
<td>62.6%</td>
</tr>
<tr>
<td>Duration, months</td>
<td>50 ± 53</td>
<td>40 ± 46</td>
</tr>
<tr>
<td>Previous treatment</td>
<td>50.8%</td>
<td>44.8%</td>
</tr>
<tr>
<td>Urgency episodes/24 h</td>
<td>8.0 ± 5.2</td>
<td>7.7 ± 5.2</td>
</tr>
<tr>
<td>Total frequency/24 h</td>
<td>14.1 ± 4.6</td>
<td>13.5 ± 4.4</td>
</tr>
<tr>
<td>Daytime frequency/day</td>
<td>10.8 ± 3.7</td>
<td>10.4 ± 3.8</td>
</tr>
<tr>
<td>Nocturia/night</td>
<td>3.5 ± 1.8</td>
<td>3.2 ± 1.6</td>
</tr>
<tr>
<td>Pad use/24 h</td>
<td>3.4 ± 2.8</td>
<td>0.1 ± 0.6</td>
</tr>
<tr>
<td>Incontinence episodes/24 h</td>
<td>4.8 ± 3.7</td>
<td>–</td>
</tr>
</tbody>
</table>

p-values result from two-tailed t-tests or χ²-tests (for gender and pretreatment).
likely to be female or to have received other prior treatment than the incontinent patients (Table 1). Despite a large patient number and hence a great statistical power, we did not detect statistically significant differences in the number of reported urgency episodes between continent and incontinent OAB patients (Table 1). While group differences in total frequency, daytime frequency and nocturia reached statistical significance, the daily number of each differed by less than 10% between the two groups (Table 1).

With regard to OAB scales, the 3-point urgency scale was slightly shifted towards more severe values in the incontinent patients (Fig. 1). Similarly, the bother rating tended to be more severe in the incontinent patients (Fig. 1). Moreover, the limitations in daily life caused by the bladder problems were also considered greater by the incontinent than by the continent patients (7.59 ± 1.65 vs. 6.66 ± 1.69 points, respectively; p < 0.0001).

3.2. Efficacy data

In the overall group, 9 months treatment with tolterodine ER lowered the number of urgency episodes from 7.9 ± 5.2 to 1.6 ± 2.8 per 24 h, total urinary frequency from 14.0 ± 4.6 to 7.5 ± 3.0 per 24 h, daytime frequency from 10.7 ± 3.7 to 6.2 ± 2.3 per day, and nocturia from 3.4 ± 1.7 to 1.4 ± 1.1 per night. Improvements of all four parameters over time were very similar in continent and incontinent patients (Fig. 2). In the group with incontinence at baseline, tolterodine ER treatment reduced the number of incontinence episodes by 3.8 ± 3.5 per 24 h and pad use by 2.4 ± 2.5 per 24 h (Fig. 3). A lack of urgency at the end
of the observation period was seen in 53.4% and 63.2% of incontinent and continent patients, respectively. The percentage of patients with a total urination frequency \( \geq 8 \) declined from 95.7% to 43.7% in the incontinent and 95.2% to 39.1% in the continent patients. With regard to qualitative OAB parameters, the distribution of urgency and bother rating was similar in both groups at the end of treatment (Fig. 1). On the 10-point scale for limitation of daily activities continent and incontinent patients had improvements of 4.10 ± 2.51 and of 4.49 ± 2.65 points, respectively.

3.3. Safety data

A total of 3416 patients (89%) completed the planned 9 months observation period. Premature treatment discontinuation in the overall group was due to lack of tolerability, administrative reasons, lack of efficacy or patient request in 2.8%, 2.6%, 2.4% and 1.2%, respectively (multiple nominations possible), and to a large variety of reasons, each occurring in less than 0.5% of patients, in the remaining group. Drop-out rates declined with time, i.e. 62% of all discontinuations occurred within the first 3 months and only 8% within the final 3 months. In the overall group adverse events were reported in 496 patients (13.0%) including dry mouth occurring in 299 patients (7.8%). Drop-out rates were similar in both groups (data not shown).

4. Discussion

4.1. Critique of methods

The present analysis is based upon an open-label, observational study. This implies certain limitations of the interpretation. Firstly, the continent and incontinent patients in our study do not necessarily reflect the prevalence and symptom severity in either group in the general population. Rather they are OAB patients who were chosen to receive treatment with tolterodine ER based on their physician’s medical judgment. Thus, these data do not allow statements in general about continent and incontinent OAB. Secondly, continent and incontinent patients differed significantly with regard to gender and age. However, we have previously demonstrated that gender has no effect upon tolerability responses and that age has only small if any effects [9]. Thirdly, there has been no assessment of the type of incontinence and hence we cannot exclude that a fraction of our patients had concomitant symptoms of stress incontinence. However, we have previously shown in a similar study that concomitant stress incontinence, except in its most severe forms, does not affect the therapeutic response to tolterodine [10]. This finding is in line with recent controlled studies demonstrating the effectiveness of tolterodine in mixed incontinence [11,8]. Therefore, the additional presence of a stress incontinence component in some of our patients is unlikely to have had a major impact on our analyses. Fourthly, in line with similar observational studies [12,9], the magnitude of treatment responses exceeded that of placebo-controlled studies with either the immediate release or the ER formulation of tolterodine [13,8,14,15,4]. Thus, due to the lack of a control group, our data do not allow statements regarding the efficacy or tolerability of tolterodine relative to placebo. Hence, they should not be abused to make such claims. Nevertheless, it appears justified to explore treatment effects in continent relative to incontinent patients based upon the present database since the efficacy and safety of tolterodine in its immediate-release and ER formulation in largely incontinent patients have previously been demonstrated in numerous placebo-controlled studies [13,8,14,15,4]. Moreover, our study also has certain advantages. It represents a large patient number and hence has great statistical power. Moreover, in contrast to most other studies in the field, our treatment data represent 9 months observation time and were obtained under real-life conditions which makes these findings likely to be applicable to general practice.

Apart from counting incontinence episodes, assessment of OAB severity has historically largely been based on counting micturition frequency and, more recently, urgency episodes. Although nocturia is as central to the definition of OAB as frequency [1], it has rarely been reported as treatment parameter, possibly due to the difficulties of its assessment [16]. The present study has quantified all three, and additionally differentiated daytime and total frequency. Since some recent data suggest that scales of OAB severity may give important information beyond counting symptoms, we have additionally used three qualitative measures of OAB; two of these had been used in previous placebo-controlled studies of tolterodine ER [7,8].

4.2. Baseline data

Continent and incontinent OAB patients differed somewhat by gender, presence of previous treatments, age and duration of disease history. Possibly some of the observed differences in baseline symptoms can be attributed to such confounding factors. Even more so it is remarkable that there was no statistically significant difference in the number of urgency episodes despite the large patient number and hence great statistical
power. Group differences in the 3-point urgency intensity scale can largely be explained by the nature of this scale [7], where I° and III° largely represent a lack of urgency and the presence of incontinence, respectively; hence this scale appears ill suited to compare continent and incontinent OAB patients at baseline and may be more suitable to address treatment effects. Group differences in frequency of urination, whether assessed as total, daytime or nighttime frequency, were statistically significant but remarkably small. Based upon previous studies [2,17,9,3], differences of this magnitude may largely be attributable to the observed minor age and gender differences between groups. Differences in overall bother by bladder symptoms and limitations in daily life caused by them, as assessed on 6- and 11-point scales, respectively, also amounted to about one point only. Since the present study was not population-based, our data do not allow to determine whether OAB with and without incontinence cause similar problems in the general population. However, these data clearly demonstrate that OAB can be similarly severe in continent patients as in those with an average of almost 5 incontinence episodes per day, irrespective whether quantitative or qualitative measures of OAB severity are used. Thus, continent and incontinent OAB do not appear to reflect two different ends of the spectrum of symptom severity but rather may at least partly result from different pathophysiological events. Population-based studies will be necessary to explore this further. From a clinical point of view, our data suggest that continent OAB may similarly merit treatment as incontinent OAB, particularly since the former group represents about two thirds of the overall OAB population [2,3]. However, to the best of our knowledge, no prospective study has ever reported treatment results in continent OAB patients.

4.3. Treatment data

The magnitude of symptom improvement in the present study was quite similar to that seen in a previous 3 months post-marketing surveillance study with immediate-release tolterodine [9] but greater than in the placebo-controlled studies with tolterodine or other anti-muscarinic agents [13,8,14,15,4]. This difference between the two types of studies is commonly seen and at least partly involves symptom improvement during the run-in phase which remains unaccounted for in most placebo-controlled studies [18]. Our data extend previous observations by demonstrating that the symptom improvement is not only maintained over 9 months but if anything continues to slightly improve. The safety findings in the present study are also in line with those of shorter post-marketing surveillance studies of immediate-release tolterodine [9], and further demonstrate that adverse events become increasingly unlikely with time. The good tolerability of tolterodine ER may also be the reason why almost 90% of patients remained in the study over a 9 months period.

The key treatment finding of our study is that continent OAB patients show similar symptom improvement as incontinent ones upon treatment with tolterodine ER. This conclusion is based on several forms of symptom assessment which include quantitative alterations in urgency, total urination frequency, daytime frequency and nocturia over the entire time course, “cure” rates for urgency and total urination frequency (the latter being defined as reaching values < 8 per 24 h) and three different scales measuring OAB intensity and impact. These data suggest that OAB patients can experience similar symptom improvement upon treatment with a muscarinic receptor antagonist as incontinent ones. Together with our above findings that symptom severity may be similar in continent and incontinent patients, these data suggest that continent OAB patients similarly deserve treatment with a muscarinic receptor antagonist such as tolterodine ER as incontinent ones. It may justly be argued that our present study does not substitute for a placebo-controlled study in continent OAB patients. However, in light of the numerous placebo-controlled studies demonstrating the efficacy of tolterodine [13,8,14,12], a very similar treatment response in a very large number of continent and incontinent patients in the present study is at least strongly suggestive of a beneficial tolterodine effect in continent OAB patients.

5. Conclusion

We conclude that OAB without incontinence does not necessarily reflect a less severe form of the syndrome than that with incontinence. Therefore, continent OAB patients merit treatment as much the incontinent ones. Tolterodine ER, and perhaps other muscarinic antagonists, appears to be similarly effective in continent as in incontinent OAB patients against the bothersome symptoms of urgency, frequency and nocturia.

Acknowledgement

This analysis is based upon a study which had been funded and performed by Pharmacia (now Pfizer).
References


Female Urology—Incontinence

Urethral Sensitivity in Incontinent Women

A.-C. Kinn\textsuperscript{a,*}, B.Y. Nilsson\textsuperscript{b}

\textsuperscript{a}Department of Surgical Science, Division of Urology, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden
\textsuperscript{b}Department of Laboratory Medicine, Division of Clinical Neurophysiology, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden

Accepted 15 February 2005
Available online 14 March 2005

Abstract

Objectives: The aim of this study was to ascertain whether frequent voiding and urge incontinence are associated with supersensitivity to electrical stimulation in the posterior urethra.

Methods: Current perception thresholds (CPT) were tested at four stimulus frequencies (1, 3, 20, and 100 Hz; duration 0.5 ms) using a square-wave constant current electrical stimulator connected to ring electrodes on a urethral catheter. The strength of the current at the first tingling sensation was regarded as the CPT. CPT analysis and cystometry were performed on 61 women (ages 28–89 years).

Results: CPTs were significantly higher at lower than at elevated stimulus frequencies, and they were also generally higher in old than in younger patients. Seven women repeated the CPT test after two months, and the thresholds were unchanged. There were no significant differences in sensitivity between patient groups with stress incontinence, urge, or mixed symptoms. Moreover, CPT was not significantly related to bladder volume at first sensation of filling.

Conclusion: Measuring CPT is an easy and reproducible method of testing urethral sensibility, but our results do not support the suspicion that urethral hypersensitivity is involved in increased voiding frequency and urge incontinence.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Current perception threshold; Quantitative sensory testing; Urethra; Incontinence; Urge

1. Introduction

The causes of bladder overactivity and sensory urgency are still only partly known, hence therapeutic efforts are in many cases unsuccessful. Symptoms of increased voiding frequency and urge incontinence are not always related to proven detrusor overactivity and indeed cystometric findings are often normal in patients who complain of increased urgency, frequent voiding and minor leakage. Therefore, it has been proposed that increased frequency and urge incontinence are induced by other factors, such as localized stretches generating periodic sensations in the bladder [1]. As early as the 1930s, Barrington [2] studied the role of urethral sensory nerves in facilitating bladder contractions in the cat and found that mechanical stimulation of the urethra could elicit reflex bladder contractions. In a more recent animal study, Jung et al. [3] found that motor activity in the detrusor waned during infusion of lidocain in the urethra. Also, in a clinical investigation [4], sensitivity of the posterior urethra was shown to be correlated with the micturition reflex when patients were subjected to cooling.

Determination of the current perception threshold (CPT) has been found to be clinically valuable for diagnosing patients with neurological diseases. Over the past few years, measuring CPT has also been used to determine the degree of urethral denervation after radical prostatectomy, cystectomy with orthotopic bladder substitution, and microwave thermotherapy for prostatic hyperplasia [5,6,7]. Nevertheless, it is not clear to what extent urethral supersensitivity to electric stimulation is linked to increased frequency
and urge incontinence, because different conclusions have been drawn from unequivocal research results. Accordingly, we conducted the present study to ascertain whether the degree of urethral sensitivity is important for urgency, and to accomplish that objective we compared perception thresholds in patients suffering from urge incontinence and those with stress incontinence. Since urologists often find that the introduction of a cystoscope is more troublesome for younger patients (both males and females), we also wanted to ascertain whether age has any impact on sensory perception in the urethra.

2. Materials and methods

A letter requesting participation in our investigation was mailed to 71 women (mean age 59.6 ± 26.7 years, range 32–89 years) with symptoms of urinary incontinence and consecutively referred for urodynamic examinations. The study was approved by the hospital ethics committee. All 71 patients who were contacted agreed to take part. However, seven women were not included because of previous treatment (three had undergone colposuspension surgery, two vaginal hysterectomy, and two irradiation for cervical carcinoma). Four women who were found to have bacteriuria were treated with antibiotics and tested for sterile urine before performing the examination. It was also necessary to exclude three women due to technical failure of the CPT equipment. Thus 61 patients could be evaluated. The Herlev Questionnaire [8] was administered to the subjects, and the responses to items about related symptoms indicated that 17 women were stress incontinent, 15 had an overactive bladder with moderately increased frequency (micturition more than ten times but only occasional leakage during 24-hour period), 16 had very frequent voidings and daily leakage, and 13 had mixed incontinence with regular leakage at least 3–4 times a week.

All patients kept a three-day micturition diary, recording the times at which they urinated and the volumes voided. Cystoscopy was performed a few weeks before urodynamic testing.

On the day of CPT analysis, the women were asked to arrive at the laboratory with a full bladder. Free flow rate was determined and residual volume measured by catheterization. A lubricating gel was injected in the urethra with the patient in lithotomy position. A 12 Ch Foley silicone catheter was fitted with two ring electrodes (21L11 Medtronic Denmark), one located 1 cm below the balloon and the other 0.5 cm further down. This catheter was inserted in the bladder, after which the bladder was emptied. The electrodes were placed in the proximal part of the urethra by pulling the balloon to the bladder neck. The electrodes were connected to a Grass stimulator (S48 Grass-Telefactor, Astro-Med Inc., USA) equipped with a constant current unit. The patients were subjected to electrical stimulation consisting of repeated square wave pulses (duration 0.5 ms), and the strength of the stimulus current was slowly increased until the patient felt a tingling sensation in the urethra. Two tests were done at each frequency before starting the actual measurements, to let the women know what the sensation felt like. Four different frequencies were used: 1, 3, 20, and 100 Hz. The order of the stimulations given was changed randomly, starting at either a low or a high frequency. The stimulation was repeated 3–4 times, and the current that gave the first sensation was registered.

For each stimulus frequency, the mean threshold value was calculated from the individual measurements. Cystometry was subsequently carried out using an 8 Ch two-channel catheter and an infusion rate of 50 ml/min. The first sensation of bladder filling (FS) and the subjective maximum bladder capacity were determined for each patient.

Two months later, CPT measurements were repeated in seven women (prior to any treatment) to study the reproducibility of the results obtained on the first occasion. These patients were randomly selected and they had symptoms of stress incontinence (n = 3) or an overactive bladder (n = 4).

The recorded sensory thresholds were compared, considering the patients in different groups according to age and clinical diagnosis. The level of electrical current that gave the first tingling sensation in the urethra at each stimulation frequency was compared with the bladder volume at the first sensation shown by cystometry, and also with the maximum bladder capacity. Urethral perception was also analyzed separately in patients with proven detrusor overactivity and in patients without phasic contractions at cystometry.

2.1. Statistics

Linear mixed models were used to evaluate the effects of electrical stimulation on sensory perception in the urethra, and the results were controlled for other important covariates. A compound symmetry covariance structure was applied to all models to deal with dependence in the observations. A p-value less than 0.05 was considered statistically significant. The value for sensory perception was log-transformed because of the highly skewed data. The statistical software SAS 8.2 was used for the analyses.

3. Results

The sensory thresholds (Fig. 1) were higher at lower stimulus frequencies (1 and 3 Hz) than at elevated frequencies (20 and 100 Hz). This difference was very significant when comparing thresholds at 1 and 20 Hz (p = 0.0042), 1 and 100 Hz (p = 0.0006), and 3 and

![Fig. 1. Box plots of median and non-outlier range values for the lowest level of current (mA) giving the first urethral perception at different stimulation frequencies (Hz). Values given for all 61 women.](image)
100 Hz ($p = 0.0012$). Fig. 2 shows that the decrease in thresholds at higher frequencies was more pronounced among middle-aged women ($n = 20, 28–49$ years) than in an older age group ($n = 14, 70–89$ years). The figure also demonstrates that the sensitivity thresholds were generally lower for younger than for older patients, and there was a statistically significant reduction of sensitivity in the oldest patients.

There were no significant differences in sensitivity between the patients with pure stress urinary incontinence, moderate or severe urge incontinence, or mixed symptoms. Furthermore, there was no significant correlation between the level of perception in the urethra and the bladder volume at first sensation of filling upon cystometry, or between urethral sensitivity and volume at maximum bladder capacity.

Detrusor overactivity was observed in 16 patients during the filling phase of cystometry, but the CPT values for these women at any stimulation frequency did not differ significantly from the corresponding values for the 45 women who had a stable detrusor at cystometry.

Sensitivity measurement repeated two months after the first analysis showed CPTs that were not significantly different from the initial values (Fig. 3). The correlation coefficient between the two measurements was 0.744.

4. Discussion

Our study revealed no differences in CPT results between patients with increased micturition frequency and urge incontinence and those with stress or mixed urinary incontinence, nor did we find any connection between a low CPT and urgency symptoms. These results do not confirm the hypothesis that symptoms of increased voiding frequency and urgency are related to a sensory dysfunction in the proximal urethra. If urgency and increased frequency do depend on abnormal sensory-motor reflexes, it seems that those reflexes do not involve receptors for conscious sensory perception.

In support of our findings Wyndaele [9] found no correlation between bladder pressure or volumes and the level of CPTs, and also noted that the CPT values did not differ between female patients with sensory urgency and normosensitive women. Wyndaele concluded that there are two separate systems of sensory innervation: one that reacts to electrical stimulation, and another that responds to bladder filling.

Furthermore our findings differ from the results reported by Powell and Feneley [10], which indicate a linear relationship between electrosensitivity and both the first desire to void and the bladder capacity in patients with normal cystometric findings. However, similar to our observations, Powell and Feneley did not detect a simple correlation between current thresholds and first sensation of bladder filling or the volume at maximum bladder capacity in patients with detrusor overactivity.

In contrast to our results and to the data reported by Wyndaele [9] and Powell and Feneley [10], Kiesswet-
ter [11] found that female patients with urge incontinence differed significantly from controls, as indicated by the observation that CPTs were <1 mA in the former group and 3–10 mA in the latter. Moreover, Hegenscheid et al. [12] observed lower sensitivity in patients with stress incontinence (CPT 14.8 mA) than in patients with urge incontinence (CPT 10.5 mA), but these authors concluded that there was no statistically significant difference due to extensively scattered CPT levels. Accordingly, Hegenscheid and coworkers considered that the CPT method is only suitable for measuring therapeutic outcome in individual patients.

We found that the current perception threshold in the posterior urethra depended on the frequency of the stimulus, or, in other words, more current was needed to give a sensation at low stimulus frequencies than was required at high frequencies (Fig. 3). In agreement with that, Brekkan et al. [13] obtained similar results using single stimuli and frequencies ranging from 1 to 20 Hz and they also reported that high frequency stimulation gave a waxing and waning sensation, which they attributed to a frequency-dependent block in thin autonomic nerves fibers. Moreover, Hansen [14] has shown that the relationship between stimulus frequency and perception thresholds is best fitted by a power function regression, thus following the general psychophysical law described by Stevens [15]. Hansen mentioned that the sensation changed character (i.e. became more burning and unpleasant) at frequencies at and above 10 Hz. Kenton and colleagues [16] observed lower CTPs during stimulation with 5 Hz than with 2000 Hz, but the thresholds were generally remarkably low in that study, and it should also be mentioned that 2000 Hz is far beyond the physiological range.

As indicated above, the results of CPT analysis vary considerably and one explanation for that variation may be the use of different stimulus parameters.

Kieswetter [11] found that CPT most efficiently distinguished urge incontinent patients from controls but, considering all the cited investigators, he was the only one to exclusively apply 100 Hz, a frequency that may block impulses in unmyelinated nerve fibers. We chose a full range of low and high frequencies in order to achieve activation of both larger myelinated A delta fibers and thin unmyelinated C fibers. Despite that, we could not repeat Kieswetter’s results, and our negative findings are more consistent with those of, for example, Powell and Feneley [10] and Hegenscheid et al. [12] which were obtained using only low to moderate frequencies.

The earlier investigations also differed with regard to pulse duration, the distance of the electrode from the bladder neck, and the volume of urine in the bladder. De Wachter and Wyndaele [17] have pointed out that standardization of the CPT method and access to reliable studies of healthy controls are needed to improve evaluation of bladder sensitivity, and the same applies to urethral examinations.

There are no previous reports indicating that older and younger age groups differ regarding sensitivity to high and low frequencies. Nevertheless, it is well known that age does affect the thresholds of several sensory modalities, such as vibration [18] and temperature [19]. Also, Solana and colleagues [20] have observed elevated thresholds for the electrical sensitivity of the mucosa of the anal canal in older people, whereas Brekkan et al. [13] did not find a relationship between age and urethral sensibility in male patients.

We studied conscious perception of nervous activity from the lower urinary tract. However, we can not rule out the presence of afferent nerve fibers which are involved in reflexes that modulate bladder motor activities but are not consciously perceived. This probably applies to afferent C-fibers that are primarily activated by low frequency stimulation, but also have high activation thresholds. Thus our results do not provide information about suprathreshold nervous dysfunction.

It is known that information originating from neural receptors in the wall of the proximal urethra is essential for normal urinary continence and voiding, but the exact mechanisms underlying this sensory function are not fully understood. The present findings show that the method used to measure electrosensitivity is easy to apply and offers good reproducibility, but it is not practical for routine clinical evaluation of urge incontinence. Nevertheless the technique may be suitable for other types of neurological dysfunction, although there seems to be poor correlation between urethral sensitivity and voiding dysfunction in incontinent women.

References


Augmentation Phalloplasty Surgery for Penile Dysmorphephobia in Young Adults: Considerations Regarding Patient Selection, Outcome Evaluation and Techniques Applied

Evangelos Spyropoulos, Charalambos Christoforidis, Dimitrios Borousas, Stamatios Mavrikos, Michael Bourounis, Sotirios Athanasiadis

Urology Department, Naval and Veterans Hospital of Athens, 24 Riga Fereou str., Paleo Faliro 17563, Athens, Greece
Plastic Surgery Department, Naval and Veterans Hospital of Athens, Athens, Greece

Accepted 22 February 2005
Available online 16 March 2005

Abstract

Objectives: To report on the efficacy and safety of augmentation phalloplasty procedures in physically normal young men, to introduce a patient selection and outcome evaluation questionnaire as well as, to propose a surgical technique modification.

Methods: Eleven (11) out of 28 psychosomatically normal men (25–35 years) who presented complaining of penile dysmorphophobia (subjective perception of small penis), were subjected to: (a) penile lengthening (suprapubic skin advancement – ligamentolysis): n = 5, (b) penile lengthening and shaft thickening (free dermal-fat graft shaft coverage): n = 3 and (c) panniculectomy – suprapubic lipectomy and penile lengthening: n = 2. A self administered questionnaire was employed in order to facilitate selection of the patients qualifying for the operation as well as to evaluate the outcome. In addition, a technical modification regarding dermal-fat graft handling was applied.

Results: The postoperative course was uneventful with minor complications. The mean penile length gain (flaccid - stretched penis) was 1.6 cm (1–2.3 cm) [p = 0.0014], the mean circumference gain was 2.3 cm [p = 0.003] at the base and 2.6 cm [p = 0.0012] subcoronaly. Significant (20%–53%) [p < 0.0001] sexual self-esteem and functioning improvement was reported by the majority (91%) of patients.

Conclusions: Although penile size alteration was not spectacular or satisfying the patients’ “great” expectations, the substantially uneventful clinical course coupled with the significant improvement in sexual self-esteem and function and the highly accepted outcome by the patients, render augmentation phalloplasty reasonable treatment modality for the management of strictly selected and thoroughly informed young adults who suffer from penile dysmorphophobia.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Penile dysmorphophobia; Small penis; Penile lengthening; Penile enlargement; Outcome evaluation

1. Introduction

Male self esteem can be affected by external genitalia image and if a man perceives his penis as inadequate, whether real or imagined, then such feelings invade his interaction with his sexual partners and social associates [1,2,3]. The perception a male has of the adequacy of his penis, does not necessarily coincide with the true dimensions of the organ. Thus, men with penile hypoplasia may declare themselves satisfied with their sexual life while others, who are considered normal may request augmentation as a result of an altered perception of the
size of the organ, a condition called “penile dysmorphophobia”. Dysmorphophobia may be an aesthetic issue: a patient whose penis is normal but who is dissatisfied with its dimensions in the flaccid state, or a functional issue: a patient with a normal penis who is dissatisfied with its size during erection [1].

Currently, as the media expose normal male figures and create interest in phallic enlargement, the demand for genital corrective surgery is increasing. However, augmentation phalloplasty procedures for penile dysmorphophobia, are still evolving and are considered a highly controversial issue, since none of the proposed methods has been unanimously approved and significant questions regarding their methodology and effectiveness exist [1–4].

In this article, we report our four year experience on performing such procedures (patient selection, surgical techniques, outcome evaluation), introduce a novel questionnaire devised by us, which aims at facilitating selection of the most suitable candidates for these operations and, present and propose a technical modification.

2. Subjects and methods

From February 2000 to November 2003, we performed augmentation phalloplasty procedures in eleven (11) men aged 25 to 35 years (median 28). They were part (39%) of a group of twenty-eight (28) patients who consulted us complaining of sexual inadequacy attributed to functional or aesthetic penile dysmorphophobia (perception of “small penis”) and seeking surgical correction. In order to select those who qualified for surgery, all were subjected to a screening protocol including: (a) medical history, physical examination and biochemical/sex hormone serum profiles, (b) psychiatric/psychosexual assessment and (c) a novel questionnaire designed by our department aiming at objectively quantifying the impact of the problem upon patients’ sexual self-esteem and the level of their desire to be subjected to penile augmentation, as well as at measuring the outcome of surgery (Table 1). This questionnaire, termed “Augmentation Phalloplasty Patient Selection and Satisfaction Inventory” (APPSSI), consists of four questions each having five possible answers graded from 0 to 4. Questions 1,2,3 were asked preoperatively while 1,2,4 postoperatively and the grades of answers of both question sets were summated to yield a total pre and postoperative score (APPSSI-score), that escalated from 0 to 12.

Invasive diagnostic workup (ICI-test) was optional as none complained of erectile dysfunction. Penile measurements were made of the length from the pubo-penile junction to the tip of the glans in the dorsal surface and of the circumference at the base

Table 1
Augmentation phalloplasty patient selection and satisfaction inventory (appssi) questionnaire

<table>
<thead>
<tr>
<th>Score</th>
<th>Very low</th>
<th>Low</th>
<th>Fairly disturbed</th>
<th>High</th>
<th>Very high</th>
<th>How would you grade your self esteem as it is impacted by your sexual body image, with respect to the size of your penis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Very low</td>
<td>1 Low</td>
<td>2 Fairly disturbed</td>
<td>3 High</td>
<td>4 Very high</td>
<td>How would you grade your self confidence in attempting sexual contacts/affairs with sexual partners, with respect to the size of your penis?</td>
</tr>
<tr>
<td></td>
<td>Extremely significant</td>
<td>I consider this operation extremely necessary to me, regardless of the final outcome.</td>
<td>1 Very significant</td>
<td>2 Moderately significant</td>
<td>3 Of little significance</td>
<td>4 Insignificant</td>
</tr>
<tr>
<td>Score</td>
<td>1 Score</td>
<td>2 Score</td>
<td>3 Score</td>
<td>4 Score</td>
<td>Total Score (Q 1-2-3)</td>
<td>How do you feel with the final outcome of the operation in relation to your preoperative condition and expectations?</td>
</tr>
<tr>
<td>Disappointed</td>
<td>Dissatisfied</td>
<td>Indifferent</td>
<td>Satisfied</td>
<td>Excited</td>
<td>Total Score (Q 1–2–4)</td>
<td></td>
</tr>
<tr>
<td>The condition is worse than preoperatively</td>
<td>Nothing changed despite the bother</td>
<td>Very little improvement</td>
<td>Significant improvement</td>
<td>The condition is much better than I expected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 Score (1–2–4)</td>
<td>1 Score (1–2–4)</td>
<td>2 Score (1–2–4)</td>
<td>3 Score (1–2–4)</td>
<td>4 Score (1–2–4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dysmorphophobia may be an aesthetic issue: a patient whose penis is normal but who is dissatisfied with its dimensions in the flaccid state, or a functional issue: a patient with a normal penis who is dissatisfied with its size during erection [1].
and coronal groove, with the penis flaccid - stretched (maximal extension) \cite{4,5,6}. The mean penile length (±SD) was 9.12 ± 1.17 cm [range: 7–11.3 cm], the mean circumference at base 6.4 ± 0.5 cm [6–7 cm] and subcoronally 6 ± 0.4 cm [5.5–6.5 cm]. In four (36.4%) patients, dysmorphophobia was of the functional type and in seven (63.6%) it was considered as aesthetic-cosmetic. The study was approved by the Hospital’s Scientific-Ethical Committee and all patients signed a consent form which thoroughly informed them on the possible complications and the likely surgery outcome. Five (45.4%) patients underwent penile elongation, four (36.4%) penile lengthening and shaft enlargement and in two (18.2%) obese patients (BMI > 35), celioplasty, suprapubic lipectomy and penile elongation were performed.

**Penile lengthening** (Fig. 1) consisted of a suprapubic Z-plasty skin incision, mobilization of the triangular flaps, dissection of the subcutaneous tissue down to the pubis, suprapubic lipectomy and incision of the fundiform and suspensory ligaments of the penis preserving the dorsal penile nerves and vessels. The subpubic space was filled with free fatty tissue and the wound was closed by moving and suturing the triangular flaps downwards and upwards \cite{2,3,6,7}.

**Penile lengthening and shaft enlargement** (Fig. 2) consisted of an elliptical groin skin incision (8–12 cm length and 4–6 cm width) followed by de-epithelialization and progressive freeing of the dermis and subcutaneous fat off underlying Scarpa’s fascia, leaving a free dermal-fat graft of approximately 1–1.5 cm in thickness. Under a Z-plasty-incision, suspensory ligamentolysis was performed as previously and through either this or a circumcision incision, the penile shaft was totally degloved and the dermal-fat graft was patched–sutured on its dorsal surface with the dermal side superficial, avoiding the spongiosal body \cite{2,3,6,7}. Before patching it, we performed four concentric, approximately 45° angled -1 cm length incisions at 2–5–7 and 10 o’clock positions of the graft, to increase its total surface area (Fig. 3).

**Panniculectomy-celioplasty, suprapubic lipectomy and penile elongation**: In cooperation with the Plastic Surgery Department, panniculectomy-celioplasty was performed followed by suspensory ligamentolysis-suprapubic lipectomy as previously described.

Postoperatively, penile weights were not routinely used but, instead, patients were instructed to manually stretch the penis several times a day. Clinical follow up was carried out at 3, 6 and 10 months after surgery and during visits the patients were subjected to physical examination, penile measurements and questions 1–2–4 were completed.

For the statistical analysis, the two-sided paired Student’s t-test was used, at 95% level of significance (p < 0.05).

### 3. Results

All patients tolerated the operations well and no major complications (severe bleeding or infection, injuries to the neurovascular bundle or urethra)
occurred. Skin ecchymoses ($n = 3$) [27.3], prepuce edema and/or paraphimosis ($n = 3$) [27.3%], sustained wound drainage ($n = 1$) [9.1%], temporary ($\frac{3}{2}$ months) dermal-fat graft sclerosis resulting in curvature and pain on erection ($n = 1$ out of 4 [25%]), transient (<2 months) pain on erection ($n = 3$) [27.3%] and hypertrophic scar formation ($n = 1$) [9.1%], were the most significant complications. Mean operative time was 102’(90’–115’) for celioplasty-penile lengthening, 115’(105’–130’) for penile lengthening and enlargement and 65’(55’–70’) for penile elongation while, median follow-up time was 14 months (6 to 24). Penile length and girth changes postoperatively are shown in Table 2. Length gain ranged between 1 to 1.4 cm in three (27.3%) patients, from 1.5–1.9 cm in six (54.5%) and was ≥2 cm in 2 (18.2%) obese patients who underwent celioplasty-penile lengthening. Measurements at 3, 6, 10 and 24 months, demonstrated sustained clinical benefit over time.

The psychosexual impact of the operations was favorable in the majority of cases. Sexual self-esteem and patient satisfaction were significantly improved as shown by the marked increase in mean APPSSI-score. Preoperatively it ranged between 0 to 3 in five (45.45%) patients, in other 5 (45.45%) from 4 to 5 and in one (9.1%) it was 6, yielding thus a mean preoperative score of 4.36 ($\pm$2.06 SD) which shifted to a mean of 7.54 ($\pm$1.4 SD) postoperatively ($p < 0.0001$). Specifically, in one patient (9%) no change occurred, in 9 (82%) the improvement rate varied between 20% to 40% (2: 20%, 3: 27%, 2:
Values are given as mean ± SD (range).

Table 2
Penile dimension measurements, before and after performance of augmentation phalloplasty surgery

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Preoperatively</th>
<th>Postoperatively</th>
<th>Gain</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length (cm)</td>
<td>9.12±1.175 (7–11.3)</td>
<td>10.75±0.88 (9.2–12.6)</td>
<td>1.645±0.4 (1–2.3)</td>
<td>0.0014</td>
</tr>
<tr>
<td>Perimeter at base (cm)</td>
<td>6.4±0.5 (6–7)</td>
<td>8.7±0.3 (8.5–9.5)</td>
<td>2.3±0.25 (2–3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Perimeter subcoronal (cm)</td>
<td>6.0±0.4 (5.5–6.5)</td>
<td>8.5±0.5 (8–9)</td>
<td>2.6±0.25 (2.5–3)</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

33%, 2: 40%) and in one patient (9%) the score increased by 53%. These figures remained essentially unchanged at 3, 6, 10 and 24 months. Sexual activities were initiated at 1(1/2) to 3 months and all patients resumed full, uneventful sexual functioning 4–5 months after surgery.

4. Discussion

Excluding cases of congenital penile hypoplasia or acquired deformity, augmentation phalloplasty is currently performed in patients who express penile dysmorphophobia, either of the aesthetic or the functional type and, therefore, the definition of this type of surgery has changed from “reconstructive urological surgery” to “aesthetic plastic surgery” [2,3,7]. As such, the basic principles of aesthetic surgery should be followed and penile dysmorphophobia surgery should guarantee a higher degree of safety concerning both functional and cosmetic results [1,3,7,8]. Men desiring genital enhancement typically suffer from feelings of penile inadequacy, in which case surgical treatment is not objectively necessary but becomes a more subjective therapy to satisfy the patient and, hence, whether or not such operation is ethical and whether or not it should be performed, is a major challenge [1–3,7,8]. Like other authors, we believe that a standardised preoperative protocol and strict patient selection criteria should be established for this type of surgery. Evaluation of the patient’s motivations and expectations is imperative and psychologic/psychiatric consultation is recommended especially in questionable cases (severely depressed, psychotic or unrealistic) [2,3,7]. To our knowledge, parameters for selecting the appropriate patients, have not yet been well defined and in order to address this issue, we devised a questionnaire aiming at objectively estimating and grading justification for this type of operation. The three first of its four questions aim at quantifying the impact of dysmorphophobia on patient’s sexual self esteem and his desire and determination to proceed with corrective surgery while, the fourth evaluates the outcome of the operation in terms of patient satisfaction–condition improvement. In the APPSSI-scaling system, score-0 characterizes a patient of extremely low sexual self esteem who desperately seeks surgical correction while, score-12 implies that the small penis perception does not adversely affect his sexual behavior and surgical treatment appears remote. Based on the questionnaire, we considered as qualifying for surgery those with a score of ≤6. Postoperatively, the score varied between 0 (dissatisfied) to 12 (excited with the result).

Since all patients claimed normal erectile function and most of them had aesthetic type dysmorphobia, they were appreciably reluctant to accept ICI-test in order to estimate erect penis size. Therefore, we decided to perform all penile measurements in the flaccid-stretched state considering as low normal limit of penile length that of 9 cm and of shaft circumference that of 6 cm [2,3,5,6]. Although the lack of data on penile size during erection can be considered limitation of our study, we believe that the above decision was appropriate, as it was based on numerous studies showing a close correlation between flaccid-stretched and erect lengths as well as circumference, rendering measurement of stretched length and girth suitable estimate of erect dimensions [2,3,5,6,9]. Austoni et al performed penile elongation in men with erect lengths from 6 to 11.2 cm, while other authors in patients with flaccid stretched length ranging between 7.5 to 10 cm [1,4,8].

Penopubic ligamentolysis and advancement of lower abdominal skin onto the penis, constitute the most popular method for penile lengthening that we applied in all our cases [2,7,10]. This technique achieves an apparent and not real lengthening since penile structures remain unchanged. Thus, any increase in penile length is noticeable when flaccid but is minimal when erect [7,8,10]. Genuine penile elongation can be obtained by the penile disassembly technique as well as by various other methods (dermo-fat free graft taken from the cruro-gluteal area, multiple incisions in the tunica albuginea combined with placement of penile prostheses and placement of intracavernous cutaneous expanders) [7,8,11–13]. All these techniques are feasible but rather aggressive and poten-
tially hazardous and achieve moderate erect length gain since the low elasticity of the neurovascular bundle is a limiting factor. Therefore, their benefits should outweigh their risks and they should be offered primarily to select patients with penile hypoplasia or deformities [8,10,12,13].

The volume of the penis can be increased either by pericavernosa (subcutaneous placement of autologous fat, dermis-fat, veins, mucous membranes, synthetic materials) or tunica albuginea enlargement phalloplasty [1–3,7,8]. Dermal-fat grafts, either free or vascularized, taken from the lateral groin or from the gluteal creases and sutured over the corpora cavernosa, provide excellent penile girth with virtually low complication rate [1,2,3,14]. This technique increases penile volume mainly in the flaccid state while, during erection the grafts are compressed by the superficial dartos fascia and the increase tends to be cancelled out [1]. Tunica albuginea enlargement phalloplasty (longitudinal albuginea incisions and incorporation of autologous dermis grafts, saphena vein grafts, synthetic materials) increases the volume of the corpora cavernosa but, it is considered experimental and the theoretical possibility of adversely affecting the erectile mechanism, should make as very sceptical when counseling young patients with cosmetic penile concerns [1]. Since our four patients sought volume increase of the flaccid penis, we proceeded with pericavernosal incorporation of free dermal-fat graft taken from the groin area. In addition, we introduced a modification of graft handling which consists of transforming the ellipsoid graft to a cruciform shaped form by making incisions sited at the 2–5–7 and 10 o’clock positions. This modification aims at expanding the graft surface area as well as at deteriorating graft shrinkage, in case fibro-elastic tissue involution appears.

Claims of 3-inch length gain after ligamentolysis and pubic skin advancement are greatly exaggerated and a 1-inch (2.5 cm) gain in the flaccid stretched state represents a success while, minimal or no gain is possible [1,2,3]. In our series, the mean total penile length gain of 1.6 cm was statistically significant but, in only two patients with marked obesity, the result was impressive (>2 cm increase). Austoni et al reported a mean erect penis length increase of 1.6 cm (1.5–2.5 cm) [1], Perovic-Djordjevic using the penile disassembly technique achieved a mean penile length gain of 3.068 (2.4–4.1) cm and 2.95 (2.3–3.6) cm in the erect and flaccid penis respectively while, Randone et al claim length gain from 2.5 to 3 cm in the erect state by using the dermo-fat free graft method [10,11,15,16]. Dermal-fat grafts achieve an average penile girth increase of 1 to 2 inches which is usually symmetric and satisfactory in appearance and texture [2,3,4]. In our cases, the average perimeter increase was 2.3 cm at the base of the penis and 2.6 cm at the coronal groove, achieving an aesthetically appreciable penile shaft volume increase in the flaccid state. Austoni et al., reported increases in the average penile diameter ranging from 2.64 cm (flaccid) to 4.82 cm (erect) by incorporating saphena vein grafts to longitudinal openings of the tunica albuginea [1,7].

Complications were of low rate and of minor severity. In general, the adverse effects of ligamentolysis are insignificant consisting mainly of inflammatory, healing problems usually conservatively treated while, dermal-fat grafting may be complicated by infection, poor graft “take” (lysis, fibrotic thickening), skin loss and penile hypoesthesia [1,2,7,10,14,17].

To our knowledge, reports on phalloplasty outcomes regarding patient satisfaction and psychosexual impact, have been limited and in most cases gross estimates are presented [1–3,7,10,12]. Kim et al. reported satisfaction varying between 69% and 77% after injecting hyaluronic acid for glans penis augmentation [18]. Based on our questionnaire, we were able to objectively estimate the psychological effects of the operation. In all but one, surgery significantly satisfied the patients and improved their sexual self esteem and function by 20% to 53%. The one patient with no improvement had a high score preoperatively while, the highest improvement rates (40–53%) occurred in patients with marked obesity. In contrast, Austoni et al. reported that a high percentage of patients who underwent dermal-fat graft penile girth enhancement, were dissatisfied [1,7]. This difference may be attributed to different patient selection criteria, as our patients were concerned on penile size in the flaccid state mainly. Regarding clinical applicability of the APPSSI-questionnaire, we realize that limitation of our study is the fact that we did not use established psychometric or quality of life tests in order to cross-match the results and measure its validity. Furthermore, due to the small number of patients, we decided not to determine its reliability and internal consistency at the present time but, instead, to address these and the above limitations of this “pilot” study, in a larger scale, multi-center study in the near future. Consequently, the introduced questionnaire is not validated yet and in order to consider this a clinically valid and widely acceptable instrument, we propose that it should be applied to a significantly larger number of patients and tested by other researchers interested in the field.
5. Conclusion

Although penile lengthening (ligamentolysis–skin advancement) and enlargement (dermal fat grafts) procedures cannot achieve spectacular phallic image alterations, they are characterized by substantially uneventful clinical course with low morbidity and a significantly positive impact on sexual self-esteem and function in the majority of patients. Therefore, this type of surgery can be considered a reasonable treatment modality for strictly selected and fully informed patients who suffer from penile dysmorphophobia and only when, conservative measures of alleviating sexual performance anxiety and feelings of sexual inadequacy, have been exhausted. Technical refinements and establishment of validated instruments facilitating proper patient selection, would further advance safety and efficacy of this type of surgery.

References


Editorial Comment

Ekkehard W. Hauck, Giessen, Germany
ekkehard.w.hauck@chiru.med.uni-giessen.de

The authors report on their experience regarding augmentation phalloplasty surgery. They performed different kinds of procedures to gain penile length and girth in young physical normal adults suffering from penile dysmorphophobia. Concerning this interesting paper some aspects should be discussed especially penile length with regard to dysmorphophobia. The mean penile length of the stretched penis in these men was 9.12 cm and assessed as normal. However, in the literature the mean penis size of the stretched penis seems to be a bit larger: In a study on 3,300 young Italian males the mean stretched length was 12.5 cm [1]. Spyropoulos et al. themselves reported on a mean total length of 12.1 cm of the stretched penis in a study on the size of the external genitalia in young adults [2]. Thus, the patients included in this series may have a penile size under the normal mean value. In medicine the normal values are usually defined by two standard deviations around the mean value. According to this definition Wessels et al. [3] recommended penile augmentation for a size of less than 7.5 cm of the stretched penis. To this opinion these patients should not have been operated.

I believe that a lot of urologists wonder why physically normal men should undergo surgery? Should subjective dysmorphophobia treated by a surgical intervention? Watching TV or reading the boulevard press it seems normal and accepted by the society that in women breasts become bigger and lips become thicker. In these cases it is the patient who decides to undergo surgery and who pays. Do normal values for the size of lips exist? A lot of plastic surgeons of more or less good quality offer these procedures. And many patients request this kind of surgery. Until today only few urologists have experience with penile augmentation. This may be the reason why the discussion on this field of surgery is very critically done among urologists. To my mind this critical point of view seems to be
justified. Hardly, no study has been published that reported on the long-term outcome and the psychological effects of these procedures. Therefore the authors of this paper should be commended for developing a questionnaire that standardizes the evaluation of the psychological outcome of these procedures. However, the series is small. It is just the beginning of using such an instrument that should be validated in even larger series.

**Editorial Comment**

Francesco Montorsi, Milan, Italy
montorsi.francesco@hsr.it

The authors report on the efficacy and safety of augmentation phalloplasty in normal young men, which is clearly a very controversial topic.

The authors have used a specifically developed questionnaire which, according to their experience, has proved to be useful when selecting patients for surgery. All patients underwent a thorough psychological/psychiatric assessment preoperatively which I believe is absolutely mandatory in this type of surgery. The surgical technique which is proposed by the authors does not look that innovative to me, especially with regards to the lengthening procedure. In this field it is interesting to note how very similar operations may held significantly different results from centre to centre.

The authors report a statistically significant improvement penile length and girth which is corroborated by their subjective improvement. The authors are aware that a major limitation of the study is related to the use of a non–validated questionnaire.

Finally readers should be stimulated to expand their knowledge in this area by running an exhaustive medline search that will pick up a number of papers reporting on disastrous complications occurring after this elongation-augmentation procedures.

**References**


Abstract

Objectives: The aim of this work was the evaluation of clinical results of the use of buccal mucosa for replacement of Peyronie’s disease plaque.

Patients and methods: Twenty-six patients with Peyronie’s disease were under observation. All the patients underwent the following investigations before and periodically within 3 years after the treatment: International Index of Erectile Function (IIEF-5), manual examination of plaque, autophotography of erect penis, conventional and power color Doppler sonography of penis, Peno-Brachial Index (PBI) before and its increase (IPBI) after the intracavernous injection of papaverin, Peak Systolic Velocity (PSV), end diastolic velocity (EDV), Resistance Index (RI), Sexual Encounter Profile questions (SEP-2 and SEP-3), measurements of penile length and curvature angle in the phase of rigidity. After stabilization in plaque’s development (mean time 2.0 ± 0.1 years) the patients underwent a surgery by means of excision of plaque and its replacement by free autograft of buccal mucosa.

Results: After the surgical treatment (with mean follow up observation of 3.2 ± 0.1 years) in 24 patients out of 26 (92.3%) the complete straightening of penis occurred, in two (7.7%) cases a residual curvature (<10°) remained, in four patients (15.4%) the shortening of penis (by 1 cm) and in two patients (7.7%) a partial reduction of erectile power were observed.

Conclusion: Buccal mucosa showed high properties of adaptation and revascularization, good anatomical and functional clinical results by replacement of indurative plaque, it kept a stable elasticity without shrinkage; the method is simple and can be recommended for wide use in clinics for surgical treatment of Peyronie’s disease. © 2005 Elsevier B.V. All rights reserved.

Keywords: Peyronie’s disease; Indurative plaque’s excision; Buccal mucosa; Clinical results

1. Introduction

Peyronie’s disease affects a considerable number of middle-aged men and is accompanied by the development of indurative plaque in tunica albuginea and corpus cavernosum of the penis. This often causes deformation and pain in the penis during erections and serious complications in sexual life [1]. Surgical methods such as plication or resection of tunica albuginea on the contralateral side of the penis without excision of plaque [2] are not always effective, especially in cases of painful and large dorsal deformation of erect penis (>30°). Besides, they are unacceptable by initially short penis [3–5]. Results of radical surgical treatment by means of excision or incision of plaque and its replacement with various bio- or alloplastic materials are controversial, as in the available literature, we could not find any randomized comparative investigations to generalize them [6–18]. Therefore, we have carried out preliminary experiments on 37 dogs using biomaterials most popular in clinics: free autopatches from skin, aponeurosis, peritoneum, vein and buccal mucosa. The best results of revascularization and adaptation after free transplantation as well as the highest indexes of elasticity and coefficient of
lengthening were obtained by using the patch of buccal mucosa [19,20]. These data became basic for the use of this material in our clinic after the approval given by Ethics Committee of Georgian State Medical Academy.

2. Material and methods

Twenty-six patients with Peyronie’s disease were under observation in the period of 1997–2003. All the patients underwent the following investigations: International Index of Erectile Function (IIEF-5) [21], manual examination of plaque for detection of its location, consistence, size and mobility; autophotography of erect penis in two projections for stating its curvature angle and direction; finding out the penile length before Peyronie’s disease; conventional B-mode and power color Doppler sonography of penis to determine the size, structure of plaque, its surrounding fields as well as the condition of penile blood vessels; Penobrachial Index (PBI) before and its increase (IPBI) after the intracavernous injection of 20 mg papaverin in the phase of full tumescence (Er-3), peak systolic velocity (PSV), end diastolic velocity (EDV), Resistance Index (RI) [23] and precise measurements of penile length, angle and direction of curvature in the following phase of full rigidity (Er-5) [22] from the middle of the penile basis up to meatus (by means of 1 mm rigid ruler and 1° gauge). Besides, the patients were asked about their ability to insert the penis into the vagina (SEP-2) and the maintenance of erection till the end of the sexual intercourse (SEP-3). The above-mentioned investigations were carried out before the surgery and then 2–6 months, 1 and 3 years later. Statistical significance was assessed by p-value ≤0.05 and Student’s t-test.

After completion of the active phase of plaque’s development and its sufficient demarcation confirmed by manual and above-mentioned sonographic investigations the patients underwent a surgical treatment. Under general anesthesia, the circumcision of this material in our clinic after the approval given by Ethics Committee of Georgian State Medical Academy.

After completion of the active phase of plaque’s development and its sufficient demarcation confirmed by manual and above-mentioned sonographic investigations the patients underwent a surgical treatment. Under general anesthesia, the circumcision of this material in our clinic after the approval given by Ethics Committee of Georgian State Medical Academy.

After completion of the active phase of plaque’s development and its sufficient demarcation confirmed by manual and above-mentioned sonographic investigations the patients underwent a surgical treatment. Under general anesthesia, the circumcision of this material in our clinic after the approval given by Ethics Committee of Georgian State Medical Academy.

The preputium was formed by knotty sutures. The penis was skin was returned towards the glans penis with subdermal drainage. When the penis was stretched manually in order to determine the most possible dimensions of defect in tunica albuginea that should be covered taking the future erections into account. After that the face and mouth cavity of the patient were prepared antiseptically by means of 0.4% chlorhexidin solution. Free autopatches of buccal mucosa of corresponding dimensions were separated (7.0 cm × 2.5 cm to 6.0 cm × 2.0 cm) and immersed in the same solution for 7–10 min. Meanwhile the defect in the cheek was sutured. The transplant was cleaned from superfluous muscular tissues, adapted to dimensions and form of the defect in tunica albuginea, placed longitudinally with its submucosal surface onto the corpus cavernosum and sutured along the circumferenc with Vicryl or Dexon 5/0 (Fig. 2). The patch was incised longitudinally in the middle by 1–2 cm in order to ensure sufficient drainage in case of possible hematoma under the patch. In case of bleeding in the process of transplantation, the basis of the penis was temporarily compressed again by tourniquet. This manipulation was also useful for evaluation of completeness of penile straightening at the final stage of transplantation by causing artificial erection. In case of residual deformation of the penis (>5°), the corresponding oval patch of tunica albuginea was resected in the middle of opposite convex part of the penis. This defect in tunica albuginea was also sutured by Vicryl or Dexon 5/0.

At the end of the operation the Buck’s fascia was sutured, the skin was returned towards the glans penis with subdermal drainage. The preputium was formed by knotty sutures. The penis was dressed circularly and the bladder catheterized for 1 or 2 days. The patients left the clinic after 4–5 days. They were prescribed Diazepam (0.01) for 7–10 days to prevent erections. The sexual life was permitted 2 months after the operation.

3. Results

The mean age of our patients was 48.3 ± 3.4 (40–62) years, 12 of them (46.1%) suffered from diabetes mellitus. The distribution of patients according to their clinical indexes is shown in Table 1. IIEF-5 was 8.1 ± 1.3; PBI before the intracavernous injection of papaverin was 0.80 ± 0.02 and 5–10 min later in the phase Er-3 it increased (IPBI) by 0.14 ± 0.02; PSV,
EDV and RI were 21.1 ± 0.6 cm/s, 5.3 ± 0.2 cm/s and 0.75 ± 0.01, respectively, and SEP-2, SEP-3—33% (8/26), 15% (4/26), respectively (Table 2). Thus, the penile vascular system of the patients and their ability to erections were quite preserved and their difficulty of coitus and pain were determined mainly by the large plaque sizes and the curvature angle. The mean duration of the disease was 2.0 ± 0.1 (<1, 1–3, >3) years. In three cases, the sufficient demarcation of plaque was observed at 11 months, but it was a rare exception from the rule.

All 26 patients underwent the surgery. There were no serious complications in the near postoperative period. Slightly expressed edema and echymosis of penis healed during a week. In one case, a small hematoma developed under the patch, which spontaneously absorbed within 2 weeks. The patients left the clinic 4–5 days after the operation with following dispensary observation.

Clinical results of the surgery within mean 3.2 ± 0.1 years are demonstrated in Table 3. In the near postoperative period (2–6 months), 24 (92.3%) patients obtained a complete straightening of penis, and 22 (84.6%) obtained restoration of its previous length; 2 (7.7%) patients with 45° and 60° curvature before the operation, retained the residual penile deformation (<10°); four (15.4%) patients with initial penile curvature of 90° after transplantation of the buccal mucosa patch (7.0 cm × 2.5 cm) and resection of tunica albuginea on the opposite side of penis obtained the shortening of penis by 1 cm as compared with the healthy period of life, which however did not bother them; two (7.7%) patients complained of the partial weakening of

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>The distribution of patients after clinical indexes before the treatment</td>
</tr>
<tr>
<td>Clinical indexes</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Duration of the disease (years)</td>
</tr>
<tr>
<td>&lt;1</td>
</tr>
<tr>
<td>1–3</td>
</tr>
<tr>
<td>&gt;3</td>
</tr>
<tr>
<td>Angle of erect penile curvature (°)</td>
</tr>
<tr>
<td>30–45</td>
</tr>
<tr>
<td>&gt;45 to &lt;90</td>
</tr>
<tr>
<td>90</td>
</tr>
<tr>
<td>Shortening of penis</td>
</tr>
<tr>
<td>0.5–1</td>
</tr>
<tr>
<td>&gt;1 to &lt;1.5</td>
</tr>
<tr>
<td>1.5–2.5</td>
</tr>
<tr>
<td>Symptoms of patients</td>
</tr>
<tr>
<td>Pain during erection with inability for coitus</td>
</tr>
<tr>
<td>Difficulty of coitus</td>
</tr>
<tr>
<td>Cosmetically disturbing penile curvature</td>
</tr>
<tr>
<td>Site of penile deformation</td>
</tr>
<tr>
<td>Dorsal</td>
</tr>
<tr>
<td>Dorsolateral</td>
</tr>
<tr>
<td>Ventrolateral</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erectile function of patients</td>
</tr>
<tr>
<td>Indexes</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>IIEF-5</td>
</tr>
<tr>
<td>PBI</td>
</tr>
<tr>
<td>IPBI</td>
</tr>
<tr>
<td>PSV (cm/s)</td>
</tr>
<tr>
<td>EDV (cm/s)</td>
</tr>
<tr>
<td>RI</td>
</tr>
<tr>
<td>SEP-2 (%)</td>
</tr>
<tr>
<td>SEP-3 (%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical results of the surgery</td>
</tr>
<tr>
<td>Indexes</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Straight penis</td>
</tr>
<tr>
<td>Restoration of previous length</td>
</tr>
<tr>
<td>Reduction in curvature</td>
</tr>
<tr>
<td>Recurrence of the curvature</td>
</tr>
<tr>
<td>Penile shortening</td>
</tr>
<tr>
<td>Decrease in erection</td>
</tr>
</tbody>
</table>
erectile power, though they were still able to realize their sexual act. These two patients belonged to the group of 12 ones suffering from diabetes mellitus. Statistically calculated mean index of IIEF-5 in that period was 20.1 ± 2.1, PBI before the intracavernous injection of papaverin was 0.93 ± 0.02, and its increase then (IPBI) 0.17 ± 0.01 (p < 0.05); PSV, EDV, RI, SEP-2 and SEP-3 became 32.3 ± 2.1 cm/s, 4.7 ± 0.1 cm/s, 0.75 ± 0.01, 92% and 85%, respectively. But the absolute indexes of two above mentioned patients with diabetes mellitus were: IIEF-5: 6, 7; PBI: 0.7, 0.73; IPBI: 0.13, 0.14; PSV: 22.0, 24.0 cm/s; EDV: 6.0, 5.6 cm/s; RI: 0.73, 0.77 (before the surgery: IIEF-5: 7, 8; PBI: 0.72, 0.74; IPBI: 0.13, 0.14; PSV: 20.0, 21.0 cm/s; EDV: 6.0, 6.5 cm/s; RI: 0.70, 0.74). Small doses of sildenafil-citrate (Viagra, 50 mg) were prescribed to these two patients from time to time.

In the following period, the physical examinations, IIEF-5, PBI, IPBI, PSV, EDV, RI, SEP-2, SEP-3 and measurements of penile length and curvature angles in the phase of rigidity (Er-5) were done after 1 year in 24 patients and after 3 years in 21 patients. The erectile function of the remaining 2 and 5 operated patients were assessed by IIEF-5, SEP-2 and SEP-3 questionnaires obtained through telephonic contacts. The state of the patients was stable (Tables 2 and 3).

4. Discussion

The results of surgical treatment of 26 patients showed a complete straightening of the penis in 24 (92.3%) cases with cessation of pain and restoration of sexual life. Two (7.7%) patients retained the penile deformation (<10°), which did not create obstacles to their sexual intercourse. These patients were operated at the first stage of our work when our technical experience was not sufficient enough. In four (15.4%) cases with initial curvature of penis 90°, which required the transplantation of a large patch (7.0 cm × 2.5 cm) of buccal mucosa and Nesbit operation on the opposite side of the penis a small shortening of penis (by 1 cm) occurred, which did not bother the patients; two (7.7%) patients complained of partial weakening of erection, which was determined mainly by diabetic vasculopathy.

In our opinion, only radical excision of plaque (neither plication nor incision) could ensure such anatomical results with following objective improvements in PBI, IPBI, IIEF-5, PSV, EDV, RI, SEP-2 and SEP-3 without significant shortening of the penis.

Summing up the results of surgical treatment with the use of buccal mucosa as biomaterial, we can assess them to be positive. In the available literature, we could not find analogous works for comparison. Therefore, our data can be compared with the results of the authors who used the venous patch for replacement of plaque (Table 4) [13].

Taking into account the fact that we had quite a complicated group of patients; among them 17 (65.4%) had a penile curvature angle >45°, 4 (15.4%): 90° and 12 (46.1%) patients were suffering from diabetes mellitus, these clinical data can be considered quite satisfactory in comparison with the results of the use of venous patch (Table 4) [13].

In view of the heated discussion concerning the optimal biomaterial for replacement of indurative plaque, we have undertaken a preliminary experimental study on dogs, using autopatches of skin, aponeurosis, peritoneum, vein and buccal mucosa. The best results of elasticity, coefficient of lengthening and morphological structure of transplant were obtained in the group of buccal mucosa [19,20]. In our opinion, the specific structure and sources of blood supply in the cheek insured such results. The majority of large, middle, small and capillary blood vessels in the cheek’s wall are located in submucosal layer. Therefore, the location of buccal mucosa graft with its submucosal surface towards the corpus cavernosum created very favorable conditions for the nourishment of graft from its whole submucosal surface. In comparison with that, in the venous wall the main net of its vaso-vasorum is located in the third external layer [24]. Therefore, the hope that the fixation of the venous patch with its endothelium towards the corpus cavernosum can ensure the nour-

<table>
<thead>
<tr>
<th>Authors</th>
<th>Used material</th>
<th>Cases (n)</th>
<th>Straight penis (%)</th>
<th>Residual curvature (%)</th>
<th>Recurrence (%)</th>
<th>Penile shortening (%)</th>
<th>Decrease in erection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>El-Sakka et al.</td>
<td>VP</td>
<td>112</td>
<td>95.5</td>
<td>4.5</td>
<td>3.5</td>
<td>16.9</td>
<td>12</td>
</tr>
<tr>
<td>Montorsi et al.</td>
<td>VP</td>
<td>50</td>
<td>80</td>
<td>14</td>
<td>6</td>
<td>40</td>
<td>6</td>
</tr>
<tr>
<td>Kadioglu et al.</td>
<td>VP</td>
<td>20</td>
<td>75</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Akkus et al.</td>
<td>VP</td>
<td>58</td>
<td>86</td>
<td>9</td>
<td>5</td>
<td>22.4</td>
<td>7</td>
</tr>
<tr>
<td>Our data</td>
<td>BM</td>
<td>26</td>
<td>92.3</td>
<td>7.7</td>
<td>0</td>
<td>15.4</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Table 4
Comparison of clinical results after use of venous patch (VP) and buccal mucosa (BM)
ishment of graft directly through endothelium [13] is, in our opinion, deceptive to a certain extent. As a matter of fact, the transplanted venous patch gets the nourishment mainly from its peripheral fields anastomosed with a tunica albuginea unrelated to it, which is itself poorly supplied with blood.

On the other hand, according to our data the use of buccal mucosa does not cause any problems with salivary duct tissues because they are removed together with remnants of muscular layer in the process of preparation of the patch before the transplantation; it does not actually create any epithelioid or subdermal cysts either, evidently because of metaplastic process in the postoperative period. One case of hematoma beneath the patch prompted us afterwards to make a small incision on it which together with suturing of Buck’s fascia, subdermal drainage and circular dressing of penis completely prevented the likelihood of hematoma and ensured the smooth healing of the wound. The incision of the patch also healed spontaneously without any problems.

It is obvious that the final clinical results depend, to a considerable degree, on a number of other factors related to operative techniques and initial conditions of the patients. In this respect, in our opinion, the surgical treatment of Peyronie’s disease can be undertaken not earlier than a year after its beginning, only in case of sufficient demarcation of plaque and considering the above recommended surgical techniques.

5. Conclusion

Buccal mucosa is a very perspective biomaterial for replacement of penile indurative plaque. It shows a high ability of adaptation and revascularization by free transplantation, gives very good anatomical and functional clinical results, and keeps the stable elasticity without shrinkage. The described method is simple and can be recommended for wide use in clinics for surgical treatment of Peyronie’s disease.

References


**Editorial Comment**

Prof. Emre Akkus, *Istanbul, Turkey*
emreak@istanbul.edu.tr

Despite having numerous therapeutic methods, the treatment of Peyronie’s Disease (PD) still has controversies. This study is an interesting one. However, there are some important points to remember, as the authors stated surgical procedures for PD are not recommended before the stabilization of the disease. This normally takes 12–18 months, but it is quite clear and evident that it may take longer. Also, even stabilization of the disease, does not result with "demarcation of the plaque" as stated by the authors to perform an easier surgery.

Penile Brachial Index (PBI) is no longer a definitive and valid diagnostic criterion for erectile function, therefore, we must be cautious when interpreting the results with PBI. Excision of the Peyronie’s plaque(s) may result with decrease in the compliance of the tunica albuginea and hence result in erectile dysfunction (ED). Therefore, incision of the plaque(s) rather than excision has gained more popularity and recommended with grafting to avoid ED. Also, valid animal models are needed before presenting clinical results of any new treatment modalities or materials.

Surgical treatment techniques of PD target to improve or straighten the penile curvature, and not to treat the disease or erectile dysfunction. Therefore, it is crucial to investigate the erectile status before the surgical treatment of PD. If there is ED with PD, the only effective and radical treatment would be penile implants. Another major issue before deciding on the surgery for PD is to consult and inform the patients and their partners about the consequences of the operations to avoid unrealistic expectations. In order to avoid medicolegal issues, a signed consent form for the surgical treatment must always be taken before such operations.

**Editorial Comment**

Luigi Cormio, *Bari, Italy*
luigicormio@libero.it

This interesting study provides robust evidence for the use of buccal mucosa as a substitute of tunica albuginea affected by Peyronie’s disease. It is noteworthy that the good results obtained in humans derive from previous meticulous work in experimental animals whereby the authors compared buccal mucosa with “conventional” (vein, derma, aponeurosis and peritoneum) autologous grafts and found it to be having better elasticity, coefficient of lengthening and capability of take than the other grafts. I enjoyed much the accurate anatomical description of grafts mechanisms of take, as it provides solid evidence for buccal mucosa easier of take and better elasticity than vein, as well as an explanation for the reported phenomenon of venous graft shrinkage. Unfortunately, no comment was made on graft harvesting, which, in my opinion, also deserves adequate counseling. Saphenous vein harvesting involves another operation, which is short-lasting, has no cosmetic impact due to the skin incision, and the theoretical disadvantage of using one potential source of vein grafting in case of aorto-coronary bypass surgery. Buccal mucosa harvesting also involves another operation which is short-lasting, has no cosmetic impact as there is no external incision, but has potential long-term complications such as oral numbness (16%) and oral tightness (32%) of which patients should be informed [1].

Obviously, the authors presented a comparison of the results of this new technique with those of vein grafting, demonstrating that buccal mucosa provides slightly better penile straightening and slightly lesser penile shortening than vein, with almost equal results in terms of postoperative ED. I expect these results to generate much enthusiasm (as it happened with me) and consequently induce most of us to present these figures when counseling candidates for surgical correction of Peyronie’s disease. After initial enthusiasm, however, one should bear in mind that the aim of this surgery is restoring a penis, which is straight, rigid and long enough for satisfactory intercourse. Thus, patients should be counseled on each of these issues, and to do this, straightness, length and rigidity should be assessed subjectively (by the patient) and objectively (by the doctor) before and after the surgery. This methodology is also essential for evaluation and comparison of different studies.

Straightening of the penis is usually the easiest issue, as subjective and objective evaluation generally match, but patients should be informed that a residual curvature below 10° is considered to be a good functional and cosmetic result, whereas a residual curvature between 10° and 30° is considered to be a fair result as it can be cosmetically disturbing but often functionally efficacious. Length is a difficult issue both in clinical practice and in scientific studies. Measurement of the short (affected) side should predict penile length after Nesbit or plication, whereas measurement of the long (normal) side should predict penile length after grafting procedures. Shortening
should always refer to length of the normal side before and after surgery. Subjective shortening is often reported by patients after all kind of procedures; thus, objective measurement is essential to determine the entity of shortening which is inevitable after Nesbit or plication but may also occur after grafting due to graft shrinkage or, more likely, to initial difficulty in resuming spontaneous (unassisted) erections, something similar to penile shortening after nerve-sparing radical prostatectomy [2]. This brings us to the third issue, i.e. erectile function. Preoperative subjective and objective (by color Doppler penile US) evaluation is essential, as grafting should be offered only to patients with absolutely normal erectile function because of the potential risk of impairment of veno-occlusive function. Consequently, postoperative vascular evaluation is mandatory to correlate objective and subjective results. This obviously applies to scientific studies, too. In the present one, the authors state that preoperative “vascular system...and ability to erections were quite preserved…” although mean PSV was 21.1 cm/s and mean RI was 0.75. Even more surprising (and unexplained) is that 1 year after surgery mean PSV raised to 34.2 cm/s and mean RI to 0.86 but there were two patients (7.7%) with postoperative ED.

Following these considerations, a great part of my enthusiasm has vanished. We know that results of 5 years of vein grafting are disappointing because of objective penile shortening in all cases, objective ED in 36%, and overall satisfaction rate of 60% [3]. Besides the advantage of a lower risk of penile shortening (which can be of clinical importance only for the minority of patients with short penis or severe obesity), this new grafting procedure continues to bear risks of postoperative ED and harvesting problems. No surprise if my patients will continue to choose our plication technique [4] which will certainly prevent harvesting problems and give them a penis shorter (like the affected side) but straight enough and rigid enough for successful intercourse.

References


Editorial Comment

Prof. Francesco Montorsi, Milan, Italy montorsi.francesco@hsr.it

This is an interesting paper reporting the use of buccal mucosa as a graft in patients with severe Peyronie’s disease treated by excision of the plaque. The authors have conducted preliminary work in the animal model, which proved buccal mucosa to be the best grafting material in terms of elasticity, coefficient of lengthening and morphological structure of the transplant (Refs. [19,20] of the article). They propose an interesting explanation for these positive results, which in their opinion is related to the specific structure and sources of blood supply in the cheek. The majority of large, middle and small capillary blood vessels in the cheek’s wall are located in submucosal layer. Therefore, the location of buccal mucosa graft with its submucosal surface towards the corpus cavernosum created very favorable conditions for the nourishment of graft from its whole submucosal surface.

The authors should be commended for using validated questionnaires to assess baseline and postoperative results. This is unusual in manuscripts dealing with Peyronie’s disease but it is certainly the most rigorous way to assess the outcome of any therapeutic procedure in this field.

The use of buccal mucosa does make sense to me, and I think that many urologists will be interested in following the take-home message of this paper. I am skeptical regarding the technique of total plaque removal as in the literature it has always been reported with significant rates of postoperative erectile dysfunction. This was typically seen at the long-term follow-up, and such data are missing in the current study. In addition, I think that the concept of real eradication of Peyronie’s disease is really not supported by any other study, and I believe that readers of European Urology should look at this concept with much caution.

I was impressed by the results reported by authors in terms of absence of postoperative penile shortening. In my personal experience with the incision and saphenous vein grafting technique, some penile shortening was always present when we compared ED with care penile length during rigid erection before and after the surgical procedure. It might well be that the particular tissue characteristics of buccal mucosa are at the bases for this favorable finding.

In summary, this is an important paper coming from an important Eastern European country, which opens the way to the use of a new material in plaque surgery. I look forward to receive a report on longer follow-up results.
Abstract

Objectives: Most of the available data on efficacy for sildenafil are based on a questionnaire and erectile dysfunction (ED) is classified with minor or severe organic factors. To better select sildenafil responders and non-responders, we have conducted a haemodynamic and morphometric study in sildenafil non-responders.

Methods: Thirty patients with ED aged from 28 to 74 years-old did not respond to 8 attempts of 100 mg of sildenafil. They underwent hormonal measurements, intracavernous injection (ICI 20 μg PGE₁) followed by Doppler examination and cavernometry. A penile biopsy was performed under local anesthesia. A quantification of the cavernous smooth muscle (SMC) was performed with a computerized image analysis after staining with actin anti-actin.

Results: Twenty-eight patients had a very poor ICI response. Five patients were diabetic and 2 had low testosterone level. Eight patients had arterial lesions, 15 had venous leak and 5 both lesions. They all had reduction of SMC (<35%). No biological and vascular abnormality was observed in two patients. They had a percentage of SMC of 38% and 42%. No complication was observed with the penile biopsy.

Conclusions: Severe vascular lesions and atrophy of SMC are mainly observed in sildenafil non-responders. The age, diabetes and low testosterone level seem not to be related with the failures.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Erectile dysfunction; PDE5 inhibitor; Sildenafil non-responder; Impotence

1. Introduction

Normal erectile function relies on the coordination of psychologic, neurologic, endocrine and vascular factors.

Treatment options for ED include oral therapy, psychogenic counsellings, vacuum device, vasoactive drug injections, transurethral drug therapy, vascular surgery, penile implants. Due to its non-invasive approach, patients with severe organic ED have demonstrated a strong preference for oral therapy even if they have lower response than with ICI injection or with penile implants [1,2]. Therefore efforts to optimise treatment of ED should target physiologic and clinical measures of improvement but also should address patient and partner satisfaction and preference.

Among the different possible drugs for oral therapy sildenafil with its longest clinical experience has demonstrated its good efficacy and safety [3,4]. The results are based mainly on a questionnaire and few studies exist concerning a better selection of the candidates for this oral therapy avoiding the unpleasant occurrences of failures for the patients [5]. Moreover, it is postulated that no particular characteristic predicts absolute failure with sildenafil [3].

We decided to conduct a study with the sildenafil non-responders by performing a complete organic assessment and morphometric examination of the intracavernous smooth muscle cells that are the key factor in the physiological mechanism of erection to
better understand the reasons of these failures and to better select the patients for the therapy.

2. Materials and methods

Thirty impotent patients aged from 28 to 74 years old (8: <39 years, 15: 40–59 years, 7: >60 years) did not respond to 8 attempts of 100 mg of sildenafil.

The laboratory tests were performed before therapy establishment. Complete blood and hormonal assays (in the morning) were performed to exclude endocrinological disease from diagnosis.

Before the vascular investigations, the patient underwent a 20 μg PGE1, intracavernous injection. After 15 min and sexual stimulation, the erectile angle was measured between the legs and the penis in the standing position [6]. Patients diagnosed with arterial disease had insufficient cavernous spongiosum blood flow velocity (<35 cm/sec). Cavernosometry confirmed that impotent patients with venous leak had a flow rate necessary to maintain artificial erection greater than 15 ml per minute.

Using the biopsy gun system, all patients underwent penile biopsy in the balanopreputial groove on the dorsolateral side of the shaft of the penis under local anesthesia. The penis is stretched by the left hand and biopsy is performed on the left corpus cavernosum [7]. The procedure was well explained to the patient before its application. Following biopsy removal, cavernous tissue specimens were immediately fixed in 10% formalin phosphate buffer solution. The fixed tissue sections were processed through xylene embedded in paraffin and sectioned at 5 μm. A tissue section was used for hematoxylin and eosin staining, and examined by light microscopy to evaluate the cavernous tissue pathologically, and 1 section was used for immunohistochemistry staining with actin anti-actin to determine the smooth muscle cells. Immunohistochemical staining was done according to the biotin-streptavidin-peroxidase technique. The sections were incubated for 15 minutes with 2% (volume in volume) hydrogen peroxide solution to block endogenous peroxidase activity, and processed for immunohistochemistry. After incubation with 1:20 normal sheep serum the sections were incubated for 30 minutes at room temperature successively with sheep antihuman immunoglobulin biotin diluted to 1:500 and streptavidin biotin for 30 minutes at room temperature successively with sheep antihuman immunoglobulin biotin diluted to 1:1000 for 30 minutes. Peroxidase activity (3,3-diaminobenzidine hydrochloride, pH 7.4, containing 0.01% (volume in volume) hydrogen peroxide for 5 to 10 minutes at room temperature.

Quantitative analysis of cavernous smooth muscle cells was done with an image analyzer system combined with a light microscope equipped with a video camera. Images were interactively discriminated and measurements were performed on the resulting binary images. The percentage of smooth muscle cells for each image results from the quantification of the difference between the gray levels on the digitised. At least 20 different fields (400×) in the cavernous tissue were examined from each tissue section. Mean percentage of cavernous smooth cells content was determined.

3. Results

Five patients were diabetic and 2 had low testosterone level (<3 ng/ml). Twenty-eight patients had poor ICI response with an erectile angle less than 45°. Eight patients had arterial lesions, 15 had venous leak and 5 both vascular pathologies. These 28 patients had a percentage of smooth muscle cells <35%; 18–30% mean 25%.

Two patients had normal biological measurements, normal ICI response, normal Doppler examination and normal cavernosometry with a percentage of smooth muscle cells of 38% and 42%.

No complication was observed after penile biopsy.

4. Discussion

All the patients had to take 8 pills with the maximum allowed dosage of 100 mg and they had received instructions on how to take the drug to optimize efficacy and treatment satisfaction before being considered as sildenafil non-responders [8].

Although men younger than 65 years appear to be more likely than older men to experience improved erections and successful sexual intercourse with sildenafil [9], we have relatively the same number of sildenafil non-responders according the different categories of age. Therefore, older impotent patients are also candidates for sildenafil therapy.

Only five patients were diabetic. Even if patients with diabetes do not respond as well as those without this pathology, they are significantly more likely to report improved erections than those treated with placebo [9].

Older patients with diabetes have a lower erectile response with sildenafil but they should not be excluded as they have an improvement of erectile function in 50% of the cases [10].

Only two patients presented lower testosterone level. Testosterone seems to have an effect on nitric oxide production [11]. As nitric oxide produced during sexual stimulation is mandatory to increase cGMP and therefore intracavernous smooth muscle relaxation, patients with lower testosterone level treated with sildenafil could have a less rigid erection.

Recently administration of testosterone with sildenafil in patients with erectile dysfunction and hypogonadism has given significant improvement of the erectile function and significant better penile arterial blood flow than sildenafil used with placebo [12,13].

Even if hypogonadism does not represent certainly the majority of the cases of sildenafil non-responders, testosterone measurement is required in the failure of PDE5 inhibitor therapy when it was not performed before treatment [14].

None of our patients had a history of spinal cord injury, pelvic trauma or surgery as it is well known that partial or complete penile neurological lesions influence sildenafil response [9,15].
Patients categorized at baseline as having severe ED appear less likely than those with mild to moderate ED to demonstrate improved erections and successful sexual intercourse when treated with sildenafil [9].

According to the guidelines in ED, complete vascular work-up is not performed routinely [14].

Severe vascular lesions as arterial insufficiency or cavernous leakage are significant factors influencing response to sildenafil treatment [5]. Twenty-eight of our patients had arterial lesion, cavernous leakage or both conditions.

Objective quantification of the smooth muscle cells in these sildenafil non-responders allowed us to demonstrate an important reduction of the smooth muscular content. This study confirms the necessity to preserve and to maintain the integrity of the smooth muscle fibers in the physiology of penile erectile mechanism. However, we must admit that the percentage of smooth muscle fibers in sildenafil responders is lacking but it is difficult to perform such an invasive procedure in patient satisfied with their treatment. However, we have already performed penile biopsy in potent patients operated on for urological operations. The percentage of smooth muscle cells in these normal potent patients is between 35 and 50% [16] which was recently confirmed by others [17]. Penile biopsy could serve as a predictive tool for orientating the therapeutical approach however it remains an invasive test and it has for the moment not its place in the arsenal of the diagnostic procedures. Intracavernous vasoactive drug injection could orientate or exclude patients to this oral therapy. Two of our sildenafil non-responders have developed a good response and confirms a previous study demonstrating that only 10% of the patients who did not achieve a full rigid lasting erection after intracavernous pharmacological testing reported good results with sildenafil therapy [5].

It has also been proved that sildenafil can give 34% good results in the patients with poor response after PGE1 intracavernous injections [18].

Patients with cavernous leakage as pure or mixed vasculogenic erectile dysfunction have lower response than those with arteriogenic erectile dysfunction [16]. Therefore, the erectile response is more dependent on the efficacy of the corporeal veno-occlusive mechanism. Our findings parallel the results already described as we have 80% of these sildenafil non-responders with this problem [5, 18].

The only reduction of smooth muscle cells observed in these patients can certainly not explain this phenomenon as it has been demonstrated that the percentage of the muscular content is lower in impotent patients with arterial lesions than in impotent patients with pure cavernous leakage [19]. Therefore, we should have more sildenafil non-responders with pure arterial lesions which is not the case in our study (only 13%) or in other studies [5, 18].

The patients may have other different deficiencies such as disturbance of penile innervation or neurotransmitter release; malfunction of the enzymes adenylate cyclase or guanylate cyclase as well as nitric oxide synthase or incompetence of the tunica albuginea. Cavernous leakage is a multifactorial problem [20].

Finally, no objective data exist to explain the non-response in two patients. Their possible anxiety could be at the origin but we have not realized a psychogenic test or a nocturnal penile erectile recording. The other possibility could be a higher penile density of PDE5 enzyme and 100 mg of sildenafil could not be sufficient to saturate the active site. We did not have any immunohistochemical agent to quantify the PDE5 enzyme density or we did not increase the dosage of sildenafil as it is forbidden on the labelling.

5. Conclusion

Patients with severe vascular lesions and reduction of intracavernous smooth muscle cells are poor candidates for sildenafil therapy. However, the same study should be performed in sildenafil responders.

Caution for sildenafil initiation treatment should be taken with patient with very poor erectile response after PGE1 intracavernous injection.

References


Editorial Comment

Edoardo S. Pescatori, Modena, Italy
pescatoriedoardo@libero.it

The authors are to be congratulated to have provided solid vascular/structural data in a strictly selected population of sildenafil non-responders.

They elegantly showed that in more than 90% of cases haemodynamic and morphometric anomalies may justify an insufficient response to pharmacologic (PDE5 inhibition and intracavernosal PGE1) intervention. We can therefore consider these patients as affected with “penile end-organ disease”; such information should further support the prosthetic surgery option (besides the vacuum option) in this patient group.

It is hoped that Authors will consider to perform a subsequent study that includes an appropriate control group (i.e. sildenafil responders), in order to unquestionably validate their conclusions.
**Pediatric and Reconstructive Urology**

**Luminal Nitric Oxide in Ileal Reservoirs for Continent Cutaneous Diversion or Orthotopic Bladder Reconstruction**

David Pazookia, Anders Kilanderb, Elisabet Lindholm, Anna-Carin Olin, Harriet Törnvist, Kjell-Arne Ung, Olof Jonsson*

*a* Department of Surgery, Sahlgrenska University Hospital, Göteborg, Sweden

*b* Department of Medicine, Section of Gastroenterology, Sahlgrenska University Hospital, Göteborg, Sweden

*c* Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, Göteborg, Sweden

*d* Department of Urology, Sahlgrenska University Hospital, SE-41345 Göteborg, Sweden

Accepted 8 December 2004

Available online 27 December 2004

**Abstract**

**Objectives:** To measure mucosal inflammation as reflected in nitric oxide (NO) production in ileal reservoirs for the storage of urine and to correlate it with the growth of bacteria as well as CRP.

**Methods:** Intraluminal gas NO concentrations were determined using the chemoluminescence technique in 25 patients with continent cutaneous ileal reservoirs (Kock pouch) and 12 patients with orthotopic bladders (hemi-Kock or T-pouch). NO concentrations were determined in both intestinal reservoir gas and silicon catheter balloon gas. Urinary culture and blood CRP determinations were performed.

**Results:** NO concentrations in reservoir gas were higher than in silicon catheter balloons. Bacteriuria was associated with approximately 20 times higher NO concentrations than sterile urine. NO concentrations did not differ between continent cutaneous reservoirs or orthotopic bladders when due attention was paid to variance in the rate of bacteriuria. Elevated CRP was associated with higher NO concentrations. Bacteriuria with acinetobacter, enterococci and pseudomonas appeared to cause comparatively lower NO concentrations. The inflammatory response of reservoir walls to bacteriuria did not decrease with time.

**Conclusions:** Urine in itself causes much less intestinal wall inflammation than bacteriuria, as reflected in NO production. High CRP values are associated with high NO concentrations. The inflammatory response varies with the bacterial specimens.

© 2004 Elsevier B.V. All rights reserved.

**Keywords:** Kock reservoir; Continent cutaneous ileal reservoir; Orthotopic ileal bladder; T-pouch; NO concentration; CRP

---

**1. Introduction**

When the intestine is used for the construction of a reservoir for the storage of urine, the mucosa is exposed to an unphysiological milieu. This could cause inflammation of the mucosa, which could damage the tissue and also provoke a systemic reaction. Upper urinary tract involvement is usually suspected when a patient with a urinary reservoir shows clinical signs of urinary tract infection. However, it is not known whether intense inflammation of the reservoir itself can cause systemic reactions similar to pouchitis in patients with faecal reservoirs [1].

Nitric oxide (NO) production is increased in both the bladder and the intestine during inflammation [2–5]. NO production can be measured by analyzing intraluminal gas since NO is a stable radical once it has emerged from the mucosa into the gas phase. During inflammation, NO is produced from arginine via the activity of inducible nitric oxide synthase (iNOS) [6].
The aim of the present study was to analyze the inflammation in ileal reservoirs exposed to urine via analysis of NO production in intraluminal air. Measurements were performed both on air collected from the catheter balloon and on air from the reservoir lumen. The correlation between the degree of inflammation as reflected in NO production and the C-reactive protein (CRP) was also analyzed.

2. Patients

Thirty-seven patients were investigated (females 20, males 17). Twenty-five patients were operated on with a continent cutaneous reservoir ad modum Kock [7]. During this operation 50 cm of the distal ileum is used for the construction of a reservoir. 15 cm of ileum proximal to the reservoir is used for construction of an antireflux intussuscepted nipple valve and 15 cm of ileum distal to the reservoir is used for a continent intussuscepted nipple valve. The reservoir is emptied regularly by catheterization of the continent nipple. Twelve patients were reconstructed with an ileal orthotopic bladder after cystectomy for bladder carcinoma. The bladder was constructed from 50 cm of the distal ileum. To prevent reflux, a similar intussuscepted nipple valve, as used in the continent cutaneous reservoir, was constructed in 10 patients. In two patients reflux protection was accomplished by performing a T-pouch [8]. During this operation an antireflux valve is constructed by anastomosing the ureters to the bladder via a subserosal ileal segment instead of via an intussuscepted nipple valve. The mean age at the time of operation (± S.D.) was 54.3 ± 12.4 years and the mean time from operation to time of analysis was 9.1 ± 5.5 years.

At the time of analysis all patients were in good general condition and none of the patients operated on following cancer diagnosis showed any signs of tumor progression. All patients in the orthotopic bladder group were operated on due to bladder carcinoma. In the continent cutaneous reservoir group 13 patients were operated on due to carcinoma and the other 12 patients due to various benign disorders.

3. Methods

A silicon catheter, range Charrière 16 (Argyle Sherwood), was introduced into the continent cutaneous reservoir or orthotopic bladder. The urine was collected for urinary culture. Even low bacterial counts were registered. After rinsing the reservoir/bladder with saline, 25 ml of air was insufflated into the catheter balloon and 300 ml into the lumen of the reservoir/bladder. After 10 minutes the gas was aspirated from the catheter balloon and the reservoir/bladder. The air was collected in plastic bags for analysis of the NO content. The procedure was repeated and gas was collected after 30 minutes of incubation. The plastic bags with the gas samples were transported to another laboratory, where the NO content was analyzed using a chemoluminescence technique (Ecophysics CLD 77, Dürnten, Switzerland).

One hundred ml of urine from five patients with bacteriuria were collected in plastic bags together with 300 ml of room air. After 30 minutes the gas was aspirated and injected into the NO analyzer.

At the time of the NO analyses a venous blood sample was collected from the last 24 of the 37 patients included in the study for determination of CRP using a turbidimetric method (Tina-quant® CRP, Roche Diagnostica GmbH, Mannheim, Germany). The upper limit for normal CRP was <5 mg/l.

3.1. Statistics

Mean values, S.D. and SEM were determined using conventional statistical techniques. The difference between paired groups was analyzed using the Wilcoxon sum rank test and between different groups using the Mann–Whitney U-test.

4. Results

Twenty-nine of the 37 patients had bacteriuria with bacterial counts of 10,000/ml or above. Nine of 24 patients had a CRP value of 5 mg/l or above. Eight of the nine patients with elevated CRP and 11 of the 15 with normal CRP had bacteriuria.

The mean NO concentration in luminal gas was approximately three times higher than the concentration in the silicon catheter balloon, both after 10 and 30 minutes of incubation when mean data for all 37 patients are given (Fig. 1). Thirty minutes of incubation yielded almost a doubling of the NO concentration compared with 10 minutes of incubation (Fig. 1). In the following figures, where data from sub-groups are presented, only NO concentrations obtained in intraluminal air after 10 minutes of incubation are given.

The NO concentrations in ileal reservoirs as well as in orthotopic bladders was 10 to 20 times higher when the urinary cultures were positive compared to negative
The NO production appeared to be similar in the two types of urinary diversion. The mean value ± SEM of NO concentrations in gas collected in plastic bags in the five ex vivo experiments was 31 ± 8 ppb after 30 minutes of incubation. The corresponding NO concentrations measured in intraluminal reservoir gas from these patients was 11446 ± 3271 ppb (p < 0.01).

Patients with an elevated CRP had higher NO concentrations than patients with CRP values below 5 mg/l (Fig. 3). Bacteriuria with Escherichia coli, Enterobacter, Klebsiella or Morganella appears to provoke higher NO production than bacteriuria with Acinetobacter, Enterococcus or Pseudomonas (Fig. 4).

There was no correlation between the time interval from the operation to the time of analysis and the production of NO for either continent cutaneous reservoirs or for orthotopic bladders (Fig. 5).

**5. Discussion**

The NO concentrations reported for the sub-groups were obtained after 10 minutes of incubation. Higher values were obtained after 30 minutes of incubation, indicating that a steady state regarding production and elimination of NO had not yet been reached after the shorter incubation time. However, the ratio between NO concentrations after the longer and shorter incubation times was fairly stable (1.80 ± 0.12, mean ± SEM), which is why the shorter time was chosen for the sake of simplicity. We do not know whether
30 minutes of incubation represents steady state values since longer incubation times have not been investigated. In urinary bladders there is a steady state concerning NO concentration between 30 and 60 minutes gated. In urinary bladders there is a steady state concerning NO concentration between 30 and 60 minutes.

Although a silicon catheter was used the recovery rate of NO was only approximately 25%. A recovery rate of up to 96% with silicon catheters was recorded by Ehre et al studying NO production in urinary bladders [2]. The same group reported a recovery rate of 45% in another study in which a thicker silicon catheter was used [9]. Obviously, it is important to use a catheter with a thin-walled balloon when that technique is used. However, we did not experience any major difficulty aspirating the majority of the insufflated gas after 10 minutes without urine contamination from the intestinal reservoirs. Since this method yielded the highest NO concentrations it was preferred in future experiments.

Bacteriuria was found to be associated with high NO production irrespective of whether an orthotopic bladder or a continent cutaneous reservoir was studied. Theoretically, NO can be produced in the urine through a reduction in nitrate by bacterial enzymes [10].

However, control experiments in the present study revealed very low NO production in urine with bacteria. The high NO production with bacteriuria must therefore come from the reservoir wall itself.

The role of NO in mucosal inflammation is not clear. A possible beneficial effect is that it may be a local defence mechanism against the invading bacteria [11]. A possible negative effect is that excess amounts of NO may have a cytotoxic effect on the host cells [11].

In recent years analysis of CRP values has yielded results from new research areas. Concentrations at the higher levels of the previously accepted normal range have been demonstrated to be predictive of further arteriothrombotic events [12]. Improved analytical methods have increased the sensitivity of the measurements and the upper limit for normal values has been reduced from 10 to below 5 mg/l. Besides being a non-specific biochemical marker of inflammation, CRP may also under various circumstances contribute to host defence against infection [12].

The significance of a correlation between elevated CRP and increased NO production in the reservoir is unclear. All patients studied were in good general condition and showed no clinical signs suggesting the equivalent of pouchitis. Similar analysis of patients with bacteriuria and fever could clarify the connection between NO production in the reservoir and the clinical situation.

In patients with continent cutaneous diversion bacteriuria is almost inevitable, which is reflected in the observation that 22 out of 25 patients had a positive urinary culture. Sterile urine is more common after orthotopic urinary reconstruction. In our study 5 out of 12 patients had a negative urinary culture. Since the present study shows that bacteriuria is associated with inflammation in the reservoir it might from a theoretical point of view be an advantage to render the urine sterile in patients with orthotopic bladders where it is possible.

The importance of the ability of macrophages to express inducible iNOS at inflammation for host defence varies with the pathogens involved [13]. The gene expressing iNOS is dispensable for host defence against infection with Pseudomonas aeruginosa but not against infection with Staphylococcus aureus. The difference regarding the effect of NO against different bacteria could explain the finding that bacteriuria with some bacteria caused higher NO production than infection with other bacteria.

Ileal mucosa exposed to urine changes with time. Decreased villus height and mucosal atrophy, as well as reduced absorption capacity of some urinary components, have been reported [14–17]. However, the ability of the intestinal mucosa to produce NO in response to bacteriuria does not change after up to 15 years post-urinary diversion.

In summary, contact between urine and intestinal mucosa causes much less inflammation than the combination of urine and bacteria reflected in NO production. NO production does not decline with an increase in time between urinary diversion and time of analysis up to 15 years. High NO production is associated with elevated CRP values. Infection with Acinetobacter and Enterococci appears to cause less inflammation than infection with other bacteria.

References


Translational Research—From Lab to Clinic

The Use of Telemetry Technology to Test the Proerectile Effect of Melanotan-II (MT-II) in Conscious Rats

F. Giuliano\textsuperscript{a,b,*}, A-S. Rössler\textsuperscript{a}, P. Clément\textsuperscript{a}, S. Droupy\textsuperscript{b}, L. Alexandre\textsuperscript{a}, J. Bernabe\textsuperscript{a}

\textsuperscript{a}Pelvipharm Laboratories, CNRS, Bat 5, 1 avenue de la Terrasse, 91190, Gif-sur-Yvette, France

\textsuperscript{b}Groupe de Recherche en Urologie, Uprés, EA 1602, University of Paris South, 63 rue du Général Leclerc, 94270 Le Kremlin Bicêtre, France

Accepted 24 February 2005
Available online 20 March 2005

Abstract

Objective: The aim of this study is to demonstrate that monitoring, by means of telemetry technology, the increases in intracavernosal pressure (ICP) in freely moving rats using melanotan-II (MT-II) as a proerectile inducer compound is a relevant experimental model to investigate the effects of pharmacological agents on erection.

Methods: Adult rats were implanted in the corpus cavernosum with a pressure sensor which permitted telemetric monitoring of ICP in freely moving animals following MT-II (0.1, 0.3 and 1 mg/kg) or saline i.v. injections. ICP was also measured after MT-II (0.1, 0.3 or 1 mg/kg) or saline i.v. delivery in anesthetized rats.

Results: In conscious rats, MT-II (1 mg/kg) significantly increased overall erectile activity compared to saline. In anesthetized rats, MT-II-induced increase in overall erectile activity was not statistically significant but displayed a similar pattern.

Conclusions: The use of telemetry technology allowed to collect quantifiable and reliable data regarding the proerectile activity of MT-II in physiological conditions. The telemetry model appears suitable for investigating the potential inducer proerectile properties of pharmacological agents.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Penile erection; Melanotan-II; Telemetry; Rat

1. Introduction

The central nervous system (CNS) is the primary control for penile erection. In addition to serotonin, dopamine and noradrenaline, other neurotransmitters such as nitric oxide, oxytocin and melanocortins take an important part in the central control of penile erection [1].

The melanocortin system represents a promising CNS target for the treatment of erectile dysfunction (ED) [2]. The melanocortin peptides α, β, γ-melanocyte-stimulating hormone (MSH) and adrenocorticotropic hormone, derived by post-translational processing of the pro-opiomelanocortin gene product, exert various physiological effects including, food intake, and sexual behavior [3]. Amongst the 5 melanocortin receptor subtypes identified to date, the subtypes 3 and 4 (MC3 and 4R) have been found located in rat spinal cord and/or brain areas well-known for their involvement in the control of male sexual behavior [4,5]. Further physiological studies have demonstrated the important role of MC3,4R in mediating the proerectile activity of α-MSH [6–8]. Early clinical trials have been conducted with two non-specific melanocortin receptor agonists, melanotan-II (MT-II) and PT-141 (the deaminated metabolite of MT-II) for the treatment of ED [9,10]. MT-II is a cyclic peptide analogue of α-MSH with an agonist activity at 4 of the 5 known melanocortin receptors: MC1R, MC3R, MC4R and MC5R. Furthermore MT-II is considered as a superagonist of MC3 and MC4R,
being 10-100-fold more potent than α-MSH [11]. MT-II has been studied in rats for its proerectile activity in different set of experiments using conscious and anesthetized animals [12]. It has been demonstrated, by using intracerebroventricular and intrathecal applications, that MT-II effects were centrally mediated.

In our previous work, telemetric monitoring of intracavernosal pressure in freely moving rats was demonstrated as an integrative approach of erection in natural physiological conditions [13,14]. The purpose of the present study was to further demonstrate telemetry technology as a suitable model to determine the proerectile activity of pharmacological agents by using MT-II as a pharmacological tool in physiological experimental conditions. The quantification of parameters characterizing erection was done in conscious rats using telemetry technology after systemic injection of MT-II and these data were compared with those obtained in anesthetized rats treated with the same compound.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (225–250 g; Charles River, St-Aubin-lès-Elbeuf, France), were housed 4 per cage at 22 °C under reversed 12L:12D photoperiodic conditions (lights off at 1:00 pm) in plastic cages and were given commercial pellets and tap water ad libitum. All animal experiments were carried out in accordance with the European Community Council Directive (86609/EEC) on the use of laboratory animals.

2.2. Telemetric recording of ICP in freely moving rats

Telemetric device implantation was performed according to the technique previously described [14]. Briefly, 10 rats were implanted under 2.5% isoflurane (Centravet, Plancoët, France) anesthesia with a telemetric device (C40, Data Sciences, St Paul, USA). The tip of a recording catheter was inserted into the proximal shaft of the right corpus cavernosum, exposed through a midscrotal incision. The pressure transducer was placed subcutaneously at the lateral aspect of the abdominal wall. The telemetric device was calibrated before and after each implantation. Rats were kept, one animal per cage, after surgical implantation in the animal facility for 7 days before testing. After completion of the tests, the rats were sacrificed by an overdose of pentobarbital and the location of the tip of the catheter within the corpus cavernosum was checked by dissection under binocular magnifying glass.

Experiments were performed between 1:00 p.m. and 6:00 p.m. MT-II was injected i.v. in the tail vein (3 min maximum) under light isoflurane anesthesia (2–3%). Saline i.v. was used as control injection. Rats, recovering from anesthesia after 1–4 min, were placed alone in a Plexiglas cage (50 × 40 × 30 cm, with sawdust on the floor) and ICP was recorded for 1 hour after awakening of the animals. The ICP signals were amplified and telemetrically transmitted to an external receiver (model RA1000, Data Sciences) placed under the observation chamber (sampling at 100 Hz). Penile erections were visually identified and manually scored by a trained observer according to behavioral criteria described by Sachs et al. [15]. Only ICP rises detected by telemetry and corresponding to penile erections that were visually scored were quantified.

Each rat was administered with vehicle (saline i.v.) or MT-II (0.1, 0.3 and 1 mg/kg; i.v.) each injection, separated by a 24-hour interval, being performed in a random order.

2.3. Intracavernous pressure recording in anesthetized rats

Rats (8–9 animals for each MT-II dose and 18 rats for saline) were anesthetized with intraperitoneal injection of urethane (1.2 g/kg, Sigma) and placed on a homeothermic blanket to maintain their temperature at 37 °C. Rats were tracheotomized to prevent aspiration of saliva. A polyethylene tubing catheter filled with heparinized saline (50 UI/ml) was inserted into the carotid artery to record arterial blood pressure and another one into the jugular vein to inject drugs intravenously (i.v.). To record ICP, the tip of a catheter filled with heparinized saline (50 UI/ml) and connected to a pressure transducer (Elcomatic EM 750, UK) was inserted into the left corpus cavernosum after the penis was denuded of skin. MT-II (Bachem, Weil am Rhein, Germany) and saline were delivered in the jugular vein after a 5-min baseline period was obtained. ICP was monitored for 1 hour after treatment. Only ICP rises with a minimal value superior to the sum of the average ± 3 standard deviations of the baseline ICP were quantified. Such ICP rises were considered as erectile events. A stimulation of the cavernous nerve (30 s overall duration, 6 V, 10 Hz, 1 ms pulse) was performed at the end of the experiment in order to certify the correct implantation of the intracavernosal catheter. Rats with an inadequate erectile response elicited by cavernous nerve stimulation were discarded from further analysis. Four groups of rats were i.v. injected with either saline or MT-II (0.1, 0.3, and 1 mg/kg).

2.4. Drugs

For experiments performed in conscious rats, MT-II was diluted in saline at a concentration of 0.05, 0.15 and 0.5 mg/ml and injected i.v. in a volume of 2 ml/kg.

For experiments in anesthetized rats, MT-II was diluted in saline at a concentration of 0.1, 0.3 and 1 mg/ml and injected i.v. in a volume of 1 ml/kg.

2.5. Data analysis and statistics

In conscious rats, penile erection was associated with ICP rise characterized by peaks superimposed on a plateau (Fig. 1). The ICP peaks resulted from contractions of the ischiocavernosus and bulbospongious muscles (striated muscles located at the penile crus). Brief contractions of these striated muscles squeezed the blood trapped in the corpora cavernosa eliciting further brief increases in ICP (usually above the systolic blood pressure). The plateau corresponded to an increase in ICP due to recruitment of the proerectile penile innervation controlling the engorgement of the erectile tissue.

In anesthetized rats erectile events elicited by electrical stimulation of cavernous nerve were associated with ICP rises in form of a plateau, without the peaks. The following parameters were used to quantify erectile activity in conscious and anesthetized rats:

- number of rats displaying at least one penile erection behaviorally scored or erectile event in conscious or anesthetized rats respectively,
3. Results

Samples of ICP recordings in conscious and anesthetized rats after treatment with MT-II are displayed on Fig. 2. The baseline ICP was 67 ± 7 and 11 ± 1 mmHg in conscious and anesthetized animals respectively.

3.1. Number of rats displaying at least one penile erection/erectile event

The number of conscious rats displaying at least one penile erection was 4 out of 5, 4 out of 6 and 6 out of 8 after 0.1, 0.3 and 1 mg/kg i.v. MT-II respectively. After i.v. saline injection 5 out of 8 rats displayed at least one erection.

Under urethane-anesthesia, the number of rats displaying at least one or more erectile event was 3 out of 8, 4 out of 8 and 7 out of 9 after 0.1, 0.3 and 1 mg/kg i.v. MT-II respectively. In comparison, 4 out of 18 animals exhibited at least one erectile event in the i.v. saline treated group.

3.2. Number of penile erections/erectile events

Two-way ANOVA did not reveal a significant effect of the vigilance state of the rat on the number of penile erections/erectile events (F[1;55] = 0.63, p = 0.43).

In the same way, no significant effect of treatment on the number of penile erections/erectile events occurring during the 1-hour observation period was evidenced by two-way ANOVA (F[3;55] = 1.83, p = 0.15). Nevertheless, there was a clear trend for MT-II 1 mg/kg to augment (2.7-fold increase when compared to saline) the number of penile erections in conscious rats (Table 1). In anesthetized rats, a 3.7-fold and 3.6-fold increase compared to saline in the number of erectile events was observed after respectively 0.3 and 1 mg/kg MT-II (Table 1).

3.3. Latency for the first penile erection/erectile event to occur

No significant effect of the vigilance state of the animals on latency was yielded by two-way ANOVA (F[1;28] = 0.62, p = 0.44).

The two-way ANOVA test showed a significant effect of treatment on the latency of the first penile erection/erectile event (F[3;28] = 3.02, p < 0.05). In conscious rats, post-hoc comparisons did not reveal statistically significant differences between MT-II and saline injections (Table 1). In anesthetized rats, a significant decrease (Bonferroni’s test: p < 0.05) in mean latency for the first erectile event to occur was found after 0.3 mg/kg MT-II injection when compared to saline (Table 1).
3.4. Quantitative evaluation of the proerectile effect of MT-II in conscious and anesthetized rats

3.4.1. Maximal ICP increases during penile erection/erectile event (ICP\textsubscript{max})

The ICP\textsubscript{max} values were found dependent on the vigilance state as revealed by the two-way ANOVA test (F[1;29] = 42.95, \(p < 0.001\)), with the conscious rats displaying higher ICP\textsubscript{max} during penile erections than the anesthetized rats during erectile events independently of the treatment they received.

No significant effect of MT-II injections on the average ICP\textsubscript{max} measured during penile erection/erectile event, whatever the dose tested compared to saline, was evidenced (two-way ANOVA: F[3;29] = 1.67, \(p = 0.20\)). In conscious rats, there was a slight increase in the average ICP\textsubscript{max} after 0.3 and 1 mg/kg MT-II (respectively 472 ± 185 and 548 ± 127 mmHg) compared to saline (261 ± 101 mmHg).

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vigilance state</th>
<th>Number of penile erections/erectile events</th>
<th>Latency (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conscious</td>
<td>Anesthetized</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>1.8 ± 0.4</td>
<td>0.7 ± 0.5</td>
<td>2410 ± 729</td>
</tr>
<tr>
<td>MT-II 0.1 mg/kg</td>
<td>1.6 ± 1.0</td>
<td>0.9 ± 0.5</td>
<td>2812 ± 909</td>
</tr>
<tr>
<td>MT-II 0.3 mg/kg</td>
<td>1.8 ± 0.9</td>
<td>2.6 ± 1.1</td>
<td>2616 ± 953</td>
</tr>
<tr>
<td>MT-II 1 mg/kg</td>
<td>4.8 ± 1.4</td>
<td>2.5 ± 0.7</td>
<td>1780 ± 556</td>
</tr>
</tbody>
</table>

The values were obtained over the 1-hour period following treatment and were statistically compared using two-way ANOVA followed, whenever \(p < 0.05\), by Bonferroni’s post-hoc test after. Asterisks (*\(p < 0.05\)) indicate a significant difference with saline.

Fig. 2. ICP recordings: typical example of 1 mg/kg MT-II-induced penile erections recorded in conscious and anesthetized rats. In conscious rats the phasic increases delimited by a plateau correspond to a penile erection scored by the behavioral observer. In anesthetized rats, the plateau phase corresponds to an erectile event.
3.4.2. Area under the curve of ICP during penile erection/erectile event (AUC)

Two-way ANOVA showed no significant effect of the vigilance state of the rats on AUC (F[1;29] = 1.64, p = 0.21).

None of the MT-II doses induced consistent changes of the AUC in both conscious and anesthetized rats (two-way ANOVA: F[3;29] = 1.06, p = 0.38; Fig. 3).

3.4.3. Sum of the area under the curve of penile erection/erectile event (SUM AUC)

SUM AUC values were superior, although not significantly, in conscious animals when compared to anesthetized ones (two-way ANOVA: F[1;29] = 1.81, p = 0.19).

There was a significant effect of treatment on SUM AUC values determined during penile erection/erectile event (two-way ANOVA: F[3;29] = 3.14, p < 0.05). In conscious rats, MT-II 1 mg/kg markedly augmented SUM AUC value compared to saline (10653 ± 2763 and 2525 ± 254 mmHg.s respectively; Bonferroni’s test: p < 0.05; Fig. 3). In anesthetized rats, there was a trend although not statistically significant (Bonferroni’s test, p > 0.05) for 0.3 mg/kg MT-II to increase the SUM AUC (2842 ± 1106 mmHg.s) when compared to saline (669 ± 452 mmHg.s; Fig. 3).

4. Discussion

We have provided a quantitative comparison of the effects of MT-II between anesthetized and conscious rats by using telemetry. Implantation of the telemetric device into the corpus cavernosum allows to reliably record ICP in freely moving rats over at least six consecutive days without altering neither erectile function (present study) nor copulatory behavior [14].

The proerectile effect of MT-II has received recent evidences in a behavioral study using the ex copula paradigm in rats [12] where i.v. injection of MT-II (1 mg/kg) significantly increased the number of penile erections. Presently, we did not find such a statistical significance with the same dose of MT-II tested, maybe because of subtle differences in experimental conditions and in strain of animals used, although a clear trend was observed (2.7-fold increase compared to saline injection vs about 4-fold increase in the cited study). However, the overall erectile activity measured through the telemetry technique was found significantly higher (4.2 times compared to saline) after i.v. MT-II 1 mg/kg, suggesting that this parameter is the most reliable to reveal significant proerectile activity. This is especially true in our study considering the limited experimental samples available (6 and 5 rats for MT-II 1 mg/kg and saline respectively).

Urethane has been reported to modestly affect multiple neurotransmitter systems at an anesthetic concentration in rats [16]. Therefore, urethane-induced modulation of the activity of neural elements constituenet of or interacting with melanocortinergic system may impair the physiological response to MT-II. However, there was no significant interaction between the various tested doses of MT-II and the vigilance state of the rats for all the parameters computed to characterize erectile activity as yielded by two-way ANOVA tests. We thus assume that the proerectile effects of MT-II are comparable between conscious and urethane-anesthetized rats. It can be noticed however that the most efficient proerectile dose of MT-II administered i.v. differs according to the vigilance state (1 mg/kg in conscious rats versus 0.3 mg/kg in anesthetized ones). This latter result may be due to the influence of anesthesia that can modify the pharmacokinetic properties of pharmacological agents and thus may explain the difference, although not statistically significant, in the latency of penile erection/erectile event between conscious and anesthetized rats. Nevertheless, we believe that the use of anesthetized animals remains essential to investigate in depth the mechanism of action of drugs for ED.

The only parameter that was significantly superior in conscious animals compared to anesthetized ones was the maximal amplitude of ICP (ICP_max). It has been shown that, in anesthetized rats, additive electrical stimulation of the motor branch of the pudendal nerve, innervating the ischiocavernosus and bulbospongiosus muscles located at the penile crus, to stimulation of the cavernous nerve led to suprasystolic ICP increases with a pattern (i.e. presence of peaks) similar to that observed in conscious rats as recorded with the telemetric device [17]. Therefore, it appears that both autonomic and somatic neural pathways activation are required for a full penile erection to occur in anesthetized rats. The fact that MT-II-induced ICP increases was in form of a plateau in anesthetized rats, with ICP_max remaining below the blood pressure value, indicates that there was no recruitment of the pudendal nerve during erectile events elicited by MT-II treatment in rats. The reasons why a synergistic recruitment of both autonomic and somatic innervations is not observed in anesthetized rats is unknown, but one may suggest the compulsory participation of integrative brain areas, not functional under anesthesia, allowing tightly coordinated activation of autonomic and somatic neural pathways. Regarding the trend for AUC and SUM AUC values to be lower in anesthetized rats compared
Fig. 3. Quantification in anesthetized and conscious rats of the erectile activity induced by MT-II (0.1, 0.3 and 1 mg/kg i.v.). ICP$_{\text{max}}$, AUC, SUM AUC (see definitions in materials and methods section) were quantified in conscious and anesthetized rats over the 1-hour period following injection. Statistical analyses were performed using two-way ANOVA followed, whenever $p < 0.05$, by Bonferroni's post-hoc test after. * ($p < 0.05$) indicates a significant difference with saline injection, # ($p < 0.001$) indicates a significant difference between conscious and anesthetized rats for the same dose of MT-II.
to conscious ones, it could be explained by the difference in systemic blood pressure according to the vigilance state (mean arterial pressure: 75 ± 4 mmHg in anesthetized rats in our conditions versus approximately 130 mmHg as measured in our previous study in conscious Sprague-Dawley rats implanted with telemetry device [14]) since ICP has been demonstrated closely correlated to mean arterial pressure [18].

In conclusion, telemetric monitoring of ICP in freely moving rats provides valid and reliable data on the proerectile activity of MT-II in physiological conditions. The determination of the overall erectile activity following pharmacological treatment (by calculating SUM AUC) allows to evidence the proerectile properties of compounds inducing erection even using small experimental sample size.

References


Editorial Comment

Francesco Montorsi, Milan, Italy
montorsi.francesco@hsr.it

This is an interesting study on melanotan II, a centrally acting compound which has shown to have proerectile activity in previous studies. From a clinical perspective there is an interest on drugs improving the erectile performance in men by using different mechanisms of action from the drugs which are currently on the market. It is known that a significant proportion of patients who do respond to PDE5-inhibitors eventually withdraw from treatment without a clearly defined reason. It might be the case that different drugs could result useful for these patients. Research is needed in this area and the authors should be commended for successfully being on the front line of basic studies in the field of sexual dysfunction in both sexes.

Editorial Comment

Xinhua Zhang, Florence, Italy
zxhmd2000@yahoo.com

The erectogenic profile of melanotan (MT-II) has already been proved in conscious and anesthetized animals, as well as in human beings. In this study,
MT-II was employed as an inducer of penile erection in rats. The main contribution of this investigation is to demonstrate that the intracavernous pressure (ICP) monitoring in conscious rats by the telemetry technique is comparable to ICP recorded in anesthetized rats by the standard, routinely used method.

The technique of ICP measurement using commercially available, small, anesthetized animals such as rats and mice, was established in the last decade. This technique has been thought to be a significant advance because it provides an objective and accurate quantitative index to evaluate erectile function. The major imperfection of this method is drug intervention after general anesthesia, while a conscious model using the telemetry technique, as in the present study, is preferable for evaluation of physiological status. However, electrical stimulation of the cavernous nerve or central nerve system cannot be performed without anesthesia. Also it is very difficult to deliver proerectile substances intracavernously, intrathecally and intracerebroventricularly to conscious animals. Contraction of the ischiocavernous and bulbocavernous muscles, which produce the burst peak pressure beyond systolic blood pressure in freely moving animals, is undesirable and creates confusion when evaluating cavernosal relaxation after pharmacological stimulation.

Hence, as mentioned in the text by the authors, the anesthetized animal models of ICP monitoring remain essential for study of experimental erectile physiology and pathophysiology, along with proerectile drug discovery and development. For the latter purpose, a combined anesthetized and conscious model for investigating proerectile activity is more desirable.
Translational Research—From Lab to Clinic

Virus Specific Immune Responses after Human Neoadjuvant Adenovirus-mediated Suicide GeneTherapy for Prostate Cancer

R.R.M. van der Linden a, B.L. Haagmans b, P. Mongiat-Artus a,f, G.J. van Doornum b, R. Kraaij a, D. Kadmon c, E. Aguilar-Cordova d, A.D.M.E. Osterhaus b, T.H. van der Kwast e, C.H. Bangma a,*

a Department of Urology, Erasmus MC, Rotterdam, The Netherlands
b Department of Virology, Erasmus MC, Rotterdam, The Netherlands
c Department of Urology, Baylor College of Medicine, Houston, USA
d Advantage Inc. Harvard Medical School, Boston, USA
e Department of Pathology, Erasmus MC, Rotterdam, The Netherlands
f University of Paris VII, Paris, France

Accepted 16 February 2005
Available online 16 March 2005

Abstract

Purpose: Neoadjuvant gene therapy potentially improves the outcome of primary treatment of prostate cancer by radical prostatectomy in patients with high risk of recurrence. We conducted a Phase I escalating dose study with a replication-defective adenovirus expressing the herpes simplex virus-thymidine kinase gene (Adv-HSV-tk vector). The primary end point was toxicity, while the evaluation of the patients’ cellular and humoral immune responses served as a secondary endpoint.

Material and methods: The Adv-HSV-tk vector was injected into the prostate in two doses (2 × 10^10 to 2 × 10^11 viral particles), followed by ganciclovir twice daily for 14 days and retropubic radical prostatectomy on day 21. Adenovirus-specific neutralizing, IgG and IgA antibodies were evaluated. Peripheral blood mononuclear cells (PBMC) were stimulated by Adv-HSV-tk and analysed for IFN-γ production and 3H-thymidine incorporation. Prostate specimens were immunostained for B (CD20 +) and for T (CD3 +) lymphocytes.

Results: Toxicity was minor in all 8 patients treated. In the prostate, no virus related cytopathic effect could be observed. Dose-dependent infiltration of T and B lymphocytes in the whole prostate and in tumor areas was observed. Boosting of adenovirus-specific antibody responses was observed in 7 patients, and an increased adenovirus-specific PBMC proliferation and IFN-γ production was seen after Adv-HSV-tk stimulation.

Conclusion: Neoadjuvant adenovirus-mediated cytotoxic gene therapy prior to prostatectomy for prostate cancer is feasible and safe in an outpatient setting for intraprostatic vector doses up to 2 × 10^11 viral particles. Activation of the immune system was observed. Application of higher vector doses may be considered.

© 2005 Elsevier B.V. All rights reserved.

Keywords: High-risk prostate cancer; Clinical trial; Neo-adjuvant therapy; Suicide gene therapy; Radical prostatectomy

1. Introduction

The widespread use of prostate specific antigen (PSA) screening has led to a greater proportion of early stage prostate cancer at diagnosis and to an
increasing number of patients being offered definitive treatment with radical prostatectomy or radiotherapy [1,2]. A considerable percentage of patients with clinical organ confined disease will have extraprostatic disease on pathological examination. Patients with a PSA < 10 ng/ml, Gleason score < 6 and T1 or T2a disease appear to be at low (6 to 20%) risk of recurrence after 5 years [3]. However, for the patients who display a different pattern of risk factors, the risk of recurrence rises from 34% up to as high as 100%.

A treatment modality combining surgery with an adjuvant therapy may conceivably improve the outcome of those high risk patients. Neoadjuvant therapy to surgery has several advantages over adjuvant therapy [4]. It provides early systemic therapy for presumed micro metastases and may reduce locally advanced tumors to allow an improvement in surgical resection. Furthermore, it provides operative specimens that allow for more rapid analysis of new therapeutic modalities for efficacy.

Gene therapy refers to the transfer of genetic material into cells and expression of this material for a therapeutic purpose. One of the experimental cancer gene therapy approaches is the transfer of herpes simplex virus thymidine kinase (HSV-tk) gene, followed by the administration of ganciclovir. This antiherpetic pro-drug is a poor substrate for mammalian thymidine kinases, but is phosphorylated into an effective cytotoxic drug by HSV-tk [5]. The phosphorylated drug is a nucleotide analogue that is incorporated into DNA during cell division, leading to termination of DNA replication and cell death [6].

Intra-prostatic adenoviral vector (Adv) transduction of the HSV-tk gene followed by ganciclovir has been extensively tested in pre-clinical and clinical studies [7–11]. The number of killed cells significantly exceeds the number of cells transduced with the HSV-tk gene, a phenomenon known as “bystander effect”. This amplification is not only mediated by the diffusion of the activated drug into cells adjacent to the transduced cells, but also by an immune reaction directed against tumor cells. In an orthotopic mouse prostate cancer model, a marked proliferation inhibition of local tumor cells upon HSV-tk gene therapy was associated with suppression of spontaneous and induced metastasis [8,9]. These results initiated this study focusing on the patients’ immune response after HSV-tk gene therapy in a neoadjuvant treatment to radical prostatectomy. In high recurrence risk prostate cancer patients a Phase I dose escalation trial of adenovirus-mediated HSV-tk gene transfer through direct intra-prostatic injection plus ganciclovir administration three weeks prior to radical prostatectomy was started in an outpatient setting. The primary end point of this study was toxicity, while the evaluation of the patients’ immune reactions, both humoral and cellular, served as a secondary endpoint. We confirmed the safety of the in situ Adv-HSV-tk gene therapy as an out-patient procedure. We also showed that the extent of infiltration of the prostate by lymphocytes is correlated with the dose of adenovirus administered.

2. Material and methods

2.1. Clinical protocol

Men with clinically confined prostate cancer, at risk for capsular penetration, were injected intraprostatically with an escalating dose of Adv-HSV-tk delivered in situ, followed by ganciclovir administration three weeks prior to radical prostatectomy. In absence of any continuous grade 3 or recurrent grade 4 toxicity (WHO standards), the vector dose was escalated in two cohorts. The protocol was approved by the Dutch Commission for Genetic Modified Agents and by the Ethics Committee of the Erasmus MC.

2.2. Inclusion criteria

Eligibility criteria included histologically proven adenocarcinoma of the prostate with one or more of the following risk factors: clinical stage ≥ T2c (1992 TNM staging), PSA ≥ 20 ng/ml or Gleason score ≥ 8. Serum acid phosphatase and bone scans had to be normal. No immunodeficiency conditions, including HCV and HIV infection, were allowed. Normal haematopoietic function (platelet count > 100,000/ml, neutrophil count > 2000/ml and hemoglobin > 6.5 mmol/l), a normal coagulation profile, and normal kidney and liver functions (serum creatinine < 1.5 mg/dl, bilirubin < 2.5 mg/dl, liver enzymes and alkaline phosphatase < 2 × normal) were required. No androgen deprivation, immunosuppressive drug or corticosteroid were accepted if not discontinued at least one month prior to inclusion. An informed consent had to be signed.

2.3. Vector

The Adv-HSV-tk vector is a replication-defective adenovirus of serotype 5 that contains the herpes simplex virus-thymidine kinase gene under the control of the Rous sarcoma virus long terminal repeat promoter in the region of the excised E1/E2 adenoviral genes. The adenoviral vector was constructed as described previously and produced in the Baylor College of Medicine Gene Vector Laboratory, in accordance with Good Manufacturing Practice [12]. The vector was characterized for purity and potency for clinical use. The infectious unit (IU) titer was calculated at 1 × 10^{12} IU/ml with a virus particle (vp) content of 2 × 10^{12} vp/ml [13]. The virus was stored at −80 °C and prior to use diluted to the dose specified for each patient cohort. The biological activity of the vector dose was confirmed in the remnant of each vial delivered using a cytotoxic effect assay with A549 cells (American Type Cell Culture Collection, Rockville, Md).

2.4. Treatment course

All patients were given an oral antibiotic prophylaxis (cotrimoxazol 960 mg) and an enema before the vector injection in the outpatient clinic. The vector was delivered in four injections of 0.5 ml each under ultrasonic guidance in four quadrants of the prostate.
The first cohort of 4 patients (A, B, C and D) received a total dose of \(2 \times 10^{10}\) vp of the vector on day 0. Thereafter they received an intravenous infusion of 5 mg/kg of ganciclovir (GCV) (Roche) twice daily from day 1 to day 14.

The second cohort of patients (E, F, G and H) received a total dose of \(2 \times 10^{11}\) vp of the vector, followed by 5 mg/kg of oral ganciclovir twice daily for two weeks.

All patients underwent a pelvic lymph node dissection and a retro-pubic radical prostatectomy on day 21.

2.5. Safety procedures

After vector administration, patients had to demonstrate complete voiding (BladderScan®). Each urine specimen was decontaminated with a chlorine tablet at home until prostatectomy.

Daily urine and pharyngeal swab specimens were inoculated on 293 and Hep2 cells (American Type Cell Culture Collection, Rockville, Md) to test for infectious adenoviral vector and replication-competent adenovirus, respectively, [14].

Viral growth was tested from a biopsy immediately after the removal of the gland.

2.6. Patient follow-up

The patients were evaluated daily in the out-patient clinic from day 0 to day 14, and during hospitalization (day 21 till 28). Follow-up was at weeks 6 and 12, months 6, 9 and 12 and every 6 months thereafter.

PSA, blood count, serum hepatic enzymes and creatinine measurements were performed twice weekly from day 0 to day 21 and at each hospital visit thereafter.

2.7. Monitoring of antibody response

Monitoring of the antibody titer of Adenovirus specific type G (Ig G: Virion/Serion, Würzburg, Germany) and type A (Ig A: in-house developed enzyme-linked immunoassay) immunoglobulins against adenoviral virus was conducted in order to illustrate the presence of pre-existing antiviral antibodies, and the response of the immune system against an adenoviral challenge.

Neutralizing antibody titers were determined by pre-incubation of serial dilutions of sera with 100 TCID<sub>50</sub> adenovirus for one hour at 37 °C and subsequent inoculation on 293 cells. The virus neutralizing titer is defined as the reciprocal of the highest dilution of serum still giving 100% reduction of cytopathic changes.

2.8. T lymphocyte stimulation

Adenovirus-specific responses of peripheral blood mononuclear cells (PBMC) following adenoviral vector stimulation were evaluated. The PBMC isolation was performed through density centrifugation of heparinised blood samples using lymphocyte separation medium (ICN, Biomedicals Inc.). PBMC were washed in PBS, frozen in RPMI 1640 with 25 mmol/l HEPES and L-Glutamine (BioWhittaker) containing 10% fetal calf serum (BioWhittaker) and 10% DMSO (Sigma) and stored at minus 135 °C. For each time point, one hundred μl containing 1 × 10<sup>5</sup> PBMC were seeded in each well of 96 well plates (Costar) and grown in RPMI 1640 with 25 mmol/l HEPES and L-Glutamine containing heat-inactivated human serum (provided by the Erasmus MC blood bank). On the following day, cells were stimulated by Adv-HSV-tk added at a multiplicity of infection (MOI) of

![Fig. 1. Serum PSA response during the gene therapy protocol (A) and follow up (B). The insert (A) shows PSA levels between day 0 (day of entry protocol) and day 21 (day of radical prostatectomy). Patients A-D were treated with a low dose of Adv-HSV-tk (\(2 \times 10^{10}\) vp) and patients E-H were treated with a higher dose of Adv-HSV-tk (\(2 \times 10^{11}\) vp).](image-url)

### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>PSA (ng/ml)</th>
<th>Clinical stage</th>
<th>Gleason score (Biopsy)</th>
<th>Pathological stage (Specimen)</th>
<th>Gleason score (Specimen)</th>
<th>Viral culture</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low dose of vector ((2 \times 10^{10}) vp)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>62</td>
<td>21</td>
<td>T2a</td>
<td>3 + 4</td>
<td>pT2c</td>
<td>3 + 4</td>
<td>Negative</td>
<td>Grade3 (Cytolysis)</td>
</tr>
<tr>
<td>B</td>
<td>66</td>
<td>22</td>
<td>T2a</td>
<td>3 + 3</td>
<td>pT2a</td>
<td>3 + 3</td>
<td>Negative</td>
<td>No</td>
</tr>
<tr>
<td>C</td>
<td>48</td>
<td>12</td>
<td>T2c</td>
<td>3 + 4</td>
<td>pT2c</td>
<td>3 + 4</td>
<td>Negative</td>
<td>No</td>
</tr>
<tr>
<td>D</td>
<td>58</td>
<td>4</td>
<td>T3a</td>
<td>4 + 3</td>
<td>pT3a</td>
<td>4 + 3</td>
<td>Negative</td>
<td>Grade 1 (Low platelets)</td>
</tr>
<tr>
<td>High dose of vector ((2 \times 10^{11}) vp)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>62</td>
<td>10</td>
<td>T2b</td>
<td>3 + 5</td>
<td>pT3c</td>
<td>3 + 5</td>
<td>Negative</td>
<td>Gradel1 (Fever)</td>
</tr>
<tr>
<td>F</td>
<td>70</td>
<td>9</td>
<td>T2b</td>
<td>4 + 4</td>
<td>pT3c</td>
<td>4 + 4</td>
<td>Negative</td>
<td>Gradel1 (Fever)</td>
</tr>
<tr>
<td>G</td>
<td>56</td>
<td>5</td>
<td>T3a</td>
<td>3 + 3</td>
<td>pT3a</td>
<td>3 + 4</td>
<td>Negative</td>
<td>Gradel1 (Fever)</td>
</tr>
<tr>
<td>H</td>
<td>62</td>
<td>17</td>
<td>T3c</td>
<td>2 + 3</td>
<td>pT3c</td>
<td>3 + 5</td>
<td>Negative</td>
<td>Gradel1 (Fever)</td>
</tr>
</tbody>
</table>

The pre-operative PSA level is indicated. The stage and grade of the prostate adenocarcinoma were determined on pre-operative material obtained through biopsy and on prostate specimens obtained after surgery. For gene therapy-related adverse events, the type of event is indicated between brackets.
5000. As a positive control, inactivated Influenza virus antigen (Pasteur-Merieux) was used. Treatment with medium only was considered as a negative control (mock stimulation). Twenty microliters of the supernatant of each well were collected on day 6 and analyzed with an IFN-γ ELISA (U-Cytech) according to the manufacturers protocol. IFN-γ production levels were normalized for production levels after mock stimulation and presented as a percentage of the production at day 0. To the remaining cells at day 6, 3H-thymidine was added to determine proliferation. Incorporation of 3H-thymidine by PBMC was measured on a radioactivity counter and presented as stimulation indexes (ratio of PBMC proliferation after mock stimulation).

2.9. Prostate specimen evaluation
The prostate specimens were gross examined, weight and inked. They were then sliced according to routine procedures [15] and paraffin embedded. A total of 36 slides were evaluated per prostate. Each cancer was staged according to 1992 TNM classification and was graded according to Gleason grade. Four randomly chosen stage matched radical prostatectomy specimens from our in hospital series were used as a control.

Slides were stained with hematoxylin and eosin (H&S) and immunostained for B lymphocytes (mouse monoclonal anti-human CD20, Dako) and for T lymphocytes (mouse monoclonal anti-human CD3, Dako).

For each slide, CD3 positive T lymphocytes were counted in 10 randomly selected fields at 400 × magnification. CD20 positive B lymphocytes were analyzed as foci. A focus was defined as a group of more than 50 CD20 positive cells. B lymphocyte foci were counted in slides and reported as number of foci per cm² slide. Foci containing very large amounts of CD20 positive cells were counted as one focus.

![Graphs](image_url)

**Fig. 2.** Anti-Adv neutralizing (A, B), IgA (C, D) and IgG (E,F) levels after treatment with Adv-HSV-tk. Data are presented as titer (A,B) or a percentage of the pre-treatment level (day 0, C-F). Patients A–D (A, C, E) were treated with a low dose of Adv-HSV-tk (2 × 10¹⁰ vp) and patients E–H (B, D, F) were treated with a higher dose of Adv-HSV-tk (2 × 10¹¹ vp); legends as in Fig. 1.
3. Results

3.1. Patients

We included 8 patients in the study, 4 patients per dose group (Table 1). The median age was 60 years (48 to 70 years). Two patients had a history of coronary disease. One patient (F) had a Chronic Lymphoid Leukaemia that was stable (leucocytes $15000 \times 10^9/l$ with 76% lymphocytes) and therefore not considered as an immunosuppressive condition. During surgery, no metastatic lymph nodes were observed. None of the patients withdrew from the study and the median follow up was 14 months (6 to 22 months), 24 months for the low dose group and 6 months for the high dose group.

3.2. Safety considerations and adverse events

All daily viral cultures of urine and pharyngeal swabs as well as the cultures of prostatectomy specimen biopsies were negative. No patient developed urinary outflow obstruction. All 4 patients of the high dose vector group experienced clinical grade 1 toxicity with fever within the 24 hours following the viral injection (Table 1). The increase in body temperature never exceeded $39^\circ C$ and never lasted longer than 72 hours.

Two patients from the low dose vector group experienced biological grade 1 and 3 toxicity. Patient D had an asymptomatic grade 1 low platelet count ($112 \times 10^9/l$) that spontaneously returned to normal and allowed for treatment completion. Patient A had a temporary hepatic cytolysis (Alanine Amino Transferase 230 U/l and Aspartate Amino Transferase 127 U/l), which peaked grade 3 thirteen days after the first ganciclovir injection. This patient was treated continuously with simvastatin (hypercholesterolemia). One patient from the high vector dose, patient G, experienced a hematological grade 1 toxicity at day 14 to 21 with a drop to $2.5 \times 10^9/l$ in leucocytes count. The patient with Chronic Lymphoid Leukaemia completed the protocol only with a short spell of fever directly after the vector injection.

3.3. PSA response

During the first days after vector injection, no PSA increase was observed (Fig. 1A). One patient (E) showed a PSA doubling from 10 to 20 ng/ml at day 7. After prostatectomy six patients displayed a complete PSA response, while patient B and patient D experienced a biological relapse (Fig. 1B). Clinical examination of these patients, including digital rectal examination, revealed no abnormalities.

3.4. Anti-adenovirus neutralizing antibody response

The antibody response was evaluated by analysing adenovirus-specific neutralizing, IgA and IgG antibody titers (Fig. 2). In the low vector dose patient group, all patients demonstrated a clear boosting of adenovirus-specific neutralizing antibody responses, paralleled by IgG and IgA responses in most patients. In the high vector dose patients group, a neutralizing antibody response was evidenced in 3 out of 4 patients (Fig. 2B). No dose dependencies were observed.

3.5. Anti-adenovirus PBMC response

PBMC were obtained during the three weeks of therapy. The presence of Adenovirus-specific lymphocytes was assessed by determining proliferation and activation of PBMC. PBMC proliferation was determined after stimulation for six days with Adv-HSV-tk (Fig. 3). Only two patients displayed a specific adenovirus response before the start of therapy. Three patients in the low dose patients group (Fig. 3A), and all of the high dose group (Fig. 3B) displayed increased PBMC proliferation upon Adv-HSV-tk stimulation, which was vector dose independent.

Fig. 3. Stimulation index (SI) of PBMC isolated at several time-points during therapy. PBMC were stimulated for 6 days with 5000 MOI Adv-HSV-tk followed by determination of $^3$H-thymidine incorporation. Data are normalized for the proliferation index of the negative control (stimulation with human serum). Patients A-D (A) were treated with a low dose of Adv-HSV-tk ($2 \times 10^{10}$ vp) and patients E-H (B) were treated with a higher dose of Adv-HSV-tk ($2 \times 10^{11}$ vp); legends as in Fig. 1. DL = detection limit.
PBMC activation was determined by Adenovirus-specific IFN-γ production after six days of stimulation (Fig. 4). In the low vector dose group, two patients (B and D) showed increased IFN-γ production after stimulation. In the high dose group, more pronounced responses were observed in three patients (F, G and H), although a clear dose dependency could not be demonstrated. No significant changes in the production of IFN-γ production after Influenza stimulation were observed, indicating a specific boost of the adenovirus specific responses.

3.6. Prostate specimen evaluation

No specific necrosis or virus related cytopathic effect could microscopically be observed in any patient. Inflammation appeared to be moderate and diffuse on H&E counterstained slides. B lymphocytes infiltrated in a diffuse pattern (Fig. 5A) in a very limited number of patients but mostly as foci (Fig. 5B). The

![Graph showing IFN-γ production](image)

Fig. 4. IFN-γ production of PBMC isolated at day 0, day 7 and day 21. PBMC were stimulated for 6 days with 5000 MOI Adv-HSV-tk (tk) or 100 MOI Influenzae vaccine (i). Data are normalised for IFN-γ production of the negative control (stimulation with human serum) and expressed as a percentage of IFN-γ production at day 0. Patients A-D were treated with a low dose of Adv-HSV-tk (2 × 10¹⁰ vp) and patients E-H were treated with a higher dose of Adv-HSV-tk (2 × 10¹¹ vp).

![Illustration of lymphocyte infiltration](image)

Fig. 5. Illustration of B (A,B) and T (C,D) lymphocyte infiltration in tumoral (D) and non-tumoral (C) areas of prostate specimens after ADV-HSV-tk gene therapy (40× magnification). B lymphocytes and T lymphocytes were visualized by immunohistochemistry using monoclonal antibodies directed against CD20 and CD3, respectively.
of infiltrating T lymphocyte in the tumoral areas in the high dose patients group with an average count of 399.25 (199 to 484) versus 100.75 (0 to 216) in low dose and 165 (114 and 216) in controls.

4. Discussion

This study of neoadjuvant in situ suicide gene therapy for local prostate cancer was safe with minimal toxicity. The immune response observed appeared related to the intra-prostatic vector dose, but its cancer specificity is unknown. PSA responses, like seen in patients with recurrence after radiotherapy, were not obvious [11].

All cancers were of high-risk according to Kattan's nomograms [17], with a substantial tumor volume as estimated by the average of carcinoma positive slides of 12.75 (6 to 21) out of 36 examined per prostate specimen. Self resolving “flu-like” symptoms were modest, like in other clinical trials, and never peaked higher than grade 2 [16,18]. No severe cytolytic effects of viral infections, as observed after intra-arterial gene therapy [19], were seen. Platelets toxicity has been recognized as a secondary effect associated with replication-competent [20,21] but also with replication-deficient adenoviral vectors [11,22] especially in immuno-compromised patients [18], while leucopenia is a common feature in viral infection. In this trial, the biological adverse events were very mild.

Vector shedding has been a major safety issue previously. No positive viral cultures of blood or urine were observed, conform the literature [11,16,18,22,23]. The value of these safety procedures has become questionable [18].

The local and systemic effects of suicide gene therapy might be supported by a cell-mediated immune response of B and T lymphocytes [16]. The optimal vector dose for an adequate immune response is unknown. In this study, no cytolytic effects were seen on histology, in line with Hassan and colleagues [23]. Others have reported apoptosis and architectural changes [24], and vector dose dependent necrosis [22]. A titer of $10^{10}$ vp represents a limit under which no necrosis can be reasonably expected. In order to obtain a therapy-supporting anti-tumor immune response, necrotic areas in the treated tumor tissue might be obligatory.

A dose dependent increase in T and B lymphocytes in the whole prostate specimen and in tumor areas was observed, independent of the titer of adenoviral antibodies. Immune responses to viral infection (including the adenoviral backbone) are predominantly antibody
mediated. [25]. In our patients the overall Ab response was comparable among low and high vector dose, like in other clinical studies [16]. Anti-Adv Ab is not a major factor in the anti-tumor effect of suicide gene therapy, as no correlation has been evidenced between anti-Adv Ab titers (neutralizing Ab) and PSA response in this study and previously [20].

B lymphocytes infiltration of the prostate specimen was clearly much higher in the high dose vector group than in the low dose group. Also T lymphocytes infiltration was more intense in those patients, especially in the tumor areas. This is in line with former work [24,16,26]. Lymphocyte activity was demonstrated in addition by proliferation index and IFN-γ production of the circulating lymphocyte under specific stimulations at different time points.

5. Conclusion

Neo-adjuvant adenovirus-mediated cytotoxic (Adv-HSV-tk plus ganciclovir) gene therapy for prostate cancer is feasible and safe in an outpatient setting in intraprostatic vector doses up to $2 \times 10^{11}$ vp. Toxicity is minimal, while no infectious vector spread has been observed. Dose-dependent infiltrations of cytotoxic T lymphocytes and B lymphocytes in the whole prostate specimen and in tumor areas were observed, illustrating the activation of the immune system. Because of the absence of cytopathic effects in the prostate and the strong immune reactivity towards the adenovirus, application of higher vector doses may be considered.

Acknowledgements

This study was completed due to the financial support of the Dutch Cancer Society ‘Nederlandse Kankerbestrijding’ grant EUR 992124 and the Erasmus MC Revolving Fund. We thank Dr M.K. Brenner (Baylor College of Medicine, and Vector Laboratories, Houston, USA) for providing the clinical grade vector batch Adv-HSV-tk. Mrs Ebelien Mulder supported the clinical program as research nurse. The study contributed to the final report of the 5th Framework Programme ‘Prostate Cancer: Initiative for Gene Therapy’ GrantQLK6-CT-2000-00271 from the European Union.

References


Translational Research—From Lab to Clinic

The Distribution of Sensory Fibers Immunoreactive for the TRPV1 (Capsaicin) Receptor in the Human Prostate

Paulo Dinis a, Ana Charrua b, António Avelino b, Istvan Nagy c, José Quintas a, Ulisses Ribau a, Francisco Cruz a,b,*

a Department of Urology, Hospital S. João, Alameda Hernâni Monteiro, 4200-319 Porto, Portugal
b Institute of Histology and Embryology, Faculty of Medicine of Porto and IBMC of University of Porto, Alameda Hernâni Monteiro, Porto, Portugal
c Department of Anaesthetics and Intensive Care, Faculty of Medicine, Imperial College London, Chelsea and Westminster Hospital, 369 Fulham Road, London, UK

Accepted 14 January 2005
Available online 27 January 2005

Abstract

Objectives: To determine the distribution of sensory fibers immunoreactive to the pain receptor TRPV1 in the human prostate.

Methods: Eight prostates were harvested from cadaver transplant donors and immediately immersion fixed. Longitudinal and transverse 20 µm sections were cut on a cryostat and immunoreacted with two anti-human TRPV1 antibodies.

Results: TRPV1-immunoreactive nerve fibers were distributed throughout the prostatic urethral mucosa, verumontanum, ejaculatory ducts and periurethral prostatic acini. In the urethral mucosa, TRPV1-immunoreactive fibers penetrated the epithelial layer up to its surface. In the transitional and peripheral zones of the gland no TRPV1-immunoreactive nerve fibers were detected.

Conclusions: The existence of a rich TRPV1 sensory innervation in the human prostate may open new therapeutic perspectives for the treatment of pain in patients with chronic prostatitis (Chronic Pelvic Pain Syndrome).

# 2005 Elsevier B.V. All rights reserved.

Keywords: Sensory fibers; Prostate; Vanilloid receptor; Chronic pelvic pain syndrome

1. Introduction

Chronic Pelvic Pain Syndrome (CPPS), also known as chronic prostatitis, is an ill-defined, highly prevalent condition characterised by pelvic pain in the absence of a clear identifiable cause. In addition to pain, patients may complain of persistent voiding symptoms [1]. In the Prospective Chronic Prostatitis Cohort conducted in the USA, this condition was shown to severely impair the quality of life, with a negative impact on mental and physical domains that surpassed that of congestive heart failure or diabetes [2]. In spite of several clinical trials conducted to evaluate different forms of treatment, no standardised directives are established for CPPS [3,4]. This situation most probably reflects our lack of knowledge of the pathology. As pain is the most important characteristic of CPPS [5], it is surprising how little attention has been given to nociceptive primary afferents in this disease. These fibers are vital in the development and maintenance of pain, as they convey noxious input generated at their peripheral terminals into the spinal dorsal horn, the first relay station in pain sensation. During the last 20 years the introduction of vanilloids in the study of primary afferent fibers has led to the recognition of a particular subset of nociceptive fibers, called capsaicin sensitive primary afferents, which are important both in somatic and visceral pain [6]. More recently, a non-specific cationic channel expressed in rodent nociceptive
primary afferents was identified as the receptor for capsaicin sensitivity. Known as the capsaicin receptor or transient receptor potential subfamily vanilloid type 1 receptor (TRPV1) [7], this molecule appears to be essential for the generation of hyperalgesia occurring in rats with chronic skin [8] and bladder inflammation [9]. TRPV1 is also present in human nociceptive primary afferents [10]. The human TRPV1 has 839 amino acids with 92% homology with the rodent equivalent. The highest homology occurs in the region of the transmembrane domains and phosphorylation sites, whereas the lowest occurs in the N- and C-terminal sequences [11]. The association between TRPV1 and human pain perception has been recently supported by the demonstration of its up-regulation in chronic painful conditions such as Crohn’s disease, ulcerative colitis [12], irritable bowel syndrome [13], and vulvodynia [14]. TRPV1 is activated by several noxious physical and chemical stimuli including heat above 43°C, pH below 6, anandamide [16], and inflammatory mediators such as eicosanoids and leukotrienes [7,15,17]. In addition, recent observations in mice suggest that TRPV1 may encode mechanical stimuli in the lower urinary tract [18].

The distribution of TRPV1 expressing nociceptive primary afferents in the human prostate has never been previously examined. Therefore, in the present study we studied the TRPV1 sensory innervation of the normal human prostate gland. Preliminary results have been presented in abstract form [19].

### 2. Materials and methods

With the permission from the Ethics Committee of the Hospital de S. João, the entire prostate gland was harvested from eight cadaver transplant organ donors. The glands were immediately immersion-fixed overnight in a mixture of 4% paraformaldehyde and 15% saturated picric acid in 0.1 M phosphate buffer, pH 7.2, at 4°C, followed by immersion in 30% sucrose in PBS for at least 24 hours. Twenty μm transverse or longitudinal sections were cut in a Microm cryostat. After air-drying, the sections were stored at −80°C until further processing.

After thawing, sections were thoroughly washed with PBS and put into PBS containing 0.3% hydrogen peroxide for 30 minutes to inhibit endogenous peroxidase activity. Sections were successively incubated in 10% normal swine serum for 2 hours, in TRPV1 antibodies (see below) for 48 h at 4°C, in biotinylated swine anti-rabbit antiserum (Dakopatts, 1:400) for 1 hour at room temperature and in avidin-biotin complex solution (Vector Elite ABC-HRP, 1:400) for another hour at room temperature. The immunoreaction was visualized by the diaminobenzidine tetrahydrochloride (DAB) reaction. Sections were cleared in xylene and mounted in plastic medium (Eukitt). For immunofluorescent staining the sections were incubated in 10% normal swine serum for 2 hours, in TRPV1 antibodies for 48 h at 4°C, in AlexaFluor™488 donkey anti-rabbit (Molecular Probes) 1:1000 for one hour, after which they were mounted in Slowfade (Molecular Probes) and examined in a Biorad 1024 confocal microscope. Antibodies were diluted in PBS containing 0.3% Triton-X (PBST) and 2% normal swine serum while the ABC complex was diluted in PBST. The specificity of the TRPV1 antibodies was verified by pre-absorption of the antibody with excess cognate peptide or substituting normal serum for the primary antibody. No fiber labeling was observed in these control experiments. In order to verify the nature of the TRPV1 immunoreactive fibers, double labelling was performed with the pan-neuronal marker PGP 9.5. Sections were incubated in a mixture of TRPV1 and PGP 9.5 antibodies for 96 h at 4°C. The immunoreactions were visualized by Alexa Fluor™488-conjugated goat anti-rabbit and Alexa Fluor 568-conjugated goat anti-mouse IgG (Molecular Probes, 1:1000), after which they were mounted in Slowfade (Molecular Probes) and examined in a Biorad 1024 confocal microscope. Controls for possible cross reactivity between the fluorescent reagents were done by incubating the sections only in one of the primary antibodies, followed by incubation in a mixture of the secondary antibodies. No cross reactivity was observed. Since the two fluorochromes used have a small degree of overlap in their emission spectra, image acquisition was made in sequential mode to exclude the possibility of color bleed from one channel to the other.

Two affinity purified anti-human TRPV1 polyclonal antibodies were used. One, custom made by Affinity Bioreagents (U.S.A.) (PA1-748), was raised in rabbit and directed against amino acid residues 7–21 of human TRPV1 protein, and was used at a dilution of 1:1000. The other antibody was also raised in rabbit. It was a kind gift from GlaxoSmithKline (C22), directed against a synthetic peptide sequence of human TRPV1 and used at 1:10000 [14,15]. It has been previously characterized by Smith et al. [20]. The anti-TRPV1 antibody was raised in guinea-pig. It was supplied by Neumonics (U.S.A.) and used at a dilution of 1:1000.

### 3. Results

TRPV1-immunoreactive (IR) nerve fibers were found in the prostatic urethral mucosa, verumontanum, ejaculatory ducts and periurethral prostatic acini (Figs. 1 and 2). In the urethral mucosa, TRPV1-IR fibers were present immediately underneath the epithelial cells and penetrated the epithelial layer up to the urethral lumen (Fig. 1A and B). They exhibited numerous varicosities and formed patchy networks within the epithelium (Fig. 1A and B). Under the confocal microscope, double immunostaining revealed that all the TRPV1-IR fibers had positive PGP 9.5-IR, whereas the opposite did not occur (Fig. 1B and C). In nerve trunks coursing the prostatic stroma, TRPV1-IR could also be observed, constituting a subset of PGP 9.5-IR fibers (Fig. 1D and E). In the verumontanum, abundant TRPV1-IR fibers were found within the epithelium and in the lamina propria (Fig. 2A). The ejaculatory ducts exhibited a particularly dense network of TRPV1-IR fibers (Fig. 2B and C). Periurethral acini were also innervated by TRPV1-IR fibers (Fig. 2D). A complete absence of TRPV1-IR fibers was observed in acini...
located in the transitional and peripheral zones of the gland (Fig. 2E).

Little DAB precipitate was found in epithelial cells of the prostatic urethra, ducts or epithelial cells lining the prostatic acini with the Affinity antibody (Figs. 1A and 2A–E). The intensity of the nerve fiber immunolabelling was similar with both anti TRPV1 antibodies (Fig. 2B and C), but upon visual inspection the DAB precipitate was almost absent in epithelial cells of sections incubated with the GSK antibody (Fig. 2C). In sections in which the primary antibodies were omitted a faint DAB-produced brownish colouring of the urothelial and acinar cells could still be observed (data not shown). No immunofluorescent staining could be observed in urothelial cells (Fig. 1B).

No other TRPV1-IR structures were identified in the prostate gland using the DAB or immunofluorescent methods in any of the eight prostate specimens.

4. Discussion

Our findings show the existence of a rich TRPV1-IR nerve fiber network in the human prostate. Immunoreactive fibers were especially numerous in the subepithelial layer of the periurethral zone, in the verumontanum, in the terminal portion of the ejaculatory ducts and around glandular acini in the vicinity of the urethra. In contrast, TRPV1-IR fibers were absent in the transitional and peripheral zones of the prostate gland.

The occurrence of TRPV1 nerve fiber immunoreactivity in the human urinary tract had been described first in the bladder [21]. Like us, the authors described a network of TRPV1-IR nerve fibers especially dense in the sub-urothelial layer [21]. In addition to TRPV1-IR fibers, other studies found TRPV1 expression in bladder urothelial cells of both humans [22] and rodents [23]. As urothelial cells lining the bladder and the prostatic urethra are very alike, we paid particular attention in the present study to any TRPV1-immunoreactivity in epithelial cells lining the prostatic urethra. An increased staining of urothelium and acinar cells was found in sections in which TRPV1 immunoreactivity was revealed by the DAB method. Although it could be tempting to conclude that this finding indicates the presence of TRPV1 receptors, two other findings in the present study refrain us from doing so. First, a similar brownish colour could also be observed in urothelial and acinar cells in sections in which the primary antibody was omitted, suggesting

Fig. 1. Expression of TRPV1 immunoreactivity in the prostate. (Affinity antibody). Thin varicose fibers can be seen in the lamina propria and penetrating the urothelium in longitudinal sections of the ventral prostatic urethra, either using the ABC (A), or immunofluorescent (B) methods. Double immunostaining for TRPV1 and PGP 9.5 show that all the TRPV1-IR fibers are positive for PGP 9.5 but the opposite does not occur (note the fibers indicated by arrowheads in (C). TRPV1-IR fibers (D) can also be seen in PGP 9.5-IR (E) nerve trunks coursing in the stroma. Magnification bars: (A, B, C), 100 μm; (D, E), 250 μm.
that it was due to non-specific DAB deposits. Second, under the confocal microscope, no urothelial staining was found in sections immunoreacted for TRPV1. There have been previous studies of TRPV1 expression in the human bladder, in which negative immunoreactivity in the urothelial cells was reported [21]. The explanation for these conflicting results may lie in the affinity of the antibodies used. Yangou and co-workers used a non-commercial antibody raised against the [21] human TRPV1 whereas Lazzeri and co-workers [22] used a commercial anti-human TRPV1 antibody. The amino-acid sequences used to raise these two antibodies were different [21,22]. Nevertheless, demonstration of the functionality of TRPV1 is more important than demonstration of its expression in human urothelium. Recently, Birder and co-workers have indeed shown that TRPV1 agonists such as capsaicin and resiniferatoxin induce calcium inflow and nitric oxide release in urothelial cells of rodent bladders, and that these effects could be blocked by the specific TRPV1 antagonist capzasepine [23]. Similar results in human bladder urothelium have also been reported, however detailed information on these findings is still not available [24]. Since our aim was to describe the distribution of TRPV1-IR fibers in the human prostate, we have not attempted to study the TRPV1-mediated responses of prostatic urothelial cells.

While a previous report claimed that TRPV1 is expressed in interstitial cells in radical and suprapubic prostatectomy specimens, it failed to identify any TRPV1-IR nerve fibers [25]. The reasons for the discrepancy between Van der Aa et al.’s [25] and the present findings may lie in the different material, techniques and antibodies used. We looked at whole prostate specimens and found immunoreactivity in the peri-urethral zone while no labelling was detected in the transitional and peripheral zones. Van der Aa and coworkers [25] limited their observation to the two latter zones [25]. In addition, the antibody they claimed to recognise TRPV1 was in fact raised against the vanilloid receptor like protein 1, presently denominated as TRPV2, which is a high threshold temperature sensor that is not sensitive to capsaicin [26]. To clarify the nature of TRPV1-IR structures, in the present study we performed double staining with the pan-neuronal marker PGP 9.5. Our results show that all the TRPV1-IR structures also express PGP 9.5, confirming their neuronal origin, that could already be expected in view of their morphology.

In humans, TRPV1 activation in the lower urinary tract results in burning pain sensation [27]. Interestingly this is the main description of pain in patients with CPPS, either upon urination or ejaculation [5].

Fig. 2. TRPV1 immunoreactivity (Affinity antibody) in the verumontanum (A). Immunoreactive fibers are present beneath and within the urothelial folds and periurethral acini. The ejaculatory ducts show a particularly rich network of varicose fibers using either the Affinity (B) or GSK antibody (C). Periurethral acini (D) exhibit abundant immunoreactive fibers, in contrast with the acini located in the periphery of the gland (E). Magnification bars: (A) 400 µm; (B, C), 200 µm; (D, E), 100 µm.
addition, TRPV1 is activated by ethanol [28] and pain sensation in CPPS patients is frequently enhanced after consumption of alcoholic beverages. Furthermore, studies in CPPS patients frequently show the presence of inflammation [29], the accompanying increased proton concentration of which may also enhance the response of TRPV1 prostatic receptors to other ligands, and decrease their temperature threshold down to body temperatures [15]. Interestingly, the pH in the prostate is much lower than in other human tissues [30], suggesting that in normal conditions TRPV1 receptors in the prostate may have lower heat threshold than in other tissues. CPPS patients have increased perineal heat sensation [31]. Finally, nerve growth factor (NGF) a neurotrophic molecule known to be increased in the semen of patients with CPPS [32], has been demonstrated to increase TRPV1 expression in primary afferents [33] and release the receptor from a tonic inhibitory control [34].

The rich TRPV1 sensory innervation in the human prostate found in the present study together with the finding that activation of TRPV1 results in burning pain sensation suggest that TRPV1-expressing prostatic sensory nerves play an important role in the development of pain in CPPS patients. Additional studies designed to compare the expression of TRPV1 in nerve fibers of normal and CPPS prostates may therefore be worth pursuing, especially as a TRPV1 nerve fiber proliferation has already been shown to occur in other chronic painful conditions, like Crohn’s disease, ulcerative colitis [35,12], irritable bowel syndrome [13], and vulvodynia [14]. The fact that TRPV1 fibers are superficially localized in the prostatic urethra suggests that TRPV1 desensitization by topical application of vanilloid substances might be useful to decrease pain sensation. Such approach has been shown to be effective in reducing pain after mastectomy or herpes zoster infections [36,37].

Acknowledgements

The authors would like to thank Dr. John Davis, GSK R&D Ltd. for the gift of antibody C22.

References


Biological Age vs. Chronological Age

We read with interest the study by Michael Froehner and colleagues [1] regarding the co-morbidity measurements in patients who underwent radical prostatectomy. We would like to congratulate the authors and the editor Bratt [2] for their comments. It is a very timely paper in 2005 when we are diagnosing more and more early and surgically curable prostate cancer in elderly patients. We however feel the discussion of this subject is not complete and would like to add the following points.

In this study, the HR for both the co-morbid and overall mortality in the age group above 70 years is less than the HR in the age group 60–69 years if the patient belonged to the ASA 3 category. This has obviously led to the conclusion that the clinical applicability of the comorbidity measurement tools seems to be limited beyond the 70th year of life. It is difficult to know from the data presented how large was the group of patients more than 70 yrs old and with ASA 3 score. It appears the outcome data was influenced by the small size of this group.

ASA scoring system has been extensively used in perioperative setting to estimate the probability of survival after a surgical procedure but as far as we know it has not been validated for its reliability and usefulness to assess long term survival over 8–10 years. Also it suffers from the drawback that it is based on subjective clinical assessment while there are scoring systems like POSSUM and APACHE which are more objective tools and found more useful for mortality prediction after major surgery [3]. Variables such as postoperative complication [4] and local institutional skills and care do play a part in long-term outcome and will have to be included in drafting local guidelines for treatment of prostate cancers.

We would like to congratulate the the editor for the succinct remark on the importance of treating a real individual rather than an abstract average (age). Clearly it is the biological age of an individual rather than his chronological age which should determine the choice of a treatment. Intensive and persistent education of both health care providers and users will eventually change the traditional fatalistic attitude for the treatment of elderly population. We hope this paper, the editorial and our discussion would promote and inspire better methods of assessment of biological age/durability of an elderly individual.

References


Shiv Mohan Bhanot*
P. Naik
R. Gopalakrishnan
D.M. Salkar
K. Naqashabandi
King George Hospital, Urology, Barley Lane
Goodmayes, Essex IG3 8YB, UK
*Corresponding author
E-mail address: bhanotsm@hotmail.com
Available online 13 April 2005
doi:10.1016/j.eururo.2005.03.028


We appreciate the comment by Dr. Bhanot and colleagues on our article [1]. Indeed, the subgroup of patients with ASA class 3 and an age of 70 or more years was small in our study [1] (five patients with two deaths observed, one of them due to comorbidity and the other one due to a second cancer). Therefore, the subgroup analyses in the oldest age group should be considered with caution. More objective perioperative risk assessment tools like the APACHE II and POSSUM scores [2] are certainly worth to be studied.
in patients with early prostate cancer. The information necessary to assign these scores may, however, hardly be derived retrospectively from patient charts with sufficient accuracy. Prospective studies on the prognostic impact of comorbidity are of considerable clinical interest in this setting. Although such studies are difficult to perform due to the long follow-up needed (ten or more years), we hope that this gap in our knowledge will be closed in the future.

References


Michael Froehner*
Sven Oehlschlaeger
Oliver W. Hakenberg
Manfred P. Wirth
Rainer Koch
Rainer J. Litz
University Hospital
“Carl Gustav Carus”, Technical University of Dresden, Fetscherstrasse 74, D-01307 Dresden, Germany

*Corresponding author. E-mail address: michael.froehner@mailbox.tu-dresden.de

Available online 7 April 2005
doi:10.1016/j.eururo.2005.03.027


We read with interest the recently published article by Froehner et al. This study assessed the predictive value of several different comorbidity classifications in relation to survival following radical prostatectomy. Two important points arise in light of their findings. Firstly, there is currently no validated comorbidity measure with general applications across different specialities and age groups. Indeed this was further highlighted by Singh et al.’s comprehensive review of the subject [1]. Secondly, clinicians appear to be poor at assessing the impact of comorbid conditions on patient life expectancy. Those patients selected for radical prostatectomy in this study would have been assumed to have at least a 10-year life expectancy. However an overall mortality of 14% was observed over the 8-year time period, with 45% of these patients dying from comorbidity-specific causes.

How do we therefore improve life expectancy prediction? Currently decisions made in multidisciplinary team cancer meetings are based largely on clinical data, with information on comorbidity, if presented at all, often being incomplete. In addition it is unclear how well clinicians are able to translate factors such as family history, age and co-morbidity into an estimation of life expectancy. Consequently, this subjective assessment, which is frequently inaccurate, may result in patients being managed inappropriately [2].

One proposed system for improving clinical accuracy, is to combine actuarial and life insurance industry data (which is evidence-based and constantly updated in line with the medical literature) with those validated clinical comorbidity measures already in use. This would provide an evidence-based system for life expectancy prediction with applicability across a range of specialities and age groups. We are currently involved in designing a computer-based tool using such information, which it is hoped will be used initially in the multidisciplinary team setting to facilitate discussion regarding which treatment is most appropriate for an individual patient.

In a healthcare system driven by consumerism and with the introduction of revalidation, it has become increasingly important for clinicians to not only distribute resources appropriately, but also to justify their practice with a sound evidence base. Therefore with an improved understanding and more objective approach to life expectancy assessment, patients will undoubtedly be managed more appropriately.

Conflict of interest

None declared.

References


in patients with early prostate cancer. The information necessary to assign these scores may, however, hardly be derived retrospectively from patient charts with sufficient accuracy. Prospective studies on the prognostic impact of comorbidity are of considerable clinical interest in this setting. Although such studies are difficult to perform due to the long follow-up needed (ten or more years), we hope that this gap in our knowledge will be closed in the future.

References


Michael Froehner*
Sven Oehlschlager
Oliver W. Hakenberg
Manfred P. Wirth
Rainer Koch
Rainer J. Litz
University Hospital
“Carl Gustav Carus”, Technical University of Dresden, Fetscherstrasse 74, D-01307 Dresden, Germany
*Corresponding author. E-mail address: michael.froehner@mailbox.tu-dresden.de

Available online 7 April 2005
doi:10.1016/j.eururo.2005.03.027


We read with interest the recently published article by Froehner et al. This study assessed the predictive value of several different comorbidity classifications in relation to survival following radical prostatectomy. Two important points arise in light of their findings. Firstly, there is currently no validated comorbidity measure with general applications across different specialties and age groups. Indeed this was further highlighted by Singh et al.’s comprehensive review of the subject [1]. Secondly, clinicians appear to be poor at assessing the impact of comorbid conditions on patient life expectancy. Those patients selected for radical prostatectomy in this study would have been assumed to have at least a 10-year life expectancy. However an overall mortality of 14% was observed over the 8-year time period, with 45% of these patients dying from comorbidity-specific causes.

How do we therefore improve life expectancy prediction? Currently decisions made in multidisciplinary team cancer meetings are based largely on clinical data, with information on comorbidity, if presented at all, often being incomplete. In addition it is unclear how well clinicians are able to translate factors such as family history, age and co-morbidity into an estimation of life expectancy. Consequently, this subjective assessment, which is frequently inaccurate, may result in patients being managed inappropriately [2]. One proposed system for improving clinical accuracy, is to combine actuarial and life insurance industry data (which is evidence-based and constantly updated in line with the medical literature) with those validated clinical comorbidity measures already in use. This would provide an evidence-based system for life expectancy prediction with applicability across a range of specialties and age groups. We are currently involved in designing a computer-based tool using such information, which it is hoped will be used initially in the multidisciplinary team setting to facilitate discussion regarding which treatment is most appropriate for an individual patient.

In a healthcare system driven by consumerism and with the introduction of revalidation, it has become increasingly important for clinicians to not only distribute resources appropriately, but also to justify their practice with a sound evidence base. Therefore with an improved understanding and more objective approach to life expectancy assessment, patients will undoubtedly be managed more appropriately.

Conflict of interest

None declared.

References


Reply to M.G. Clarke, R. MacDonagh

We thank Dr. Clarke and Dr. MacDonagh for their comment on our article [1]. It is certainly true that there is currently no generally accepted comorbidity measure [2,3] and that the clinical estimation of the individual life expectancy is difficult. A computer-based life expectancy assessment incorporating actuary and life insurance data as well as validated comorbidity measures could be a promising step to increase the information available in counselling patients with early prostate cancer. The prediction of an individual patient’s destiny will, however, remain challenging particularly when ten or more years are to be considered and age exceeds the 70th year of life.

Yours sincerely,

References


Michael Froehner*
Sven Oehlschlaeger
Oliver W. Hakenberg
Manfred P. Wirth
Rainer Koch
Rainer J. Litz

University Hospital
“Carl Gustav Carus”, Technical University of Dresden, Fetscherstrasse 74
D-01307 Dresden, Germany
*Corresponding author
E-mail address: michael.froehner@mailbox.tu-dresden.de

Available online 20 April 2005
doi:10.1016/j.eururo.2005.04.003


Despite the large body of literature investigating different aspects of this issue (i.e. benefits, risks, clinical use), there seems to be no long-term strategy of testosterone-replacement. Should men be treated for two, three, five or ten years?

One may hypothesize that endogenous androgen levels are supressed by altering physiological feedback and production mechanisms and therefore symptoms associated with low testosterone levels are not only delayed but potentially worsened after short-time replacement therapy.

Regarding long-term treatment or even life-time treatment, we should strongly keep in mind and learn from the results of the “Woman’s health initiative study” [1], revealing after decades of accumulated clinical evidence, that the balance of risks and benefits for hormone use still remained uncertain.

In fact most of the discussed symptoms including loss of libido and decreased erectile function [2] are at least partly reversible by lifestyle modifications (physical activity, weight loss, dietary habits).

References

Reply to M.G. Clarke, R. MacDonagh

We thank Dr. Clarke and Dr. MacDonagh for their comment on our article [1]. It is certainly true that there is currently no generally accepted comorbidity measure [2,3] and that the clinical estimation of the individual life expectancy is difficult. A computer-based life expectancy assessment incorporating actuary and life insurance data as well as validated comorbidity measures could be a promising step to increase the information available in counselling patients with early prostate cancer. The prediction of an individual patient’s destiny will, however, remain challenging particularly when ten or more years are to be considered and age exceeds the 70th year of life.

Yours sincerely,

References


Michael Froehner*
Sven Oehlschlaeger
Oliver W. Hakenberg
Manfred P. Wirth
Rainer Koch
Rainer J. Litz

University Hospital
“Carl Gustav Carus”, Technical University of Dresden, Fetscherstrasse 74
D-01307 Dresden, Germany
*Corresponding author
E-mail address: michael.froehner@mailbox.tu-dresden.de

Available online 20 April 2005
doi:10.1016/j.eururo.2005.04.003
Anton Ponholzer*
Michael Rauchenwald
Stephan Madersbacher

Department of Urology and Andrology
Danube hospital SMZO, Langobardenstrasse 122
1220 Vienna, Austria
*Corresponding author

E-mail addresses: antonponholzer@hotmail.com
anton.ponholzer@wienkav.at

Available online 17 March 2005
doi:10.1016/j.eururo.2005.03.001

Reply to Anton Ponholzer, Michael Rauchenwald, Stephan Madersbacher

Ponholzer and co-workers present important thoughts concerning testosterone replacement therapy in elderly men. Their points are well taken and are in agreement with the policy of the authors. It is a matter of course that symptoms of androgen deficiency in many patients can successfully be treated by lifestyle modifications. However, there still remains a proportion of patients whose symptoms can only be relieved by elevating serum testosterone levels. We don’t know what Ponholzer et al. mean with “short-time replacement”, but we agree that treatment for e.g. 4 weeks or 3 months won’t improve the patient’s situation. The duration of therapy has not been defined yet.

The comparison between estrogen/gestagen replacement in women and testosterone therapy in men is inappropriate for various reasons:

- Hormone replacement in 60-year old women always leads to supraphysiological levels of serum hormones. This is not true in males, where testosterone production does not usually stop at a certain age.
- The biological effects (e.g. cardiovascular side effects) of estrogen are different from testosterone in both males and females.
- The interpretation of the WHI study is extremely difficult. It seems very hard to draw any evidence-based conclusion from this study for testosterone replacement in men.
- The WHI is under persistent scrutiny and its conclusions are been revised.
- Ponholzer et al should be aware that the Institute of Medicine Report (IOM)¹ regarding the issues involved in testosterone replacement promoted the development of a clinical trial similar to the WHI study. Until those results are available, treatment of late onset male hypogonadism should continue by experienced physicians following well publicized guidelines and recommendation from learned societies.

Again, we would like to underline that testosterone therapy is indicated for a limited proportion of elderly men with symptoms of hypogonadism. The IOM report and all guidelines available unambiguously indicate that testosterone administration for prevention of disease or for performance enhancement are clearly not indicated.

As with any therapy, side effects have to be weighed against benefits. But, there is no effective therapy for any disease without the possibility of side effects!

Thomas Ebert
Euromed Clinic, Urology, Europa-Allee 1
90763 Fuert, Germany
E-mail address: ebert@euromed.de

Available online 19 March 2005


Bryan and Chapple illustrate clearly the reproducibility of 3 day urinary frequency charts (FVC) in the majority of patients who complete them. Test-retest reliability is as important for FVCs as it is for any other clinical investigation. However, 8 out of 51 patients had a difference in their mean voided volume or their 24 hour frequency showing that urinary symptoms may indeed vary from day to day or over a period of days. In keeping with this we have found that many of our patients state that the tested period was not typical of their voiding pattern. This has prompted us to regularly ask patients if their completed chart is “normal for them” before placing too much weight on its interpretation. Those patients who describe day to day variability of symptoms are asked to indicate ‘good’ and ‘bad’ days over the charted period.

The reliability of this approach has not been tested but is similar to the practical way in which we use

Anton Ponholzer*  
Michael Rauchenwald  
Stephan Madersbacher  
Department of Urology and Andrology  
Danubehospital SMZO, Langobardenstrasse 122  
1220 Vienna, Austria  
*Corresponding author  
E-mail addresses: antonponholzer@hotmail.com  
anton.ponholzer@wienkav.at  
Available online 17 March 2005  
doi:10.1016/j.eururo.2005.03.001  

Reply to Anton Ponholzer, Michael Rauchenwald, Stephan Madersbacher  

Ponholzer and co-workers present important thoughts concerning testosterone replacement therapy in elderly men. Their points are well taken and are in agreement with the policy of the authors. It is a matter of course that symptoms of androgen deficiency in many patients can successfully be treated by lifestyle modifications. However, there still remains a proportion of patients whose symptoms can only be relieved by elevating serum testosterone levels. We don’t know what Ponholzer et al. mean with “short-time replacement”, but we agree that treatment for e.g. 4 weeks or 3 months won’t improve the patient’s situation. The duration of therapy has not been defined yet.

The comparison between estrogen/gestagen replacement in women and testosterone therapy in men is inappropriate for various reasons:

- Hormone replacement in 60-year old women always leads to supraphysiological levels of serum hormones. This is not true in males, where testosterone production does not usually stop at a certain age.
- The biological effects (e.g. cardiovascular side effects) of estrogen are different from testosterone in both males and females.
- The interpretation of the WHI study is extremely difficult. It seems very hard to draw any evidence-based conclusion from this study for testosterone replacement in men.
- The WHI is under persistent scrutiny and its conclusions are been revised.
- Ponholzer et al should be aware that the Institute of Medicine Report (IOM) regarding the issues involved in testosterone replacement promoted the development of a clinical trial similar to the WHI study. Until those results are available, treatment of late onset male hypogonadism should continue by experienced physicians following well publisized guidelines and recommendation from learned societies.

Again, we would like to underline that testosterone therapy is indicated for a limited proportion of elderly men with symptoms of hypogonadism. The IOM report and all guidelines available unambiguously indicate that testosterone administration for prevention of disease or for performance enhancement are clearly not indicated.

As with any therapy, side effects have to be weighed against benefits. But, there is no effective therapy for any disease without the possibility of side effects!

Thomas Ebert  
EuromedClinic, Urology, Europa-Allee 1  
90763 Furth, Germany  
E-mail address: ebert@euromed.de  
Available online 19 March 2005  


Bryan and Chapple illustrate clearly the reproducibility of 3 day urinary frequency charts (FVC) in the majority of patients who complete them. Test-retest reliability is as important for FVCs as it is for any other clinical investigation. However, 8 out of 51 patients had a difference in their mean voided volume or their 24 hour frequency showing that urinary symptoms may indeed vary from day to day or over a period of days. In keeping with this we have found that many of our patients state that the tested period was not typical of their voiding pattern. This has prompted us to regularly ask patients if their completed chart is “normal for them” before placing too much weight on its interpretation. Those patients who describe day to day variability of symptoms are asked to indicate ‘good’ and ‘bad’ days over the charted period.

The reliability of this approach has not been tested but is similar to the practical way in which we use...
Anton Ponholzer*
Michael Rauchenwald
Stephan Madersbacher

Department of Urology and Andrology
Danubehospital SMZO, Langobardenstrasse 122
1220 Vienna, Austria

*Corresponding author
E-mail addresses: antonponholzer@hotmail.com
anton.ponholzer@wienkav.at

Available online 17 March 2005
doi:10.1016/j.eururo.2005.03.001

Reply to Anton Ponholzer, Michael Rauchenwald, Stephan Madersbacher

Ponholzer and co-workers present important thoughts concerning testosterone replacement therapy in elderly men. Their points are well taken and are in agreement with the policy of the authors. It is a matter of course that symptoms of androgen deficiency in many patients can successfully be treated by lifestyle modifications. However, there still remains a proportion of patients whose symptoms can only be relieved by elevating serum testosterone levels. We don’t know what Ponholzer et al. mean with “short-time replacement”, but we agree that treatment for e.g. 4 weeks or 3 months won’t improve the patient’s situation. The duration of therapy has not been defined yet.

The comparison between estrogen/gestagen replacement in women and testosterone therapy in men is inappropriate for various reasons:

- Hormone replacement in 60-year old women always leads to supraphysiological levels of serum hormones. This is not true in males, where testosterone production does not usually stop at a certain age.
- The biological effects (e.g. cardiovascular side effects) of estrogen are different from testosterone in both males and females.
- The interpretation of the WHI study is extremely difficult. It seems very hard to draw any evidence-based conclusion from this study for testosterone replacement in men.
- The WHI is under persistent scrutiny and its conclusions have been revised.
- Ponholzer et al should be aware that the Institute of Medicine Report (IOM)1 regarding the issues involved in testosterone replacement promoted the development of a clinical trial similar to the WHI study. Until those results are available, treatment of late onset male hypogonadism should continue by experienced physicians following well publicized guidelines and recommendation from learned societies.

Again, we would like to underline that testosterone therapy is indicated for a limited proportion of elderly men with symptoms of hypogonadism. The IOM report and all guidelines available unambiguously indicate that testosterone administration for prevention of disease or for performance enhancement are clearly not indicated.

As with any therapy, side effects have to be weighed against benefits. But, there is no effective therapy for any disease without the possibility of side effects!

Thomas Ebert
EuromedClinic, Urology, Europa-Allee 1
90763 Fuertth, Germany
E-mail address: ebert@euromed.de

Available online 19 March 2005


Bryan and Chapple illustrate clearly the reproducibility of 3 day urinary frequency charts (FVC) in the majority of patients who complete them. Test-retest reliability is as important for FVCs as it is for any other clinical investigation. However, 8 out of 51 patients had a difference in their mean voided volume or their 24 hour frequency showing that urinary symptoms may indeed vary from day to day or over a period of days. In keeping with this we have found that many of our patients state that the tested period was not typical of their voiding pattern. This has prompted us to regularly ask patients if their completed chart is “normal for them” before placing too much weight on its interpretation. Those patients who describe day to day variability of symptoms are asked to indicate ‘good’ and ‘bad’ days over the charted period.

The reliability of this approach has not been tested but is similar to the practical way in which we use

urinary flow rates in the clinical setting—ie if possible, those with an unrepresentative flow rate are asked to repeat the test if it is felt to influence clinical management. The same reasoning should be applied to FVCs.

As the results of FVC analysis are regularly presented as an end point in trials of treatment of lower urinary tract problems, it is important that their format is standardised. In those that do have variability of their FVC results (and in particular the mean individual voided volume, as suggested by the authors) we need to decide whether we should take the ‘bad’ day(s) as the baseline before treatment or an overall average of all the days recorded. While taking an overall average may hide a significant response for the symptomatic days, conversely, selecting the bad days for analysis exaggerates it.

S.K. Govindaraju
D. Neilson
Department of Urology
Blackburn Royal Infirmary
East Lancashire NHS Trust, 55 Infirmary Road, Blackburn BB2 3LP, UK

Available online 25 February 2005

doi:10.1016/j.eururo.2005.01.019

Reply to S.K. Govindaraju, D. Neilson

Thank you very much for your comments. Certainly there is variability in test-retest reliability as evidenced by this data. It must be remembered however that 43 out of 51 patients had a very reproducible result. The approach which you have tested is certainly commonly used in real life clinical practice and we would strongly encourage you to carry out a controlled study to demonstrate whether your approach makes a significant difference to the clinical outcome of patients.

With regard to the use of frequency volume analysis in randomised clinical trials your comment emphasises the importance of placebo control and appropriate randomisation of patients which will allow for this factor.

Christopher R. Chapple
Urology Research, J Floor
Sheffield Teaching Hospitals NHS Trust
Royal Hallamshire Hospital
Glossop Road, Sheffield
South Yorkshire S10 2JF, UK
E-mail address: c.r.chapple@sheffield.ac.uk

Available online 8 March 2005

urinary flow rates in the clinical setting-ie if possible, those with an unrepresentative flow rate are asked to repeat the test if it is felt to influence clinical management. The same reasoning should be applied to FVCs. 

As the results of FVC analysis are regularly presented as an end point in trials of treatment of lower urinary tract problems, it is important that their format is standardised. In those that do have variability of their FVC results (and in particular the mean individual voided volume, as suggested by the authors) we need to decide whether we should take the ‘bad’ day(s) as the baseline before treatment or an overall average of all the days recorded. While taking an overall average may hide a significant response for the symptomatic days, conversely, selecting the bad days for analysis exaggerates it.

S.K. Govindaraju
D. Neilson
Department of Urology
Blackburn Royal Infirmary
East Lancashire NHS Trust, 55 Infirmary Road, Blackburn BB2 3LP, UK

Available online 25 February 2005

do:10.1016j.eururo.2005.01.019

Reply to S.K. Govindaraju, D. Neilson

Thank you very much for your comments. Certainly there is variability in test-retest reliability as evidenced by this data. It must be remembered however that 43 out of 51 patients had a very reproducible result. The approach which you have tested is certainly commonly used in real life clinical practice and we would strongly encourage you to carry out a controlled study to demonstrate whether your approach makes a significant difference to the clinical outcome of patients.

With regard to the use of frequency volume analysis in randomised clinical trials your comment emphasises the importance of placebo control and appropriate randomisation of patients which will allow for this factor.

Christopher R. Chapple
Urology Research, J Floor
Sheffield Teaching Hospitals NHS Trust
Royal Hallamshire Hospital
Glossop Road, Sheffield
South Yorkshire S10 2JF, UK
E-mail address: c.r.chapple@sheffield.ac.uk

Available online 8 March 2005

do:10.1016j.eururo.2005.01.020
### Congress Calendar

**European Urology 48 (2005) 173–177**

<table>
<thead>
<tr>
<th>Event Date</th>
<th>Event Name</th>
<th>Location</th>
<th>Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.6–1.7.2005</td>
<td>BAUS Annual Meeting</td>
<td>Glasgow, Scotland</td>
<td>E-Mail: <a href="mailto:admin@baus.org.uk">admin@baus.org.uk</a></td>
</tr>
<tr>
<td>3.7–8.2.2005</td>
<td>17th World Congress of Sexology</td>
<td>Montreal, QC</td>
<td>E-Mail: <a href="mailto:pierre.assalian@muhc.mcgill.ca">pierre.assalian@muhc.mcgill.ca</a></td>
</tr>
<tr>
<td>8.7–9.7.2005</td>
<td>Vaginal Surgery &amp; Dissection</td>
<td>Boston, Massachusetts USA</td>
<td>IMET 132 Kings Highway East Haddonfield, NJ 08033, USA Tel.: 800-690-IMET (4638) or +1 856-427-6200 Fax: +1 856-427-6202 Website: <a href="http://www.imetcme.com">www.imetcme.com</a></td>
</tr>
<tr>
<td>8.7–10.7.2005</td>
<td>Hands-on Urological Laparoscopy</td>
<td>Boston, Massachusetts USA</td>
<td>23rd World Congress on Endourology 2005 SWL 21st Basic Research Symposium</td>
</tr>
<tr>
<td>17.7–22.7.2005</td>
<td>Doubletree Hotel</td>
<td>Rockville, MD</td>
<td>35th Annual Meeting of the International Continence Society (ISC)</td>
</tr>
<tr>
<td>23.8–26.8.2005</td>
<td>39th Annual Dr. F.K. Mostofi Urological Pathology and Radiology Course</td>
<td>Amsterdam, Netherlands</td>
<td>1st International Conference on Urogenital Disorders: From Gene to Clinics</td>
</tr>
<tr>
<td>29.8–2.9.2005</td>
<td>EUREP III Residents Education Programme</td>
<td>Prague, Czech Republic</td>
<td></td>
</tr>
<tr>
<td>1.9–3.9.2005</td>
<td>6th Royan International Research Award – Congress (Reproductive Biomedicine)</td>
<td>Tehran, Iran</td>
<td></td>
</tr>
<tr>
<td>7.9–9.9.2005</td>
<td>3rd Urological Alpe-Adria Meeting</td>
<td>Rijeka, Opatia</td>
<td></td>
</tr>
<tr>
<td>10.9.2005</td>
<td>Contact: Armed Forces Institute of Pathology's Department of Medical Education Tel.: +1-202-782-2637 Fax:+1-202-782-5020 Email: <a href="mailto:sutton@afip.osd.mil">sutton@afip.osd.mil</a> Website: <a href="http://www.afip.org/Departments/edu/upcoming.htm">http://www.afip.org/Departments/edu/upcoming.htm</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.9–10.9.2005</td>
<td>EUREP III Residents Education Programme</td>
<td>Prague, Czech Republic</td>
<td></td>
</tr>
<tr>
<td>10.9.2005</td>
<td>3rd Urological Alpe-Adria Meeting</td>
<td>Rijeka, Opatia</td>
<td></td>
</tr>
</tbody>
</table>

**References**

doi:10.1016/S0302-2838(05)00344-1
<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
<th>Contact</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.9-17.9.2005</td>
<td>PSA : Past, Present and Future, First International Course</td>
<td>E-Mail: <a href="mailto:imm2005@colloquium.fr">imm2005@colloquium.fr</a></td>
<td>Milan, Italy</td>
</tr>
<tr>
<td>21.9-24.9.2005</td>
<td>Croatian Urological Association, 3rd Croatian Urological Congress</td>
<td>Contact: Congress office</td>
<td>Osijek, Croatia</td>
</tr>
<tr>
<td>21.9-24.9.2005</td>
<td>57th Annual Meeting of the German Urological Society</td>
<td>Contact: Scientific secretariat</td>
<td>Düsseldorf, Germany</td>
</tr>
<tr>
<td>24.9-27.9.2005</td>
<td>Arab Urological Congress</td>
<td>E-Mail: <a href="mailto:balroche@siu-urology.org">balroche@siu-urology.org</a></td>
<td>Tripoli, Lebanon</td>
</tr>
<tr>
<td>29.9-1.10.2005</td>
<td>SIU Meeting on Prostatic Disease: Recent Advances and New Technologies</td>
<td>E-Mail: <a href="mailto:simone.viertler@wcmh.info">simone.viertler@wcmh.info</a></td>
<td>Bariloche, Argentina</td>
</tr>
<tr>
<td>30.9-1.10.2005</td>
<td>4th Biennial World Congress on Men’s Health &amp; Gender</td>
<td>E-Mail: <a href="mailto:beate.ruloff@arcor.de">beate.ruloff@arcor.de</a></td>
<td>Vienna, Austria</td>
</tr>
<tr>
<td>7.10.2005</td>
<td>Focus on kidney disease (with live surgery)</td>
<td>E-Mail: <a href="mailto:urologie@med.uni-marburg.de">urologie@med.uni-marburg.de</a></td>
<td>Marburg, Germany</td>
</tr>
<tr>
<td>7.10-8.10.2005</td>
<td>5th Central European Meeting</td>
<td>E-Mail: <a href="mailto:info@congressconsultants.com">info@congressconsultants.com</a></td>
<td>Budapest, Hungary</td>
</tr>
<tr>
<td>8.10-9.10.2005</td>
<td>4th Meeting of the European Society of Andrological Urology (ESAU)</td>
<td>E-Mail: <a href="mailto:info@congressconsultants.com">info@congressconsultants.com</a></td>
<td>Budapest, Hungary</td>
</tr>
</tbody>
</table>
10.10–11.10.2005  
**EAU Balkan Meeting**  
**Location:** Belgrade  
**Contact:** Congress Consultants  
Tel.: +31 26 3891 751  
Fax: +31 26 3891 752  
E-Mail: info@congressconsultants.com  
Website: www.uroweb.org.org

12.10–14.10.2005  
**Annual Meeting of the Czech Urological Society**  
**Location:** Prague  
**Contact:** Nucleus  
Tel.: +420 495 217 146  
Fax: +420 495 211 630  
E-mail: info@nucleus.cz  
Website: www.nucleus.cz

13.10–14.10.2005  
**32nd Munich Endourological Symposium with Nursing Seminar**  
**Location:** Munich  
**Contact:** Drs. R. Paul van Randenborgh, and H. Kübler  
Department of Urology, Technische Universität München  
Klinikum rechts der Isar, Ismaninger Str. 22  
D-81675 Munich, Germany  
Chairman: Prof. Dr. R. Hartung  
Tel.: +49 89 4140 2507  
Fax: +49 89 4140 2585  
E-Mail: mriu@lrz.tu-muenchen.de  
Website: http://www.mriu.de/kongresse

14.10–15.10.2005  
**15 years of oncologic laparoscopy in urology**  
**Location:** Paris  
**Contact:** Chairman: Prof. Guy Vallancien  
Tel.: +33 1 44 64 15 15  
Fax: +33 1 44 64 15 16  
E-Mail: imm2005@colloquium.fr

15.10–16.10.2005  
**ESPU Educational Committee Course with International Meeting on Paediatric Urology**  
**Location:** Prague  
**Contact:** Nucleus  
Tel.: +420 495 217 146  
Fax: +420 495 211 630  
E-Mail: info@nucleus.cz  
Website: www.nucleus.cz

20.10–22.10.2005  
**International Course on Urology: Madrid 2005**  
**Location:** Madrid  
**Contact:** Prof. Dr. R. Vela-Navarrete (Scientific Information)  
Tel.: +34 91 549 66 56  
Fax: +34 91 544 70 94  
E-Mail: rvela@fjd.es, rvelan@telefonica.net  
**Contact:** Alcandora (Registration)  
Tel.: +34 91 576 32 20  
Fax: +34 91 435 52 88

16.11–18.11.2005  
**Second International Urologic Workshop on Laparoscopy Congress website: www.urolap.com**  
**Location:** Madrid  
**Contact:** Scientific Secretariat:  
Urology Unit, Biomedical Research Foundation Hospital Universitario La Paz  
Paseo de la Castellana 261, E-28046 Madrid, Spain  
Tel.: +34 91 727 73 05  
E-Mail: martinez-pineiro@telefonica.net & cisnerosledo@telefonica.net  
**Contact:** Technical Secretariat:  
Unicongress  
Bárbara de Braganza, 12-3/’D, E-28004 Madrid, Spain  
Tel.: +34 91 310 43 76  
Fax: +34 91 310 43 76  
E-Mail: unicongmad@unicongress.com and urolap2005@urolap.com  
Website: www.unicongress.com

16.11–19.11.2005  
**99th Congress of the French Urological Association (AFU) Palais des Congrès, Paris**  
**Location:** Paris  
**Contact:** Colloquium – AFU 2005  
Tel.: +33 1 44 64 1515  
Fax: +33 1 44 64 15 16  
E-Mail: afu@colloquium.fr  
Website: www.urofrance.org
17.11–19.11.2005
Istanbul
Turkey
First World Congress on Hypospadias and Intersex Disorders

Contact: Sefef Etker, MD (ISHID Vice-President)
serefe@superonline.com
Secretariat: Cnidus PCO
Tel.: +90 212 291 19 06
Fax: +90 212 219 05 88
E-Mail: cnidus@cnidus.com
Website: www.ishid.org and www.hypospadias-intersex.org

1.12–3.12.2005
Punta del Este
Uruguay
VIII Congress of the Latinamerican Society for Sexual
and Impotence Research

Contact: Tel.: (+54 11) 4325 1273 (1290)
Fax: (+54 11) 4326 8577
E-Mail: info@slais2005.org
Website: www.slais2005.org

4.12–7.12.2005
Copenhagen
Denmark
8th Congress of the European Society for Sexual Medicine (ESSM)

Contact: Tel.: +39 (0)27004 8577
Fax: +39 (0)27004 8577
E-Mail: admin@essm.org
Website: www.essm.org

Santa Cruz de Tenerife
Spain
4th European Urological Winter Escape Meeting

Contact: ESU Office
Tel.: +31 26 3890 680
Fax: +31 26 3890 686
E-Mail: esu@uroweb.org
Website: www.uroweb.org

Urology Calendar for 2006

Brussels
Belgium
3rd Meeting of the European Society of Oncological Urology (ESOU)

Contact: Congress Consultants
Tel.: +31 26 3890 680
Fax: +31 26 3890 674
E-Mail: info@congressconsultants.com
Website: www.uroweb.org.org

13.4–16.4.2006
Antalya
Turkey
8th Mediterranean Video-Endoscopic Urology and European Society of Urological Technology (ESUT) Workshop

Contact: Scientific Secretariat:
Assoc. Prof. Dr. Tibet Erdogru
Tel.: +90 242 2274326
Fax: +90 242 2274326
E-Mail: terdogru@akdeniz.edu.tr
Contact: Congress Organizing Bureau:
SymCon Tourism
Tel.: +90 216 3473535
Fax: +90 216 3477850
E-Mail: bilhan@symcon.com.tr
Website: www.urovizyon.org

25.1–27.1.2006
Herlev (Copenhagen)
Denmark
14th Copenhagen Symposium on Endoscopic Urological Surgery (in collaboration with the ESUT)

Contact: Secretary Susanne Lenskjold,
Dept. of Urology
Tel.: +45 44 88 36 44
E-Mail: suslen01@herlevhosp.kbhams.dk
Website: www.seus2006.dk

5.4–8.4.2006
Paris
France
XXIst Congress of the European Association of Urology

Contact: Congress Consultants BV
Tel.: +31 26 3890 689
Fax: +31 26 3890 686
E-Mail: info@congressconsultants.com
Website: www.uroweb.org

20.5–25.5.2006
Atlanta, GA
USA
The American Urological Association Annual Meeting

Contact: Karen Goodall
E-Mail: Kgoodall@auanet.org

27.9–1.10.2006
Rhodes
Greece
18th Panhellenic Urological Congress
The Rodos Palace Hotel
Organizer: The Hellenic Urological Association

Contact: Tel.: +30 210 7223 126
Fax: +30 210 7245 959
E-mail: hua@huanet.gr
Website: www.huanet.gr
Announcement and rules C.E. Alken Prize

1. The C.E. Alken Foundation supports clinical and experimental research through an annual prize for outstanding scientific work.

2. The C.E. Alken Prize is awarded for the best unpublished scientific work in the field of urology and may be divided. The prize comprises a certificate and the sum of SFr. 10,000.

3. Manuscripts can be submitted in either English or German. Seven copies of the manuscript should be sent to the following address and marked for the attention of Mr. E. Hauser, Advocate:
   C.E. Alken Foundation
   Dr. F. Kellerhals & Partner
   Marktgasse 55
   CH-3011 Bern
   Switzerland

   The deadline for submission of manuscripts is 1 September 2005. Each paper has to be marked with a code word and must not include the name of the author. An additional sealed envelope (marked on the outside with the code word) has to be enclosed and must contain the following:
   - Curriculum vitae of the author (max. 3 pages)
   - Structured summary of the manuscript (aim of study, material/methods, results, conclusions)

4. The award winner will be determined by the Foundation Council, whose decision is final.

C.E. Alken Foundation Council
Announcement and rules C.E. Alken Prize

1. The C.E. Alken Foundation supports clinical and experimental research through an annual prize for outstanding scientific work.

2. The C.E. Alken Prize is awarded for the best unpublished scientific work in the field of urology and may be divided. The prize comprises a certificate and the sum of SFr. 10,000.

3. Manuscripts can be submitted in either English or German. Seven copies of the manuscript should be sent to the following address and marked for the attention of Mr. E. Hauser, Advocate:

   C.E. Alken Foundation
   Dr. F. Kellerhals & Partner
   Marktgasse 55
   CH-3011 Bern
   Switzerland

4. The award winner will be determined by the Foundation Council, whose decision is final.

C.E. Alken Foundation Council

The deadline for submission of manuscripts is 1 September 2005. Each paper has to be marked with a code word and must not include the name of the author. An additional sealed envelope (marked on the outside with the code word) has to be enclosed and must contain the following:

- Curriculum vitae of the author (max. 3 pages)
- Structured summary of the manuscript (aim of study, material/methods, results, conclusions)