EMJ EUROPEAN MEDICAL JOURNAL

EUROPEAN MEDICAL JOURNAL

ISSN 2397-6764 Vol 1.1 • January 2016 • emjreviews.com

CONTENTS

EUROPEAN MEDICAL JOURNAL

Editorial Board

European Medical Journal

EMJ EUROPEAN MEDICAL GROUP
Published by:

European Medical Journal
EMJ Vol 1.1
January 2016

The MedBIC,
Anglia Ruskin University,
Chelmsford, CM1 1SQ

Welcome

Welcome to the first edition of the *European Medical Journal* for 2016.

We have compiled an assortment of research from across medicine that includes original peer-reviewed articles, reviews from recent symposia, and feature articles from our esteemed editorial board.

We begin this journal with three features on the theme of 'How I Diagnose, How I Treat'. This includes a piece entitled 'How I Treat: Multiple Sclerosis?', and a feature which dissects the current guidelines in oncology and how evidence could be improved to advance treatment.

An insightful review of a symposium from 2015's Congress of the European Society for Paediatric Urology covers the most recent developments in nocturnal enuresis. This review summarises a selection of studies from the areas of sleep and genetics, and provides suggestions for digital patient care initiatives.

A number of innovative authors have provided peer-reviewed articles from a variety of medical fields. Bush and Nagakumar, for example, discuss the diagnosis of wheezing in young children and the requirement for more specific phenotyping criteria. Amodu et al. review the current state of exosomes containing genetic material as diagnostic and therapeutic tools in the management of pancreatic adenocarcinoma. Another review in the field of oncology from Sternberg and Tombal focusses on the use of hormonal therapies, such as androgen deprivation therapy, in the care of prostate cancer patients.

In our Editor's pick, Caliskan reviews the pathogenesis of various nephrological diseases with the spotlight on the role of the complement system and the use of complement inhibitor use. In addition, Avery and Stocks provide a comprehensive literature review within the field of urinary incontinence and its psychological effects, utilising 25 years of research.

We hope that you enjoy this edition of the *European Medical Journal* and that it provides you with the most up-to-date advances in your field of medicine. Our thanks go to the EMJ editorial board who have continued to deliver an exceptional service to us for this publication. We look forward to the research that will be presented in our publications as the year progresses, and wish you all the best with your continuing work.

Spencer Gore

Director, European Medical Journal

European Medical Journal is published four times a year.
For subscription details please visit www.emjreviews.com

Front cover and contents artwork: Stacey Rivers/EMJ

Ph+ CML

Bosulif®▼(bosutinib): In the sea of choice, anchor the appropriate TKI to your patient's profile

Bosulif® is indicated for the treatment of adult patients with chronic phase, accelerated phase, and blast phase Philadelphia chromosome-positive chronic myelogenous leukaemia previously treated with one or more tyrosine kinase inhibitor(s) and for whom imatinib, nilotinib and dasatinib are not considered appropriate treatment options.

Bosulif® was granted 'conditional approval' by the EMA. A confirmatory phase IV safety and efficacy study is underway and will be submitted to the EMA when possible.

This advert is intended to be viewed by healthcare professionals only.

Bosulif® ▼(bosutinib)
Prescribing Information:
Please refer to the Summary of Product Characteristics (SmPC) before prescribing Bosulif 100mg or 500mg film-coated tablets.

This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions. See section 4.8 of the SmPC for how to report adverse reactions.

Presentation: Film-coated tablet containing 100mg or 500mg bosutinib (as monohydrate). **Indications:** Bosutinib is indicated for the treatment of adult patients with chronic phase (CP), accelerated phase (AP), and blast phase (BP) Philadelphia chromosome positive chronic myelogenous leukaemia (Ph+ CML) previously treated with one or more tyrosine kinase inhibitor(s) and for whom imatinib, nilotinib and dasatinib are not considered appropriate treatment options. **Dosage:** Therapy should be initiated by a physician experienced in the diagnosis and the treatment of patients with CML. The recommended dose of bosutinib is 500mg taken orally once daily with food. In clinical trials, treatment with bosutinib continued until disease progression or until it was no longer tolerated by the patient. Dose escalation to 600mg once daily was allowed in the phase 2 clinical trial of adult patients with previously treated Ph+ CML who did not experience severe or persistent moderate adverse reactions, and who did not meet certain early efficacy criteria. For details of dose escalation and dose reduction guidelines for non-haematologic adverse reactions and for haematologic adverse reactions, refer to SmPC section 4.2. Patients with serum creatinine >1.5 x ULN were excluded from CML studies. Increasing exposure (AUC) in patients with moderate and severe renal impairment during studies was observed. For details of dosage in patients with moderate and severe renal impairment please refer to SmPC section 4.2. Caution should be exercised in patients with relevant cardiac disorders and in patients with recent or ongoing clinically significant gastrointestinal disorder (see section 4.4 of SmPC). No specific dose recommendation is necessary in the elderly (≥65 years). Since there is limited information in the elderly, caution should be exercised in these patients. The safety and efficacy of bosutinib in patients under 18 years of age has not been established. **Contraindications:** Hypersensitivity to the active substance or to any of the excipients. Hepatic impairment. **Special warnings and precautions for use:** Treatment with bosutinib is associated with elevations in serum transaminases (ALT, AST). Transaminase elevations generally occurred early in the course of treatment. Patients receiving bosutinib should have liver function tests prior to treatment initiation and monthly for the first 3 months of treatment, and as clinically indicated. Treatment with bosutinib is associated with diarrhoea and vomiting, therefore patients with recent or ongoing clinically significant gastrointestinal disorder should use this medicinal product with caution and only after a careful benefit-risk assessment. Patients with diarrhoea and vomiting should be managed using standard-of-care treatment, including an antidiarrhoeal or antiemetic medicinal product and/or fluid replacement. In addition, these events can also be managed by withholding bosutinib temporarily, dose reduction, and/or discontinuation of bosutinib (see SmPC sections 4.2 and 4.8). The antiemetic agent, domperidone, has the potential to increase QT interval prolongation and to induce "torsade de pointes"- arrhythmias; therefore, co-administration with domperidone should be avoided. It should only be used if other medicinal products are not efficacious. In these situations an individual benefit-risk assessment is mandatory and patients should be monitored for occurrence of QT prolongation. Treatment with bosutinib is associated with myelosuppression, defined as anaemia, neutropenia, and thrombocytopenia. Complete blood counts should be performed weekly for the first month and then monthly thereafter, or as clinically indicated. Treatment with bosutinib may be associated with fluid retention including pericardial effusion, pleural effusion and pulmonary oedema. Patients should be monitored and managed using standard-of-care treatment. Elevation in serum lipase has been observed. Caution is recommended in patients with previous history of pancreatitis. Bosutinib may predispose patients to bacterial, fungal, viral or protozoan infections.
Automated machine-read QTc prolongation without accompanying arrhythmia has been observed. Bosutinib should be administered with caution to patients who have a history of or predisposition for QTc prolongation, who have uncontrolled or significant cardiac disease including recent myocardial infarction, congestive heart failure, unstable angina or clinically significant bradycardia, or who are taking medicinal products that are known to prolong the QT interval. Monitoring for an effect on the QTc interval is advisable and a baseline ECG is recommended prior to initiating therapy with Bosutinib and as clinically indicated. Hypokalaemia or hypomagnesaemia must be corrected prior to bosutinib administration and should be monitored periodically during therapy. Treatment with bosutinib may result in a clinically significant decline in renal function in CML patients. A decline over time in estimated glomerular filtration rate (eGFR) has been observed in patients treated with bosutinib in clinical studies. It is important that renal function is assessed prior to treatment initiation and closely monitored during therapy with bosutinib, with particular attention in those patients who have preexisting renal compromise or in those patients exhibiting risk factors for renal dysfunction, including concomitant use of medicinal products with potential for nephrotoxicity, such as diuretics, ACE inhibitors, angiotensin receptor blockers and nonsteroidal anti-inflammatory drugs (NSAIDs). The concomitant use of bosutinib with strong or moderate CYP3A inhibitors/inducers should be avoided as an increase/decrease in bosutinib plasma concentration will occur. Grapefruit products, including grapefruit juice and other foods that are known to inhibit CYP3A should be avoided. **Drug interactions:** The concomitant use of bosutinib with with strong CYP3A inhibitors (including, but not limited to itraconazole, ketoconazole, posaconazole, voriconazole, clarithromycin, telithromycin, nefazodone, mibefradil, indinavir, lopinavir/ritonavir, nelfinavir, ritonavir, saquinavir, boceprevir, telaprevir, grapefruit products including grapefruit juice) or moderate CYP3A inhibitors (including, but not limited to fluconazole, ciprofloxacin, erythromycin, diltiazem, verapamil, amprenavir, atazanavir, darunavir/ritonavir, fosamprenavir, aprepitant, crizotinib, imatinib) should be avoided, as an increase in bosutinib plasma concentration will occur. Refer to section 4.5 of the SmPC for further details. If a strong or moderate CYP3A inhibitor must be administered during bosutinib treatment, an interruption of bosutinib therapy or a dose reduction in bosutinib should be considered. The concomitant use of bosutinib with strong CYP3A inducers (including, but not limited to carbamazepine, phenytoin, rifampicin, St. John's Wort), or moderate CYP3A inducers (including, but not limited to bosentan, efavirenz, etravirine, modafinil, nafcillin) should be avoided, as a decrease in bosutinib plasma concentration will occur. Caution should be exercised when administering bosutinib concomitantly with proton pump inhibitors (PPIs). Short-acting antacids should be considered as an alternative to PPIs and administration times of bosutinib and antacids should be separated (i.e. take bosutinib in the morning and antacids in the evening) whenever possible. Bosutinib should be used with caution in patients who have or may develop prolongation of QT, including those patients taking anti-arrhythmic medicinal products or other medicinal products that may lead to QT prolongation. Refer to sections 4.4 and 4.5 of the SmPC for further details. **Fertility, pregnancy and lactation:** Not recommended in pregnancy or whilst breast feeding. Bosutinib has the potential to impair reproductive function and fertility. **Driving and operating machinery:** Bosutinib has no or negligible influence on the ability to drive and use machines. **Undesirable effects:** Very common adverse events are: respiratory tract infection, thrombocytopenia, neutropenia, anaemia, leukopenia, decreased appetite, headache, cough, diarrhoea, vomiting, nausea, abdominal pain, alanine aminotransferase increased, aspartate aminotransferase increased, rash, arthralgia, pyrexia, oedema, fatigue. Commonly reported adverse events are: pneumonia, influenza, bronchitis, nasopharyngitis, febrile neutropenia, drug hypersensitivity, dehydration, hyperkalaemia, hypophosphataemia, dizziness, dysgeusia, pericardial effusion, electrocardiogram QT prolonged, hypertension, dyspnoea, pleural effusion, gastritis, hepatotoxicity, hepatic function abnormal, blood bilirubin increased, gamma-glutamyltransferase increased, urticaria, acne, pruritus, myalgia, back pain, renal failure, chest pain, pain, asthenia, lipase increased, blood creatinine increased, blood amylase increased, blood creatine phosphokinase increased. Refer to section 4.8 of the SmPC for further information on side effects, including description of selected adverse reactions. **Legal category:** POM. **Basic NHS price:** Bosulif 100mg, 28 tablets [EU/1/13/818/001] £859.17. Bosulif 500 mg, 28 tablets [EU/1/13/818/003] £3436.67. **Marketing authorisation holder:** Pfizer Ltd, Ramsgate Road, Sandwich, Kent, CT13 9NJ, UK.

Further information is available on request from: Medical Information at Pfizer Limited, Walton Oaks, Dorking Road, Tadworth, Surrey, KT20 7NS, UK. Tel: +44 (0) 1304 616161
Last revised: 11/2015
Ref: BO 6_0

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard. Adverse events should also be reported to Pfizer Medical Information on 01304 616161.

Bosulif® bosutinib tablets

BOS172 • Date of preparation: November 2015

Pfizer

Foreword

Prof Markus Peck-Radosavljevic

Professor of Medicine,

Klinikum Klagenfurt am Wörthersee, Austria

Dear Colleagues,

I am delighted to warmly welcome you to the very first edition of the *European Medical Journal*, a new namesake venture by EMJ that will provide you with a selection of important updates from a variety of medical fields. A tremendous amount of growth has been occurring over the last year in all areas of medicine, so it stands to reason that there is more to report on than ever. As research is increasingly carried out, great leaps and bounds have been made in transforming ideas into treatments. From innovative solutions in urology to new oncological therapies, I hope that these developments, presented to you in this eJournal, will inspire you and all medical professionals to keep researching, diagnosing, caring for patients, and doing everything in 2016 and beyond to build upon these medical successes.

> “As research is increasingly carried out, great leaps and bounds have been made in transforming ideas into treatments.”

The papers within this issue are a testament to the aforementioned accelerating medical progress. One such example includes an overview of the current techniques that are available to preserve human sperm. In the midst of increasing demand for assisted reproductive techniques, depending of course upon sperm and oocytes, this will prove useful both now and in the coming years. Another paper addresses the issue of the umbrella term ‘preschool wheezing’, which currently applies to children of a preschool age with respiratory problems. In an effort to correctly diagnose the extent of the child’s symptoms and stop the practice of over-treatment, the author proposes utilisation of the ‘Hargreave phenotyping’ method. These papers and many more will make the first edition of the *European Medical Journal* a fascinating read. I would like to thank all of the authors and my fellow editorial board members for the hard work they have put into this eJournal, and wish all of you the greatest pleasure in reading it.

Yours sincerely,

Markus Peck-Radosavljevic

Professor of Medicine, Chairman, Department of Gastroenterology and Hepatology, Endocrinology and Nephrology, Klinikum Klagenfurt am Wörthersee, Klagenfurt, Austria; Fellow of the Austrian College of Physicians; Member of the American Association for the Study of Liver Disease (AASLD), the European Association for the Study of the Liver (EASL), the Austrian Transplant Association, the Austrian Society for Infectious Diseases and Tropical Medicine (OEGIT), the Austrian Association for Gastroenterology and Hepatology, and the Austrian Society for Internal Medicine (ÖGIM); Past Secretary-General of EASL.

EMJ EUROPEAN MEDICAL JOURNAL

Subscribe for FREE!

Don't miss out on any of the recent developments in these therapeutic areas and many more.

Review of the European Cancer Congress 2015, held in Vienna, Austria, 25th-29th September 2015

Symposium Reviews:

Intelligent Application of Breast Cancer Trials Data in the Clinic

Incorporating PARP Inhibition in Cancer Therapy: Key Questions, Expert Answers

Cabozantinib Versus Everolimus in Patients with Advanced Renal Cell Carcinoma: Results of a Randomised Phase III Trial (METEOR)

Review of the European Respiratory Society International Congress 2015, held in Amsterdam, Netherlands, 26th–30th September 2015

Symposium Reviews:

Treating Inadequately Controlled Asthma: Exploring the Potential of Phenotype-Targeted Therapy

Inhaler Devices: The Past, the Present, and the Future

Therapy for Alpha-1 Antitrypsin Deficiency: The Evidence for Efficacy

Advances in Ambulatory Oxygen Workshop and Long-Term Oxygen Therapy in Real-Life Practice

Long-Term NIV in COPD – Who and When?

Review of the European Renal Association - European Dialysis and Transplant Association Congress held in London, UK, 28th-31st May 2015

Symposium Reviews:

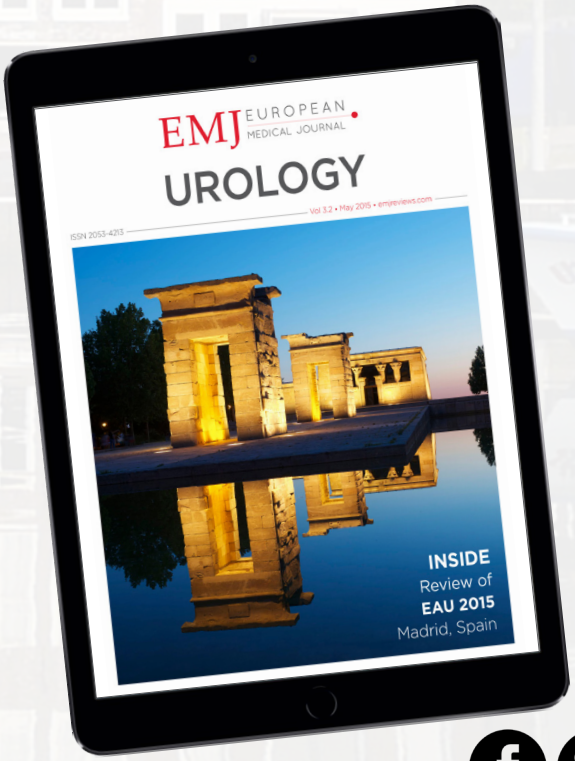
Supporting CKD Patients at Home

The Ongoing Management of Hyperkalaemia in Chronic Kidney Disease Patients: Cases for Clinical Decisions

Review of the 31st Annual Meeting of the European Society of Human Reproduction and Embryology, held in Lisbon, Portugal, 14th-17th June 2015

Best Abstract Award Winners at ESHRE 2015

Interviews with EMJ Reproductive Health Editorial Board



Review of the European Association of Urology Congress held in Madrid, Spain, 20th-24th March 2015

Symposium Reviews:

A New Approach for the Patient with Erectile Dysfunction

Navigating the mCRPC Landscape: Exploring Key Clinical Decision Points

Follow us:

www.emjreviews.com

EUROPEAN UNION
Investing in Your Future
European Regional Development Fund 2007-13

*László Vécsei

Albert Szent-Györgyi Clinical Center, University of Szeged, Szeged, Hungary
**Correspondence to vecsei.laszlo@med.u-szeged.hu*

Multiple sclerosis (MS) is a neurological disorder, characterised by inflammation and neurodegeneration. Though originally viewed as an illness of white matter of the central nervous system, advanced imaging methods have shown early and ongoing grey matter damage. Patients diagnosed with MS usually have several fluctuating and disabling symptoms (impaired mobility, fatigue, mood and cognitive alterations, pain, visual disturbances, etc.) with a significant influence on quality of life. Most MS patients have relapses and remission of the symptoms, particularly in the early stages of the disease. However, gradual progression independent of acute inflammatory attacks, known as progressive or degenerative changes, may take place early on and increase in incidence over time.

Table 1: 2014 RADS committee guidelines for multiple sclerosis (MS) treatment strategies.

			Drug choice				
Disease type	**Disease activity**		**1st**	**2nd**	**3rd**	**4th**	**5th**
Clinically isolated syndrome	Mild		Interferon beta 1-alpha*	Glatiramer acetate	Interferon beta 1b	-	
	Active		Interferon beta 1-alpha†	Glatiramer acetate	Interferon beta 1b	-	
RRMS	Mild-to-moderate		Teriflunomide	Interferon beta 1-alpha*	Glatiramer acetate	Interferon beta 1-alpha†	Interferon beta 1b
	Active	JC -ve	Natalizumab	Fingolimod	-		
		JC +ve	Fingolimod	No previous immunosuppressive treatment: Natalizumab for 12 (max. 24) months	-		
				Previous immunosuppressive treatment: Alemtuzumab	-		
	Breakthrough	JC -ve	Natalizumab	Fingolimod	-		
		JC +ve	Fingolimod	No previous immunosuppressive treatment: Natalizumab for 12 (max. 24) months	-		
				Previous immunosuppressive treatment: Alemtuzumab	-		
	With significant side effects on IFN-β or glatiramer acetate		Teriflunomide	-			
	With significant side effects on teriflunomide		Interferon beta 1-alpha*	Glatiramer acetate: Interferon beta 1-alpha†	Interferon beta 1b	-	
Secondary progressive MS	With superimposed relapses		Interferon beta 1-alpha†	Interferon beta 1b	-		
	With rapid disease progression but without superimposed relapses		Interferon beta 1b	-			

*Avonex, †Rebif.
RRMS: relapsing remitting multiple sclerosis; JC: John Cunningham virus antibody.

Table 2: 2015 RADS committee guidelines for multiple sclerosis (MS) treatment strategies.

			Drug choice				
Disease type	**Disease activity**		**1st**	**2nd**	**3rd**	**4th**	**5th**
Clinically isolated syndrome	All		Teriflunomide	Interferon beta 1-alpha*	Interferon beta 1-alpha†	Glatiramer acetate	Interferon beta 1b
RRMS	Mild-to-moderate		Teriflunomide	Dimethyl fumarate	Peginterferon beta-1a	Interferon beta 1-alpha*	Glatiramer acetate‡
	Active	JC -ve	Natalizumab	Fingolimod	-		
		JC +ve	Fingolimod	No previous immunosuppressive treatment: Natalizumab for 12 (max. 24) months	-		
				Previous immunosuppressive treatment: Alemtuzumab	-		
	Breakthrough	JC -ve	Natalizumab	Fingolimod	-		
		JC +ve	Fingolimod	No previous immunosuppressive treatment: Natalizumab	-		
				Previous immunosuppressive treatment: Alemtuzumab	-		
	With significant side effects on IFN-β or glatiramer acetate		Teriflunomide	-			
	With significant side effects on teriflunomide		Dimethyl fumarate	-			
	With significant side effects on teriflunomide and dimethyl fumarate		Peginterferon beta-1a	Interferon beta 1-alpha*	Glatiramer acetate	Interferon beta 1-alpha	Interferon beta 1b
Secondary progressive MS	With superimposed relapses		Interferon beta 1-alpha†	Interferon beta 1b	-		
	With rapid disease progression but without superimposed relapses		Interferon beta 1b	-			

Treatment of patients with clinically isolated syndrome should be offered if the following four criteria are met:
1) Other diagnoses are ruled out after relevant examinations
2) The severity of the relapse is such as to interfere with daily living
3) The requirement for dissemination in space according to the McDonald criteria are met
4) Oligoclonal band in cerebrospinal fluid
*Avonex; †Rebif.
‡ 6th and 7th line options are Interferon beta 1-alpha (Rebif) and Interferon beta 1b, respectively.
RRMS: relapsing remitting multiple sclerosis; JC: John Cunningham virus antibody.

There are several considerations related to the therapy of MS:

1. Early treatment is essential, since inflammation and degeneration occur early on in the disease (treatment should occur as soon as possible following the diagnosis of relapsing MS)
2. Patients showing early clinical symptoms together with magnetic resonance imaging results consistent with MS who are not treated have a high probability of further disease activity
3. Depression, fatigue, and cognitive impairment occur in the early stages of the disease

4. Treatment with the medication should be continued indefinitely with the following exceptions: sub-optimal treatment response, serious side effects, inadequate adherence to the treatment regimen, and availability of a more appropriate drug

To date, the following disease-modifying agents have been approved (and result in a relative decrease in annualised relapse rate compared with placebo):

- Self-injected agents: glatiramer acetate (29%), interferon beta 1-alpha (Avonex, 32%; Rebif, 18%), interferon beta 1b (34%)
- Oral agents: dimethyl fumarate (44–53%), fingolimod (54%), teriflunomide (31%)
- Intravenous agents: mitoxantrone (67%), natalizumab (68%), alemtuzumab (55%)

Concerning the treatment strategies of MS patients, the RADS Committee suggested the following guidelines in 2014 (Table 1) and an update in 2015 (Table 2), which we prefer to follow in our everyday clinical practice.

Factors affecting the choice of treatment at any point in the disease course are complex and most appropriately analysed collaboratively by the patient and his or her treating neurologist.

In the future, however, an interesting approach may be the combination of immunmodulatory agents with neuroprotective agents like LINGO-1. Further research is required for profiling patients; for example, research into T or B cell targeted approaches or susceptibility for specific infectious side effects.

FURTHER READING

1. Ingwersen J et al. Advances in and algorhitms for the treatment of relapsing-remitting multiple sclerosis. Neurotherapeutics. [Epub ahead of print].

2. MS Coalition. The Use of the Disease-Modifying Therapies in Multiple Sclerosis: Principles and Current Evidence. Available at: http://www.nationalmssociety.org/getmedia/5ca284d3-fc7c-4ba5-b005-ab537d495c3c/DMT_Consensus_MS_Coalition_color. Last accessed: 13 January 2016.

3. RADS Committee for the Disease Modifying Treatment of Multiple Sclerosis. Treatment guidelines, including product recommendations for the diseases modifying treatment of multiple sclerosis. 2014.

DO THE GUIDELINES IN ONCOLOGY NEED TO EVOLVE?

***Ahmad Awada, Gil Awada, Alain Hendlisz**

Jules Bordet Institute, Brussels, Belgium
**Correspondence to ahmad.awada@bordet.be*

Therapeutic guidelines in oncology, such as those from the National Comprehensive Cancer Network (NCCN) or the European Society for Medical Oncology and the European Cancer Organisation (ESMO/ECCO), are designed as tools intended to help oncologists to manage their patients according to the best available clinical data. In the metastatic setting, these guidelines are based upon individual clinical trials and/or meta-analyses, as well as upon the opinion of key experts. Nevertheless, these important tools are often not fully applicable in clinical practice because they suffer from many drawbacks. These drawbacks are not only related to the design of the clinical trials supporting these guidelines, but also to patients' characteristics and the studies' eligibility criteria, the evolution of tumour biology over time, and finally the mechanism of action of the therapeutic interventions. These issues will be detailed in the sections following.

Most of the available clinical trials in metastatic research involve first and second-line therapies but rarely much more. Consequently, beyond these therapeutic lines, the available guidelines are either not available, or based on scanty data or expert opinions. Moreover, most clinical trials have included a highly selected homogeneous population of patients with good performance status, who are compliant, have volunteered, and are able to understand the principles of clinical trials necessary to give a proper consent. Overall, these patients' profiles are not representative of the whole population of patients seen routinely in clinical practice. This means that the translation of published data to daily clinical practice can be potentially misleading. The patients who are seen in clinical practice are extremely different on various levels from those seen in clinical research, starting from age and performance status, and ending at the level of tumour extension and biology. Consequently, the tumour response, patient outcome, and treatment tolerability observed in reality may be very different and less positive from the results obtained from clinical trials. Additionally, in clinical research as well as in clinical practice, nothing is static but rather globally, every parameter, patient or disease-related, may quickly change and therefore guidelines agreed to at one time should be updated regularly.

Guidelines, as previously mentioned, are often based on Phase III clinical trials, but the landscape and methodology of clinical trials are changing rapidly. For example, the frontier between Phase I, II, and III has become less stringent. The drugs and therapeutic approaches to development are very variable ranging from cytotoxics, to the new formulations of cytotoxics (like antibody drug conjugates), to molecular-targeted agents, and finally to new immunotherapeutics. The clinical research for each of these categories might be different and the result obtained (ranging from outstanding to borderline clinical significance) will have different interpretations and powers and thus influence the strengths or limitations of the guidelines. Therefore, the guidelines should evolve in parallel to reflect this move in clinical research methodology and on the mechanisms of action of available therapies.

In conclusion, the guidelines currently cover a selection of patients who are largely unrelated to what is seen in clinical practice and do not take into account the evolution of tumour biology, the therapeutic classes under experimentation, and the need to adapt to rapidly evolving clinical research methodology. Future guidelines should not only include the lines of therapy but also important patient characteristics, tumour biology subtyping, the availability of biomarkers if any, and the presence or absence of companion diagnostics etc., with the ultimate aim of adequately taking into account real clinical practice.

DIAGNOSIS AND TREATMENT OF BLADDER CANCER: FROM ESTABLISHED PARADIGMS TO RECENT ADVANCES AND NEW PERSPECTIVES

***Riccardo Autorino**

Case Western Reserve University School of Medicine, Cleveland, Ohio, USA; Second University, Naples, Italy
**Correspondence to ricautor@gmail.com*

INTRODUCTION

Bladder cancer (BC) certainly represents a challenging disease for the patient, given the significant impact on health-related quality of life, and for the urologist, given the broad spectrum of clinical scenarios that can be presented. Epidemiology studies suggest that this is a common malignancy worldwide, with an estimated 330,400 new cases diagnosed and 123,100 attributable deaths in 2012.[1] In most cases, BC presents as non-invasive into the detrusor muscle, which initially and frequently allows bladder-sparing therapies. Nevertheless, non-muscle invasive BC (NMIBC: tumours staged as Ta, T1, or carcinoma *in situ*) tends to recur (≤80% of cases) and progress to muscle-invasive BC (MIBC) (≤30% of cases), so that close patient follow-up is always needed, as well as more aggressive treatment options.[2,3] In the setting of this quite complex clinical entity, currently available guidelines are extremely helpful to provide evidence-based guidance to the urologists in their daily practice. Four major evidence-based guidelines are available and most widely used, including those published by the American Urological Association (AUA),[2] the European Association of Urology (EAU),[3] the International Consultation on Urological Disease (ICUD),[4] and the National Comprehensive Cancer Network (NCCN).[5] Over the years, these guidelines have been regularly updated whenever evidence supporting the implementation of new diagnostic tools, technologies, and techniques have become available. However, from other points of view, one can reasonably argue that the management of BC patients has been based on the same pillars and concepts over the past two decades.

DIAGNOSIS

The first and most important step in the diagnostic protocol of BC is still represented by the cystoscopy, and subsequent histologic evaluation of the tissue obtained by transurethral resection (TUR). Refinements in technique and surgeon experience are critical for the performance of a thorough, complete, 'high-quality' TUR. Recent technological advances including bipolar electrocautery and regional anaesthetic techniques may help to reduce the risk of complications.[6] Advances in endoscopic imaging technology may improve sensitivity for the detection of BC and ultimately lead to improved cancer control. Fluorescence cystoscopy requires intravesical administration of a photosensitising agent (5-aminolevulinic acid or hexaminolevulinate) and imaging with a blue-light endoscopy system demonstrably improves the detection of papillary and flat bladder lesions compared with conventional white-light cystoscopy.[7] In my daily clinical practice I do prefer to use narrow band imaging (NBI) technology (Olympus, USA), which does not require the use of any dye or agent as it works by providing increased contrast between normal and abnormal tissue on the basis of neovascularity. Evidence shows that NBI is effective in the identification of abnormal lesions including carcinoma *in situ*, and it can provide higher diagnostic precision of BC than white light cystoscopy.[8] Other novel technologies such as optical coherence tomography and confocal laser endoscopy hold promise as useful tools in better characterising bladder tumours.[7] Whenever a TUR procedure is deemed to be 'incomplete' (no muscle is identified in the specimen), or when a high-grade tumour is detected, it is a well-established principle that a second TUR should be performed within 2–6 weeks.[9]

Besides endoscopy, another 'traditional' diagnostic tool is represented by urine cytology, which can be useful for the diagnosis of a high-grade tumour. On the other hand, several urine markers have been extensively studied to aid in the diagnosis of BC, and possibly decrease the need for cystoscopy.[10] However, none of these markers, despite having a higher sensitivity, demonstrated a higher specificity than simple urine cytology; this should be considered in addition to the cost associated with the use of these markers. Therefore, their use in daily clinical practice remains to be determined.

TREATMENT OF BLADDER CANCER

Non-Muscle Invasive Disease

The risks of both recurrence and progression of NMIBC can be estimated for individual patients by using the EORTC scoring system, which is a practical ready-to-use tool to plan further treatment. However, controversies exist regarding this and other available predictive tools.[11] A consolidated concept is that the use of intravesical chemotherapy administered immediately after TUR is likely to reduce the risk of recurrence in NMIBC.[12]

The stratification of patients into low, intermediate, and high-risk groups is pivotal to determining the adjuvant treatment:[3]

- For low-risk patients, one immediate instillation of chemotherapy is recommended
- For intermediate-risk cases, one immediate instillation of chemotherapy should be followed by 1 year of full-dose Bacillus Calmette-Guérin (BCG) intravesical immunotherapy or by further instillations of chemotherapy for a maximum of 1 year
- In patients with high-risk tumours, full-dose intravesical BCG for 1–3 years is indicated
- In tumours at highest risk of progression and those that are BCG-refractory, radical cystectomy (RC) should be considered

The administration of BCG immunotherapy has certainly become the standard of care for high-grade NMIBC and carcinoma *in situ* in terms of prevention of recurrence and progression. Nevertheless, despite its wide usage over the past two decades and the wide implementation of the standard 6-week induction course, the optimal duration of BCG treatment remains unknown, and should be the subject of further studies.[13]

All guidelines advocate the use of cystoscopy during follow-up of NMIBC:

- In the case of Ta tumours: the AUA2 and the ICUD4 panel do not make recommendations regarding the timing. The EAU3 recommend that patients should have cystoscopy at 3 months. If negative, subsequent cystoscopy is advised 9 months later and then yearly for 5 years (Grade C). No recommendations are made about cytology or imaging. The NCCN panel recommends cystoscopy at 3 months and then at "increasing intervals as appropriate."[5] Neither cytology nor upper tract imaging are advocated
- In the case of high-grade Ta, T1, and clinically isolated syndrome (CIS): the EAU recommends cystoscopy and urine cytology at 3 months. If negative, subsequent cystoscopy and cytology should be repeated every 3 months for 2 years, then every 6 months until 5 years, and then yearly. For CIS, the ICUD panel recommends cystoscopy and cytology every 3 months for 2 years, every 4 months in the 3rd year and every 6 months in the 4th and 5th years, and then yearly. Moreover, the ICUD does recommend "periodic" imaging of the upper urinary tract for patients with CIS. Similarly, the NCCN panel recommends cystoscopy and urine cytology every 3–6 months for 2 years, followed by increasing intervals "as appropriate", and they also recommend to consider upper tract imaging every 1–2 years for high-grade Ta, T1 tumours, and CIS

Muscle-Invasive Disease

When BC presents or progresses to muscle invasive disease, there is a major shift in the treatment paradigm, as a more aggressive approach is needed to optimise the oncological outcome. Neoadjuvant chemotherapy followed by RC with bilateral pelvic lymph-node dissection currently represents the standard of care for patients with MIBC.[14,15] Timely diagnosis and prompt surgical treatment are keys in the management of MIBC, as multiple studies have shown that a delay in diagnosis or treatment adversely impacts outcomes.[16]

A complete metastatic workup and medical evaluation considering age, nutrition, and performance status are necessary before starting treatment. For patients with T2 disease on TUR, staging studies of the chest, abdomen, and pelvis, including upper-tract evaluation, must be

performed to rule out metastases. Currently, the gold standard for staging in BC is computed tomography, although more recently multiparametric magnetic resonance imaging and positron-emission tomography imaging have shown promise.[17]

Over the past 5 years, there has been an ongoing discussion with regard to the best surgical approach for RC (open versus robotic), and the optimal extent of lymph-node dissection. With the increasing role of robotic surgery, many centres have adopted robot-assisted RC (RARC), and there has been growing evidence in the literature comparing open RC with RARC. In a recent systematic literature review, Novara et al.[18] found that RARC can be performed safely with acceptable perioperative outcome, although complications are common. Operative time was shorter with RC, whereas RARC may provide some advantages in terms of blood loss and transfusion rates and, more limitedly, for postoperative complication rates. In another recent systematic review, Yuh et al.[19] examined oncologic and functional outcomes following RARC from 87 studies, and they concluded that data remain immature. Their cumulative analyses demonstrated that lymph node yields and surgical margin rates are similar between RARC and open RC, but long-term survival outcomes for RARC are still limited. High volume robotic surgeons are also implementing intracorporeal urinary diversion following RARC,[20] and the debate in this field is still ongoing as data are maturing. Besides the surgical technique, innovations have also been implemented in the perioperative management of BC patients undergoing RC. Several enhanced recovery protocols have been developed with the aim of expediting bowel function recovery and shortening hospital stay after RC and urinary diversion.[21] Last but not least, the role of systemic chemotherapy represents another field of ongoing debate. Despite available evidence supporting its role as standard of care for MIBC, neoadjuvant chemotherapy remains underused, and strategies to fill this gap should be investigated.[22]

CONCLUSIONS

BC represents an exciting field of clinical research, as many questions remain unanswered, and diagnosis and treatment of the disease remain suboptimal. Recent innovations might pave the way for future paradigm shifts in the management of this very challenging multifaceted disease. As clinicians and investigators, it is our role to stay tuned and updated with the ultimate aim of providing the best care to our patients.

REFERENCES

1. Torre LA et al. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87-108.

2. Hall MC et al. Guideline for the Management of Nonmuscle Invasive Bladder Cancer (Stages Ta, T1, and Tis): 2007 Update. J Urol. 2007;178(6): 2314-30.

3. Babjuk M et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: Update 2013. Eur Urol. 2013;64(4):639-53.

4. Burger M et al. ICUD-EAU International Consultation on Bladder Cancer 2012: Non-muscle-invasive urothelial carcinoma of the bladder. Eur Urol. 2013;63(1): 36-44.

5. Clark PE et al. Bladder cancer. J Natl Compr Cancer Netw. 2013;11(4):446–75.

6. Richards KA et al. The importance of transurethral resection of bladder tumor in the management of nonmuscle invasive bladder cancer: a systematic review of novel technologies. J Urol. 2014;191(6):1655-64.

7. Lerner SP, Goh A. Novel endoscopic diagnosis for bladder cancer. Cancer. 2015;121(2):169-78.

8. Zheng C et al. Narrow band imaging diagnosis of bladder cancer: systematic review and meta-analysis. BJU Int. 2012;110(11 Pt B):E680-7.

9. Dalbagni G. Bladder cancer: Restaging TUR reduces recurrence and progression risk. Nat Rev Urol. 2010;7(12):649-50.

10. Xylinas E et al. Urine markers for detection and surveillance of bladder cancer. Urol Oncol. 2014;32(3):222-9.

11. Xylinas E et al. Accuracy of the EORTC risk tables and of the CUETO scoring model to predict outcomes in non-muscle-invasive urothelial carcinoma of the bladder. Br J Cancer. 2013;109(6):1460-6.

12. Perlis N et al. Immediate post-transurethral resection of bladder tumor intravesical chemotherapy prevents non-muscle-invasive bladder cancer recurrences: an updated meta-analysis on 2548 patients and quality-of-evidence review. Eur Urol. 2013;64(3):421-30.

13. Ehdaie B et al. Maintenance bacillus Calmette-Guérin treatment of non-muscle-invasive bladder cancer: a critical evaluation of the evidence. Eur Urol. 2013;64(4):579-85.

14. Gakis G et al. ICUD-EAU International Consultation on Bladder Cancer 2012: Radical cystectomy and bladder preservation for muscle-invasive urothelial carcinoma of the bladder. Eur Urol. 2013;63(1):45-57.

15. Sternberg CN et al. ICUD-EAU International Consultation on Bladder Cancer 2012: Chemotherapy for urothelial carcinoma-neoadjuvant and adjuvant settings. Eur Urol. 2013;63(1):58-66.

16. Hollenbeck BK et al. Delays in diagnosis and bladder cancer mortality. Cancer. 2010;116:5235–42.

17. McKibben MJ, Woods ME. Preoperative imaging for staging bladder cancer. Curr Urol Rep. 2015;16(4):22.

18. Novara G et al. Systematic review and cumulative analysis of perioperative outcomes and complications after robot-assisted radical cystectomy. Eur Urol. 2015;67(3):376-401.

19. Yuh B et al. Systematic review and cumulative analysis of oncologic

and functional outcomes after robot-assisted radical cystectomy. Eur Urol. 2015;67(3):402-22.

20. Canda AE et al. Robot-assisted nerve-sparing radical cystectomy with bilateral extended pelvic lymph node dissection (PLND) and intracorporeal urinary diversion for bladder cancer: initial experience in 27 cases. BJU Int. 2012;110(3):434-44.

21. Mir MC et al. Enhanced Recovery After Surgery protocols for radical cystectomy surgery: review of current evidence and local protocols. ANZ J Surg. 2015;85(7-8):514-20.

22. Sfakianos JP, Galsky MD. Neoadjuvant chemotherapy in the management of muscle-invasive bladder cancer: bridging the gap between evidence and practice. Urol Clin North Am. 2015;42(2):181-7.

GREENLIGHT™ LASER SYSTEM FOR DAY-CASE SURGERY

This symposium took place on 2nd October 2015 at the 33rd World Congress of Endourology and SWL 2015 in London, UK

Chairperson
Ian Jackson[1]
Speakers
Ian Jackson, Andrea Tubaro,[2] Gordon Muir[3]

1. York Teaching Hospitals NHS Foundation Trust, York, UK
2. Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy
3. King's College Hospital, London, UK

Disclosure: Ian Jackson has received funding for speaker engagement from Boston Scientific. Andrea Tubaro has served as a consultant for Allergan, AMS/Boston Scientific, Astellas, GSK, and Pfizer, as an investigator for AMS, Astellas, GSK, Pierre Fabre, and Shionogi, and has received a research grant from Astellas. Gordon Muir has received speaker and mentoring fees from Boston Scientific and Neotract.
Acknowledgements: Writing assistance was provided by Dr Juliane Moloney, ApotheCom.
Support: The publication of this article was funded by Boston Scientific. The views and opinions expressed are those of the authors and not necessarily of Boston Scientific.
Citation: EMJ. 2016;1(1):20–23.

MEETING SUMMARY

In light of recent economic challenges, reductions in healthcare spending are vital to sustain the level of care in health services across the world. The British Association of Day Surgery (BADS) recommendations highlight the need to revise post-surgery care and consider short-stay surgery as a viable option in patients undergoing treatment for benign prostatic hyperplasia (BPH).

A comparative study of the GreenLight XPS™ laser system and transurethral resection of the prostate (TURP) reported significant benefits with GreenLight™ Laser Therapy across Europe, and specifically in the UK, in terms of duration of catheterisation, time to stable health, and duration of hospitalisation. In addition, fewer post-surgery complications and morbidity highlight the suitability of GreenLight for day-case treatment in 70% of patients. When based on day-case GreenLight surgery, the direct cost of the GreenLight system is significantly more cost-effective than TURP. Although GreenLight is suitable across the entire patient spectrum, with particular benefits to high-risk patients, a pragmatic approach to assessing the best treatment management for each individual patient is required to allow for adequate care and ultimately patient quality of life.

Introduction

Doctor Ian Jackson

In light of recent economic challenges, there has been a global requirement for healthcare services to minimise costs. As the economic crisis continues to have an impact, both public and total healthcare spending across the Organisation for Economic Co-operation and Development (OECD) countries have fallen sharply since 2009,[1] a trend that has been reported around the globe.[2] In many regions, this drop has been primarily driven by a collapse in the growth of government health spending, whereas in some countries, such as the UK, a level health spending or a slight increase in health spending translated into reduced funding as a result of health service inflation.[3] Therefore, it is vital for health service economies to minimise costs in the acute hospital setting by maximising the use of day-case surgery and short-stay surgery with enhanced recovery.

The BADS Directory[4] has been developed as a benchmark for day-case and short-stay surgical procedures and provides a focus for clinicians and managers involved in the planning and provision of short-stay elective surgery. Now in its fourth edition,

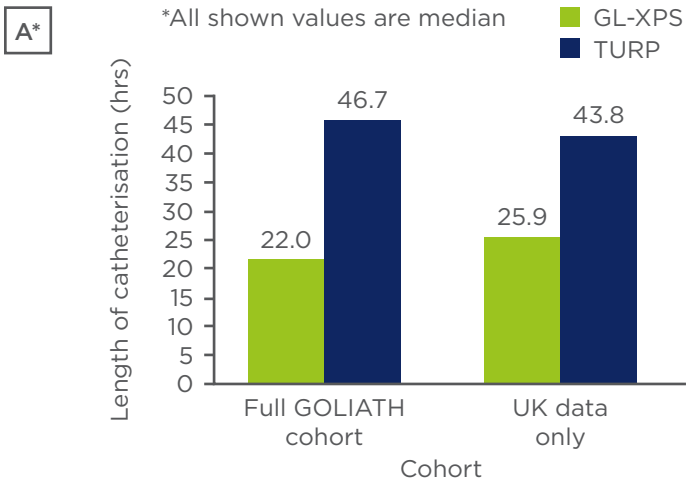
recent additions to recommendations highlight the viability of performing TURP by laser in a day-case surgery setting. Currently a limited number of patients are treated as day cases, but evidence suggests that up to 75% of patients who require this procedure could be managed on an ambulatory care basis.[4]

GreenLight Laser System for Day-Case Surgery

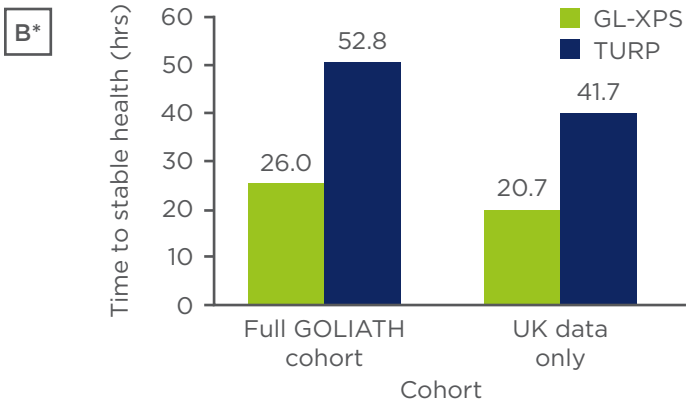
Professor Andrea Tubaro and Mister Gordon Muir

TURP is the standard of care for BPH in Europe. However, the recent pan-European GOLIATH study has demonstrated non-inferiority of the GreenLight XPS laser system compared with TURP in terms of symptoms, flow rate, and residual urine. Furthermore, the study has demonstrated a significantly lower incidence of adverse events when using GreenLight.[5-7] In addition, the GreenLight system demonstrated significant benefits with regard to length of catheterisation, time to stable health, and hospitalisation (Figure 1), factors that greatly support shorter-term stay of patients undergoing surgery for BPH.

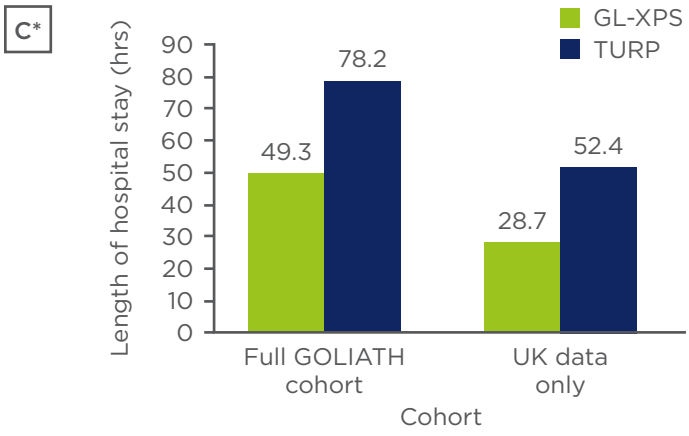
Current regulations in some European countries do not mandate early discharge of patients after surgery, which may lead to resistance against short-stay management by clinicians and to unnecessarily extended hospitalisation. However, practical influences and economic incentives allow UK-based clinicians to discharge patients within the first day of surgery if regarded safe. This behaviour and the significant results observed for the GreenLight system in the main trial were reflected in a UK-based sub-analysis of the GOLIATH study (Figure 1) (data on file, Boston Scientific). All patients were released from hospital earlier than their counterparts in the main trial. When comparing treatments, patients receiving surgery with the GreenLight system reported reduction in hospital stay by 1 day and significant benefits in terms of stable health, defined as the ability to void without an indwelling catheter, a post-void residual urine of <100 mL, as well as a reduction in the duration of catheterisation.[5] Early discharge of patients receiving treatment with the GreenLight system has been demonstrated across Europe due to lower rates of complications/morbidity seen with his technique.



King's Practice - "fast" TWOC at 2 hours for majority of patients 70% void on day of surgery, others take small catheter home.



Local reimbursement may have skewed overall data.



Some centres limited in ability to discharge patients.

Figure 1: Secondary outcomes of the GOLIATH trial and the GOLIATH UK-specific sub-analysis comparing the GreenLight XPS™ (GL-XPS) system with transurethral resection of the prostate (TURP) for the treatment of benign prostatic hyperplasia: (A) duration of catheterisation following surgery, (B) time to stable health, and (C) duration of hospitalisation.[5]
TWOC: trial without catheter.

Despite the fact that the GreenLight system has been used primarily in patients with severe comorbidities, in the UK up to 70% of patients had recovered to an acceptable level for discharge the morning after surgery[8] and, although the direct cost of the GreenLight system is slightly higher compared with the cost of TURP, cost-effectiveness studies have demonstrated that a discharge rate of 32% establishes GreenLight as economically viable.[9] There are no advantages with GreenLight from a cost point of view when treatments are performed as inpatient procedures. However, when estimated on a day-case basis, the GreenLight system is significantly more cost-effective than TURP based on a 25% reduction in procedural cost, overall lower indirect costs to treat complications and reoperate, and lower financial burden as a result of efficacy and adverse event outcomes. These values could be further improved upon with increasing rates of day-case surgery,[8] a practice that is incentivised in the UK healthcare service.

It is worth noting that the GOLIATH study was based on the average patient seen in clinical practice, excluding patients experiencing urinary retention or with an enlarged prostate (>100 g), patients over 80 years of age, or patients with bleeding disorders or cardiovascular comorbidities <180 days prior to consent. Therefore, the suitability of short-stay management requires careful consideration of individual patient background and needs.

The degree of debulking of the prostate required to claim successful treatment has remained undefined and, although a larger debulking ratio can lead to a longer-lasting treatment effect, the risk of developing complications may increase proportionally. In clinical practice, GreenLight provides a tool to achieve effective and reproducible debulking of the prostate comparable to TURP even in large prostates. Additional benefits of GreenLight include low rates of bleeding and a lack of TURP syndrome, erectile dysfunction, stress urinary incontinence, and death.[10] Another study assessing higher-risk patients previously excluded from the GOLIATH study treated with or without anticoagulants and antiaggregants demonstrated comparable outcomes for patients receiving GreenLight or TURP in terms of flow rate, International Prostate Symptom Score, and residual urine, thus supporting the case for day-case surgery with GreenLight.[10]

Consideration of patient background plays a vital role when deciding the length of patient stay following surgery. Patients, especially elderly patients, may feel more comfortable and safer in their home environment. Home-stays potentially reduce the risk of delirium in elderly patients and the risk of contracting infections in a hospital setting.

Short-stay procedures with GreenLight have been performed in a multitude of high-risk patients, and a pragmatic approach to assessing patient comorbidities, assessing a patient's social environment, reaffirming treatment safety, and adjusting the timing of the procedure to allow monitoring if required, contributes to a prompt discharge within the first 24 hours following surgery.

Q&A Session

All questions were answered by Mr Muir.

What was the degree of irritative symptoms reported and are there studies assessing these effects?

Two clinical trials comparing GreenLight and TURP demonstrated slightly lower levels of dysuria with GreenLight, based on patients reporting pain and the use of pain medication. Dysuria may have occurred more frequently in the past when treatment methods were new and there was little standardisation of technique. Initial marginal differences in storage symptoms disappeared shortly after surgery. It is important to reassure patients undergoing bladder/urethral surgery and raise awareness that symptoms may worsen before any improvements become apparent. Reoperation rates were low for both GreenLight and TURP.

Given the constraints of transport in busy commuter regions, would you recommend a geographical and/or time cut-off in terms of how long it takes for a patient to get to your hospital?

This issue needs to be assessed on an individual basis for each patient and depends on age, the level of social support, homecare service availability, familiarity with GPs, and comorbidities. Patients living independently are generally suited for short-stay surgery. Patients usually live within a 1-hour journey time from the hospital, but rules may vary between day care units.

If you think that GreenLight is such a wonderful treatment, why it is not more widely adopted, for example, in the UK?

Although GreenLight is now widely adopted in the UK, acceptance of the method when it was first marketed may have been slow due to lack of

training and bad experiences using other lasers in the past. In addition, the National Institute for Health and Care Excellence (NICE) required clinical evidence, which carries some difficulty in urology, bearing in mind the patient numbers required for a randomised clinical trial and the exclusion of high-risk patients. Currently, GreenLight represents the safest method of laser surgery for the average BPH patient and is straightforward for clinicians to learn and teach.

Do you think NICE is going to change its opinion?

Mr Muir commented, in his opinion, that the GOLIATH study was the best randomised study assessing patients with lower urinary tract symptoms and/or BPH, but has not been included in the most recent NICE assessment.

If patients with large glands had been included in the GOLIATH study, outcomes would have demonstrated more of a separation between TURP versus GreenLight. Are any studies in this patient population planned?

The exclusion of patients with large glands (>100 g) in the GOLIATH study was mandated by the external safety committee, as well as current guidelines which recommend treatment with TURP in prostates 30–80 mL in size.[11] In addition, a rigid approach towards clinical trials by NICE further complicated the comparison of GreenLight and TURP in patients on blood thinning medication, in patients with severe cardiovascular problems, and in elderly patients. While many cases of laser surgery in these patient groups have been reported, it appears that no recommendations will be made by NICE without evidence from randomised clinical trials, which may never be performed.

The limitation for the gland size in the GOLIATH study was based on the use of TURP. The trial design was based on that of Peter Gilling, which is similar to the GOLIATH trial design. However, NICE did not accept the resulting publication as it was published outside the UK.

Mr Muir agreed and highlighted that, traditionally, clinicians in the UK will treat BPH in larger prostates with TURP, whereas this is not the case in the majority of other countries, where clinicians will use TURP for debulking in prostates up to a weight of 80 g but resort to prostatectomy in larger glands.

Click below to view the following videos:

- **Q&A Session - Impact of the GreenLight TM Laser System for Day-Case Surgery Mr Gordon Muir**
- **Results of the GOLIATH Study Prof Andrea Tubaro**
- **The Importance of Day-Case Surgery Dr Ian Jackson**

REFERENCES

1. OECD. Health spending continues to stagnate, says OECD. Available at: http://info-oecd.blogspot.co.uk/2013/06/health-spending-continues-to-stagnate.html. Last accessed: 14 January 2016.

2. OECD. Health Statistics 2015. Available at: http://www.oecd.org/els/health-systems/health-data.htm. Last accessed: 14 January 2016.

3. Nuffield Trust. The funding pressures facing the NHS from 2010/11 to 2021/22: A decade of austerity? Available at: http://www.nuffieldtrust.org.uk/sites/files/nuffield/121203_a_decade_of_austerity_full_report_1.pdf. Last accessed: 14 January 2016.

4. British Association of Day Surgery. BADS Directory of Procedures 4th Edition. 2006.

5. Bachmann A et al. 180-W XPS GreenLight laser vaporisation versus transurethral resection of the prostate for the treatment of benign prostatic obstruction: 6-month safety and efficacy results of a European Multicentre Randomised Trial--the GOLIATH study. Eur Urol. 2014;65(5):931-42.

6. Thomas JA et al. A multicenter randomized noninferiority trial comparing greenlight-XPS laser vaporization of the prostate and transurethral resection of the prostate for the treatment of benign prostatic obstruction: two-yr outcomes of the GOLIATH Study. Eur Urol. 2016;69(1):94-102.

7. Bachmann A et al. A European multicenter randomized noninferiority trial comparing 180 W GreenLight XPS laser vaporization and transurethral resection of the prostate for the treatment of benign prostatic obstruction: 12-month results of the GOLIATH study. J Urol. 2015;193(2):570-8.

8. Thomas JA et al. Cost effectiveness of XPS vs TURP, a UK analysis. Eur Urol Suppl. 2014;13:e129.

9. Thomas JA et al. The continuing story of the cost-effectiveness of photoselective vaporization of the prostate versus transurethral resection of the prostate for the treatment of symptomatic benign prostatic obstruction. Value Health. 2015;18(4):376-86.

10. Bachmann A et al. 180-W XPS GreenLight laser therapy for benign prostate hyperplasia: early safety, efficacy, and perioperative outcome after 201 procedures. Eur Urol. 2012;61(3):600-7.

11. Gratzke C et al. EAU Guidelines on the treatment and follow-up of non-neurogenic male lower urinary tract symptoms including benign prostatic obstruction. Eur Urol. 2013;64(1):118-40.

NEW INSIGHTS IN NOCTURNAL ENURESIS: GOING DIGITAL, SLEEP, AND GENETICS

This symposium took place on 16th October 2015 as part of the 26th Congress of the European Society for Paediatric Urology in Prague, Czech Republic

Chairperson

Serdar Tekgül[1]

Speakers

Guy Bogaert,[2] Johan Vande Walle,[3] Søren Rittig[4]

1. Department of Pediatric Urology, Hacettepe University, Ankara, Turkey
2. Department of Urology and Pediatric Urology, UZ Leuven, Leuven, Belgium
3. Department of Paediatric Nephrology, Ghent University, Ghent, Belgium
4. Department of Paediatrics, Aarhus University, Aarhus, Denmark

Disclosure: Serdar Tekgül has received research funding for trials supported by Pfizer and Sanofi. Guy Bogaert has received speaking honoraria from Ferring Pharmaceuticals. Johan Vande Walle has acted as a safety board advisor for Astellas and Ferring Pharmaceuticals, has received research funding for trials supported by Astellas and Ferring Pharmaceuticals, and has received speaking honoraria from Ferring Pharmaceuticals. He is a board member for the European Society for Paediatric Nephrology, a liaison officer for the International Children's Continence Society, and has acted as the project coordinator for the SafePedrug project. Søren Rittig has received consultancy fees from and has participated in studies conducted by Astellas and Ferring Pharmaceuticals, and has also received speaking honoraria from Ferring Pharmaceuticals.
Acknowledgements: Writing assistance was provided by Dr Tom Priddle, ApotheCom.
Support: The Congress of the European Society for Paediatric Urology 2015 received sponsorship from Ferring Pharmaceuticals. Speakers for this symposium received honoraria from Ferring Pharmaceuticals. Medical writing assistance for this summary was funded by Ferring Pharmaceuticals. The views and opinions expressed are those of the authors as presented during the symposium and not necessarily those of Ferring Pharmaceuticals.
Disclaimer: The Drydawn website and app are owned and produced by the International Children's Continence Society, who are responsible for all content. The costs of production and maintenance are supported by Ferring Pharmaceuticals.
Citation: EMJ. 2016;1(1):24–29.

MEETING SUMMARY

Prof Tekgül opened this symposium on nocturnal enuresis (NE). Prof Bogaert described new digital initiatives for engaging with children, parents, and physicians, which included a description of the Drydawn app and reference to the Bedwetting Resource Centre (BRC). Prof Vande Walle discussed recent studies in bedwetting, demonstrating that children with nocturnal polyuria (NP) are not simply deep sleepers. The meeting concluded with a summary of recent advances in the genetics and treatment of NP by Prof Rittig.

Introduction

Professor Serdar Tekgül

Prof Tekgül welcomed the audience to the Ferring-sponsored satellite symposium on NE.

Bedwetting Going Digital – Drydawn App?

Professor Guy Bogaert

The inaugural World Bedwetting Day, a collaborative initiative between the International Children's Continence Society (ICCS) and the European Society of Paediatric Urology (ESPU),

took place on 17th October 2015. World Bedwetting Day 2015's slogan, 'Time to Take Action', recognises that much more can be done to diagnose and treat children who suffer from bedwetting. A key theme of World Bedwetting Day is engagement through digital channels with the children themselves, parents, and other caregivers such as teachers and healthcare professionals (HCPs). Bedwetting is still a highly stigmatised medical condition, which is distressing for children and their families. Furthermore, there is a paucity of primary care education on the subject of enuresis, and the ICCS and ESPU have identified a need to provide a single, high-quality repository of internet-based information, a unified voice, and a platform to facilitate cooperation between stakeholders Drydawn.com, the Drydawn app, and the BRC are central to this digital initiative. These digital resources have three main goals: 1) to provide reassurance, education, and advice; 2) to engage with children who suffer from bedwetting, in a fun yet discreet manner, and 3) to help HCPs make an informed diagnosis, particularly those in primary care.

Drydawn.com revolves around a central character called Ingolf, who suffered from bedwetting in the past but has been cured. Ingolf is used as a device to communicate with children and provide information to them and their carers. The drydawn.com website features easy-to-understand tips, advice, and background information about bedwetting (Figure 1).

Many activities can be completed by children alone, whilst others are designed for parents or carers to work through with the child. There are a number of downloadable resources, including diaries, mood meters, and checklists. The video section includes a number of specially designed videos for children and there is a list of 'frequently asked questions'.

The Drydawn app was created to engage with children and motivate them to record information that is useful for carers and HCPs. It was important that the app did not include the word bedwetting in its name, was fun but not childish, and was easy to use. The Drydawn app includes a simple interface for recording the number of 'wet' and 'dry' nights to enable the HCP to monitor bedwetting. The system encourages children to record data by rewarding them with items that can be used to personalise the character Ingolf. With each entry the child is rewarded with a medal that can be exchanged for an item of clothing to dress Ingolf.

Figure 1: The drydawn.com homepage showing Ingolf, his parents, and his doctor.

The app also includes a more detailed diary feature, which records fluid intake and output by monitoring drinking volume and urine output by weighing nappies. As these data are more complex, children may require help from an adult to input the information but medals are still awarded for completing entries. All the information entered into the app will allow the HCP to receive emailed or printed reports containing the child's expected bladder capacity, the maximum voided volume, fluid intake up to 18:00 hrs and over a 24-hour period, and calculation of the average nightly urine production. The report also includes general 'rules of thumb' to assist the HCP in interpreting the data. The app is currently being beta tested and will be initially released in English, with French, Dutch, Danish, and other language versions planned.

Information for HCPs is hosted on the BRC in conjunction with Elsevier (http://bedwetting.elsevierresource.com). Each month, the BRC provides a convenient round-up of relevant articles published in the field. Each article is placed into a specific category for convenience and is accompanied by a brief editorial highlight. In addition to this, the BRC hosts a number of useful tools, such as the Clinical Management Tool for the Diagnosis and Treatment of Nocturnal Enuresis.[1] The BRC also features a number of video presentations and interviews with key opinion leaders.

The next generation of bedwetting information (Drydawn.com, the Drydawn app, and the BRC) will facilitate better compliance, better information exchange, and better communication, leading to more motivated children, which is crucial to bringing us closer to the goal of more 'dry nights' for children.

Recent Sleep Studies in Bedwetting

Professor Johan Vande Walle

Until very recently, NE was considered 'normal' in childhood and the affected children were simply viewed as deep sleepers with abnormal arousal, although early studies failed to demonstrate electroencephalogram abnormalities during deep sleep.[2] Historically, abnormal arousal was inferred from questionnaire data[3] and demonstrated in a study of enuretic boys.[4]

Current evidence for abnormal arousal thresholds is unclear. However, an abnormal circadian rhythm is certainly involved, as are the bladder, kidney, and psychological factors. There is a clear association between attention deficit and NE, with evidence for subjective[5] and objective[4] increases in arousal thresholds. The idea that nocturia is normal in children stems from the adult viewpoint that most clinicians hold: namely that waking up to urinate is normal. However, it is becoming apparent that, unlike adults, children do not wake up to become continent but start to wake up if they are incontinent. This phenomenon is related to the maturation of melatonin, which reaches a peak at the age of 10 years and begins to decrease from the age of 25 years.

The first studies to suggest that children with NE had disrupted sleep were performed in patients with overactive bladders[6] and could not be generalised to monosymptomatic enuresis. A subsequent study of refractory monosymptomatic enuresis in a group of 15 desmopressin-resistant and 14 desmopressin-dependent children showed that 28 of the 29 children had abnormal sleep architecture.[7] These children had a high incidence of 'restless legs' symptoms and their periodic limb movement score (PLMS) index was >5. A follow-up study in an extreme–refractory population demonstrated that PLMS and arousal indices were significantly higher in children with NE than those with other sleep disorders without NE.[8] The above studies suggest that NP, enuresis, sleep disorders, and attention deficit are comorbid conditions. However, they may also have a causal relationship. To investigate this relationship, a recent prospective study has examined the effect of desmopressin on children with monosymptomatic enuresis and NP.[9] The population consisted of 30 children (6–16 years of age) assessed before and after 6 months of desmopressin treatment. Data were gathered on a number of measures, including sleep quality, renal function, circadian rhythm of diuresis, and a range of psychological tests. Psychological tests were administered using questionnaires completed by the child and their parents and teachers. Significant improvements were seen in a number of domains, as shown in Table 1.

Effective treatment of NP leads to improvement in enuresis, sleep quality, and increased daytime cognitive function, suggesting that NP is the underlying cause of enuresis, disturbed sleep, and attention deficit in these patients.

In conclusion, NP is not a benign disorder, and there is mounting evidence which suggests that it causes sleep disruption and cognitive dysfunction.

Table 1: Results of psychological tests following 6 months of desmopressin treatment.[9]

Test result		p value	Respondent
Reduced	Attention problems	p<0.01	Parents
	Internalising problems	p<0.05	Parents
	Externalising problems	p<0.01 p<0.05	Parents Teacher
Improved	Quality of life	p<0.01	Child, parents
	Executive functioning	p<0.05–<0.01	Child, parents, teacher
	Auditory memory	p<0.01	Child

Desmopressin melt is an effective treatment for NP and enuresis, which therefore reduces sleep disruption and leads to improvements in both cognitive function and quality of life. A 'wait and see' approach to NE is no longer appropriate; NP should be taken seriously, and children and their families should be offered treatment.

Nocturnal Enuresis – From Genes to Treatment of Nocturnal Polyuria

Professor Søren Rittig

Twenty years ago, the third International Children's Continence Symposium in Sydney marked the beginning of the modern classification of NE (Figure 2).[10] An important distinction was made between those patients who only wet the bed, now known as monosymptomatic, and those who had additional daytime symptoms. It was also apparent that there was a subset of monosymptomatic patients who had an increased urine production (polyuric), and a subset who produced urine in usual amounts and who also failed to respond to desmopressin (non-polyuric). Data from linkage studies provided evidence that further subgroups of these patients could be discerned according to their genetic dispositions. The ICCS concluded that "a differentiated approach to NE is, therefore, essential for a better understanding of the pathogenesis of this disorder and for designing successful treatment strategies."

This article will briefly review the progress that has been made in striving toward a differentiated approach. Studies of night-time urine production in bedwetting children have shown that these children produce more urine on a 'wet night' than on a 'dry night'. Patients with NP have a normal bladder capacity, but produce an abnormally high volume of urine on a 'wet night'. What constitutes abnormally high urine production in NP has been a matter of some debate. NP was defined by the ICCS in 2006 as production of urine that exceeded 130% of the child's expected bladder capacity, giving the formula: Nuvol equals >130% MVV_{age}, where Nuvol represents night-time urine volume and MVV_{age} is the maximum voided volume for that age.[11] A study of bladder volume in 148 children aged between 3 and 15 years who were not experiencing bedwetting determined that the ICCS 2006 definition only applies well to children between the ages of 4 and 7 years.[12] Based on these data, a population-based definition of childhood NP was proposed: Nuvol = 20 × (age + 9) mL.[12] This definition has been used to predict desmopressin response,[12] but most research continues to use the ICCS 2006 definition. For comparison, the definition of adult NP is night-time urine production >20% of the total daily output for younger adults, and >33% for older adults. A joint International Continence Society and ICCS working group has been proposed to investigate the plausibility of producing a definition that would accommodate all ages.

Urine production can be simply divided into the amount of water and the amount of osmoles (urine osmolarity) that a person excretes.[13] The amount of water excreted is principally determined by the function of the aquaporins in the distal tubules of the kidneys.[14] The amount of aquaporins is determined by the level of vasopressin.[14] Urine osmolarity is dependent on levels of sodium, potassium, calcium, urine, and many other solutes.[13]

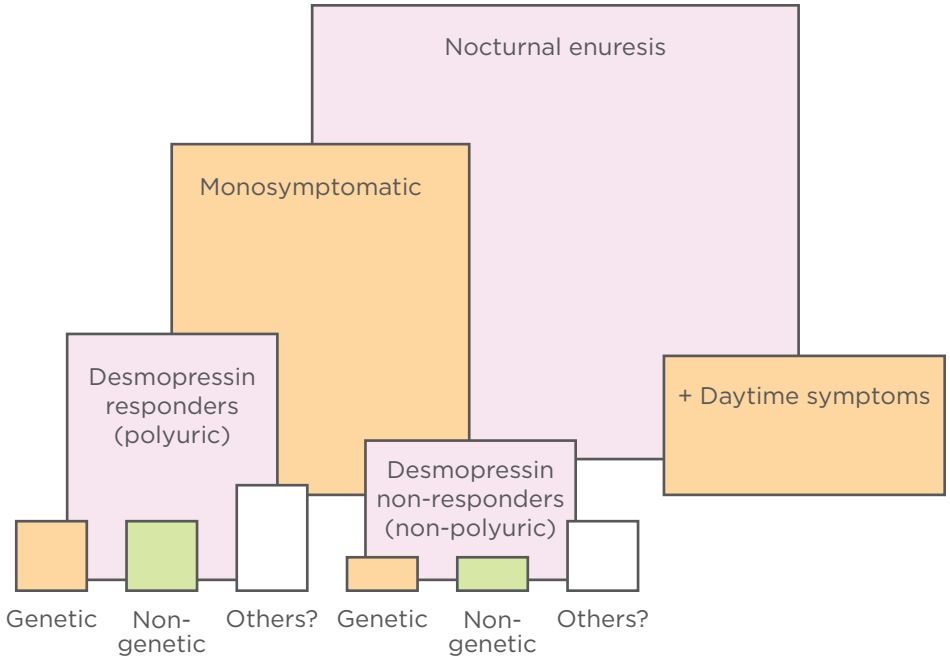


Figure 2: Classification of patients with nocturnal enuresis.[10]

As described above, urine production is only one of a number of factors that contribute to the disturbed sleep seen in NP. The intrinsic circadian rhythm and genetic factors also play a role. PLMS and fragmented sleep may increase blood pressure, which has a direct effect on the kidneys. Renal excretion of sodium and potassium is increased, glomerular filtration rate rises, and vasopressin secretion is suppressed, causing polyuria.

A promising area of research is the discovery of biomarkers that could identify polyuric patients. It would be particularly useful to identify those patients who secrete low amounts of vasopressin, as they are likely to respond to desmopressin. Vasopressin is produced as a preprohormone made up of a signal peptide, large carrier protein, vasopressin itself, and copeptin, a protein of unknown function.[14] Copeptin is a promising biomarker for polyuria because it correlates well with vasopressin levels in the blood and has a longer half-life. The long half-life allows measurement in a morning blood sample, which is far more convenient than measuring urine production.

Our current understanding of the pathophysiology of NP has improved the available treatment options. For instance, we now know that patients who fail to respond to desmopressin have problems with osmotic excretion and a number of treatment options are now available. Indomethacin inhibits prostaglandin production which, in turn, inhibits sodium excretion and potentiates the effects of vasopressin. Imipramine also reduces osmotic excretion. The use of diuretics during the day may deplete sodium levels, thereby preventing increased sodium excretion during the night. Studies are currently ongoing to investigate whether a low-solute diet is an effective treatment in these patients. A promising new development in the treatment of NP is combining one of the above treatment strategies with desmopressin to maximise the effect on urine production.

Advances have been made in elucidating the molecular mechanism of enuresis. Vasopressin attaches to the V2 receptor and activates cyclic adenosine monophosphate (cAMP) production in renal cells. The rise in cAMP stimulates the insertion of aquaporins into the cell membranes of the collecting duct, allowing water to be reabsorbed and subsequently enter the bloodstream. Aquaporin, the V2 receptor, and vasopressin have been sequenced. Linkage analysis has identified five main chromosomal areas of interest: 4p,[15] 8p,[16] 12q,[15] 13q,[17] and 22q,[18] and several candidate genes have been proposed. However, many affected families do not share these markers and so additional loci must be involved.

In conclusion, great progress has been made in our understanding of NP over the last 20 years. Nevertheless, a more robust definition of NP is required. The increasing body of knowledge of the

pathophysiology of NP will provide the basis for personalised treatment. Biomarkers and improved measures of NP will facilitate directed treatments. The genetic basis of NP will soon be determined, and there is hope for a 'dry' future for all children.

Click below to view the following websites:

- **World Bedwetting Day**
- **Drydawn**

REFERENCES

1. Vande Walle J et al. Practical consensus guidelines for the management of enuresis. Eur J Pediatr. 2012;171(6):971-83. Errata. Eur J Pediatr. 2012;171(6):1005; Eur J Pediatr. 2013;172(2):285.

2. Nørgaard JP et al. Nocturnal studies in enuretics. A polygraphic study of sleep-EEG and bladder activity. Scand J Urol Nephrol Suppl. 1989;125:73-8.

3. Nevéus T et al. Depth of sleep and sleep habits among enuretic and incontinent children. Acta Paediatr. 1999;88(7): 748-52.

4. Wolfish NM et al. Elevated sleep arousal thresholds in enuretic boys: clinical implications. Acta Paediatr. 1997;86(4):381-4.

5. Chandra M et al. Prevalence of diurnal voiding symptoms and difficult arousal from sleep in children with nocturnal enuresis. J Urol. 2004;172(1):311-6.

6. Yeung CK et al. Sleep disturbance and bladder dysfunction in enuretic children with treatment failure: fact or fiction? Scand J Urol Nephrol Suppl. 1999;202: 20-3.

7. Dhondt K et al. Abnormal sleep architecture and refractory nocturnal enuresis. J Urol. 2009;182(4 Suppl):1961-5.

8. Dhondt K et al. Sleep fragmentation and increased periodic limb movements are more common in children with nocturnal enuresis. Acta Paediatr. 2014;103(6):e 268-72.

9. Van Herzeele C et al. Desmopressin melt improves sleep and psychological functioning in patients with monosymptomatic nocturnal enuresis. Annual Meeting of the International Continence Society, Rio de Janeiro, Brazil, 20-24 October, 2014.

10. Nevéus T et al. The standardization of terminology of lower urinary tract function in children and adolescents: report from the Standardisation Committee of the International Children's Continence Society. J Urol. 2006;176(1):314-24.

11. Rittig S et al. Age related nocturnal urine volume and maximum voided volume in healthy children: reappraisal of International Children's Continence Society definitions. J Urol. 2010;183(4):1561-7.

12. Gwinnutt D, Thorburn J. Body Fluids - Part 1. Anaesthesia Tutorial Of The Week 184. Available at: http://www.aagbi.org/sites/default/files/184-Body-fluids-part-1.pdf. Last Accessed: 30 November 2015.

13. Ikeda M, Matsuzaki T. Regulation of aquaporins by vasopressin in the kidney. Vitam Horm. 2015;98:307-37.

14. Eiberg H et al. Linkage study of a large Danish 4-generation family with urge incontinence and nocturnal enuresis. J Urol. 2001;166(6):2401-3.

15. Eiberg H. Nocturnal enuresis is linked to a specific gene. Scand J Urol Nephrol Suppl. 1995;173:15-6; discussion 17.

16. Eiberg H et al. Assignment of dominant inherited nocturnal enuresis (ENUR1) to chromosome 13q. Nat Genet. 1995;10(3):354-6.

17. Eiberg H. Total genome scan analysis in a single extended family for primary nocturnal enuresis: evidence for a new locus (ENUR3) for primary nocturnal enuresis on chromosome 22q11. Eur Urol. 1998;33 Suppl 3:34-6.

EDITOR'S PICK

Our understanding of pathophysiology across the continuum of renal diseases is rapidly increasing, not only in terms of rare renal disease, but also in relation to more common diseases. Complement activation is associated with a wide number of kidney disorders, and recent advances in this area of research mean that it is quickly becoming a key target for treatment. As the range of available treatments for glomerulopathies widens, it becomes more and more important that both renal pathologists and general practitioners have the most up-to-date knowledge available at their fingertips. This is a niche that is well filled by the following paper by Dr Caliskan, who provides a great summary of a range of complement pathway associated glomerulopathies.

COMPLEMENT PATHWAY ASSOCIATED GLOMERULOPATHIES

***Yasar Caliskan**

Division of Nephrology, Department of Internal Medicine,
Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey
**Correspondence to ykcaliskan@yahoo.com*

Disclosure: The authors have declared no conflicts of interest.
Received: 16.11.15 **Accepted:** 07.12.15
Citation: EMJ. 2016;1[1]:30-38.

ABSTRACT

The complement system causes kidney injury in a variety of different diseases, and clinical evaluation of the complement system is an important part of the diagnostic workup of patients with glomerulonephritis. In cases of ongoing, uncontrolled complement activation, the kidney is susceptible to complement hyperactivation, and thrombotic microangiopathy associated kidney injury can occur. Two principle modes of complement-mediated kidney injury have been proposed: classical pathway mediated injury in immune complex diseases and/or alternative pathway mediated renal injury causing atypical haemolytic uraemic syndrome (aHUS) and C3 glomerulopathy in patients with abnormalities in alternative pathway regulation. Recent advances have also provided new insights into the pathogenesis of glomerular and tubulointerstitial injury associated with aberrant complement activation. Complement inhibition is effective for treatment of aHUS, and there is growing evidence of the favourable effect of the anti-C5 monoclonal antibody eculizumab. Measurement of *ex vivo* serum-induced endothelial C5b-9 deposits is supposed to be a sensitive tool to monitor complement activation and eculizumab effectiveness. Although understanding the role of the complement system in the pathogenesis of many kidney diseases is improved, there is not a simple algorithm for identifying which patients should be treated with complement inhibitors or for how long complement inhibition should be continued.

Keywords: Atypical haemolytic uraemic syndrome (aHUS), complement system, C3 glomerulopathy (C3G), eculizumab, glomerular diseases.

INTRODUCTION

Complement activation plays a major role in several renal pathophysiological conditions, and the spectrum of complement system associated renal diseases is constantly expanding.[1] Two principle modes of complement-mediated kidney injury have been proposed: classical pathway mediated injury

in immune complex diseases and/or alternative pathway mediated renal injury causing atypical haemolytic uraemic syndrome (aHUS) and C3 glomerulopathy (C3G) in patients with abnormalities in alternative pathway regulation.[1,2] Recent advances have also provided new insights into the pathogenesis of glomerular and tubulointerstitial injury associated with aberrant complement activation.[1,2]

The Complement System

The main effector mechanisms of complement activation are: recruitment of immune cells to sites of infection, labelling of the invading pathogens via opsonisation for uptake and destruction by phagocytes, and/or direct lysis of susceptible pathogens.[3] There are three pathways recognised for complement activation: the classical pathway, the alternative pathway, and the more recently discovered lectin pathway.[3] All three pathways converge to cleave complement component C3, which subsequently initiates activation of the terminal complement pathway and formation of the membrane attack complex (MAC) (Figure 1). In order to prevent damage to self-tissues, the complement system contains membrane-bound and fluid-phase proteins that regulate complement activation. They act by promoting decay of the convertase complexes, being cofactors for the enzymatic degradation of the active proteins, and by preventing the assembly of the MAC. An abnormal functioning of the regulatory system, due to either genetic or acquired causes, can shift the balance between regulation and activation towards the latter and lead to complement system associated tissue injury.[1-3] This kind of imbalance occurs on susceptible surfaces that lack complement regulators or do not support the binding of such regulators that normally occur on host cells.

The Complement System and Glomerular Diseases

The complement system is involved in the pathogenesis of kidney disease as a key mediator (Table 1).[4] Activation of complement can cause direct glomerular injury in glomerulonephritis characterised by immune complex deposition, and facilitates the recruitment of leukocytes into the glomerulus.[5] There is also growing evidence that activation of plasma complement proteins leaking into the tubular lumen during proteinuria, followed by strong activation of locally synthesised complement, leads to progressive tubulointerstitial damage.[4] The tendency of the kidney for complement system associated injury is incompletely understood. The following reasons are suggested: the presence of the fenestrae in the glomerular basement membrane (GBM) continuously exposing the acellular subendothelial tissues to complement activators, a lower baseline expression of complement regulators, and/or differences in the composition of the glycocalix.[5] Complement activation products can be found in the urine of patients with a wide variety of proteinuric diseases, and proteinuria provides a source of complement proteins to a host cell surface that is unable to control activation.[4]

Alternative Complement Pathway Associated Glomerulopathies

In recent years, the identification of disease-causing mutations and genetic variants in complement regulatory proteins has brought up the concept of alternative complement pathway-associated glomerulopathies (ACPAG). C3G and aHUS are the main disorders characterised by excessive activation of the alternative complement pathway. aHUS is characterised by microangiopathic haemolytic anaemia, low platelet count, and acute renal failure.[1] A similarly rare disease entity termed 'C3G' is characterised by isolated C3 deposition in glomeruli without positive staining for immunoglobulins and immunofluorescence.[1,3] Although the impact of family history and consanguineous unions on the development of ACPAG in different populations is unknown, there is ample evidence for familial aggregation of aHUS and C3G cases.[1,2,6,7] Genetic and functional studies have demonstrated that mutations and/or autoantibodies affecting proteins implicated in regulating complement biology are central to these diseases' pathophysiology.[6,7]

Membranoproliferative Glomerulonephritis - C3 Glomerulopathy

Membranoproliferative glomerulonephritis (MPGN) denotes a general pattern of glomerular injury characterised by an increase in mesangial cellularity and matrix with thickening of glomerular capillary walls. This is secondary to subendothelial deposition of immune complexes and/or complement factors, cellular entrapment, and new basement membrane formation.[8] Recently, improved understanding of the role of the alternative complement pathway in MPGN has illuminated the field and led to a paradigm shift in the disease classification.[2,8] MPGN has now been reclassified into immunoglobulin-

mediated disease (driven primarily by the classical complement pathway) versus non-immunoglobulin-mediated disease (fuelled by the alternative complement pathway overactivity).[2,8,9] C3G is a unifying description of glomerular changes, particularly of glomerular deposits of C3 without immunoglobulins that represent the hallmark of alternative pathway dysregulation through inherited or acquired defects.[9-11] C3 glomerulopathies encompass dense deposit disease (DDD) and C3 glomerulonephritis (C3GN).

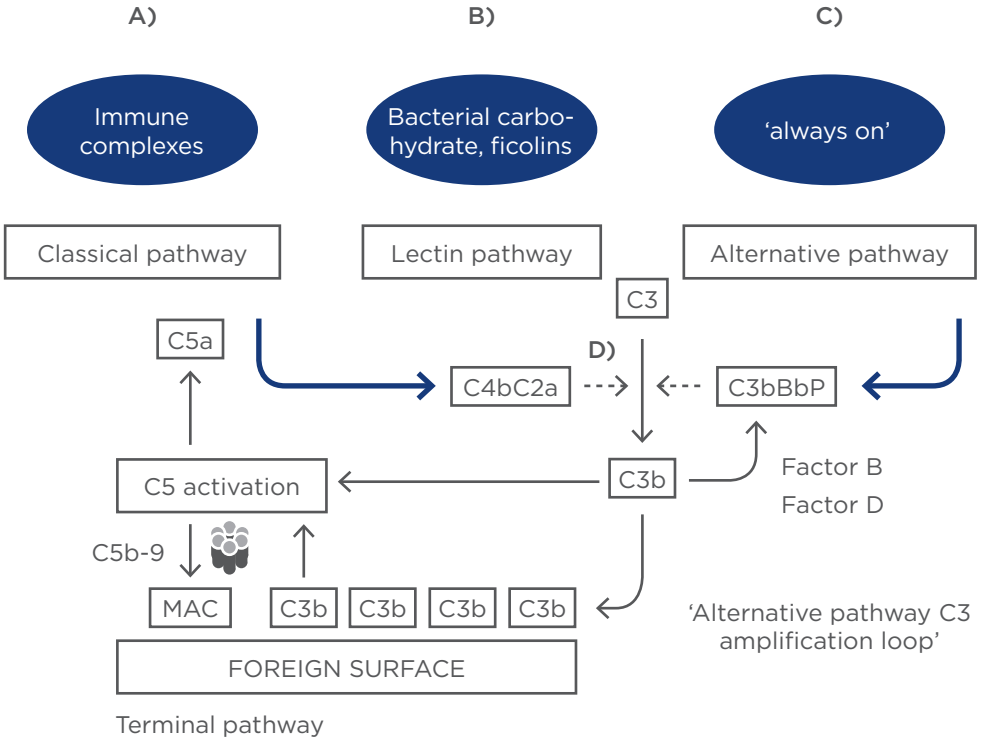


Figure 1: Activation of the complement system.

There are three pathways recognised for complement activation: classical, alternative, and lectin pathways. The classical and lectin pathways are similar, differing only in the initiator molecular complexes and the triggering signals. a) Complement C1q in association with two molecules of each of the serine proteases C1r and C1s makes the initiator complex of the classical pathway. The C1qrs complex is activated on binding to the antigen–antibody complex. b) The lectin pathway is initiated upon recognition of certain sugar patterns on surfaces of microbial pathogens by the broad-spectrum carbohydrate recognition molecules of mannose binding lectin, ficolins, or collectin-11. Binding of recognition complexes from either classical or lectin pathways to their target structures leads to activation of the attached serine proteases: C1r/C1s and MASP-1/MASP-2, respectively. The activated serine proteases C1s and MASP-2 cleave C4 into C4a and C4b. C4a is released into the fluid phase, whereas C4b attaches to the target surface. C2 binds to the attached C4b and is cleaved by C1s or MASP-2 releasing C2b into the fluid phase, whereas C2a remains attached to C4b. The resulting complex C4b2a represents the C3-convertase of the classical and lectin pathways then activates C3. c) The alternative pathway remains constitutively active, as a result of spontaneous low-level hydrolysis of the internal thioester bond of C3 to C3(H2O) in a process known as 'tick-over'. The cleavage of C3 allows the binding of the C3(H2O) to plasma protein Factor B, rendering it susceptible to cleavage by Factor D to Ba and Bb. This produces a limited amount of the fluid phase alternative pathway C3-convertase (C3[H2O]Bb) that is able to cleave C3 into C3a and C3b. C3-convertase cleavage of additional C3b and formation of C3bBb forms an amplification loop leading to the exponential production of additional C3 convertase. d) The C3 convertase cleaves C3 resulting in assembly of the C5 convertase and sequential binding of C6, 7, 8, and 9 to form C5b-9, the membrane attack complex (MAC), which inserts into target membranes, forming channels that disrupt membrane function and lead to lysis of target cells. Complement activation also results in production of the small, biologically active anaphylatoxins: C3a and C5a.

Table 1: Complement pathway-related glomerulopathies.

Alternative complement pathway associated glomerulopathies
Membranoproliferative glomerulonephritis – C3 glomerulopathy Atypical haemolytic uraemic syndrome
Other complement-associated glomerulopathies
Anti-glomerular basement membrane glomerulonephritis Anti-neutrophil cytoplasmic antibody-associated vasculitis C1q nephropathy Focal segmental glomerulosclerosis IgA nephropathy Lupus nephritis Membranous nephropathy Post-infectious glomerulonephritis

IgA: immunoglobulin A.

Although DDD and C3GN are morphologically distinguishable by the appearance and location of deposits on electron microscopy, these diseases are now considered to be part of the same aetio-pathological spectrum.[9-11] However, adult patients with DDD appear to have more aggressive disease and worse outcomes compared with C3GN.[12] Distinguishing C3G from immunoglobulin-mediated MPGN, and also further differentiation between DDD and C3GN may help for treatment algorithms and long-term prognostication. Complement system associated mutations can be found in patients with C3G. Examples include familial DDD with C3 mutation and familial C3GN with mutations in the complement factor H related (CFHR) genes.[13,14] The treatment algorithm for C3G is not well established yet. The efficacy of the C5 inhibitor eculizumab is tested in patients with persistent C3G under immunosuppressive therapy, and some patients responded to this treatment.[15] Factors affecting response to therapy are poorly understood, although acute lesions and high circulating MAC levels have been proposed to indicate higher chances of response to therapy.[15] The efficiency of eculizumab in rapidly progressive or crescentic C3G has also been reported recently.[16] However, eculizumab for the treatment of patients with C3G, or prevention of recurrence in a transplanted kidney is still outside the approved indications. Results of ongoing prospective studies of eculizumab in C3G are expected.[17,18]

Although previous studies reported a failure in response to glucocorticoid, mycophenolate mofetil, and rituximab therapy,[15,19] a recent study showed that immunosuppressive treatments, particularly corticosteroids plus mycophenolate mofetil, are found to be beneficial in C3GN.[20] Soluble CR1, a potent regulator of complement activity, is another novel therapeutic agent, and its membrane-bound form inhibits the conversion of C3 to C3b and promotes the breakdown of active C3b. A recent study suggested the effectiveness of soluble CR1 as a treatment for DDD and C3GN.[21]

Atypical Haemolytic Uraemic Syndrome

aHUS is an extremely rare disease and the annual incidence is about 0.5–2.0 per million adults and 3.3 per million children or adolescents. A differential diagnosis among the different forms of HUS is often not feasible based only on age of onset or clinical symptoms. HUS caused by *Escherichia coli* strains that produce Shiga toxins (STEC-HUS) largely predominates in infants and children aged between 6 months and 5 years, but may occur at any age. The presence of diarrhoea, a characteristic symptom of STEC-HUS, cannot be used to differentiate between STEC-HUS and aHUS because approximately one-third of patients with aHUS experience diarrhoea.[22,23] Extra-renal findings including cardiac and neurological abnormalities present in up to 20% of patients, and pancreatitis and/or hepatitis have also been reported.[22,23] Signs and symptoms of renal failure at presentation can include haematuria, oedema, hypertension (malignant in up to 8%), and electrolyte abnormality.[22] Most of the children (59%) and adults (81%) require dialysis at presentation.[23] The clinical presentation of aHUS associated with the coagulation protein, diacylglycerol

kinase epsilon (DGKE), is very similar to that of complement-mediated aHUS, although all aHUS patients associated with DGKE present in the first year of life.[24,25]

Laboratory evidence of haemolysis, thrombocytopenia, and renal failure is present in the overwhelming majority of patients. Serum C3 levels may vary during acute versus chronic disease, but are low in up to 35.9% of patients,[22] occurring most frequently in the presence of a CFH gene mutation. Historically, the onset of aHUS in only 40–60% of aHUS patients has been explained by mutations in genes encoding complement-related proteins, and 25–30% of these mutations were CFH mutations.[6,26] Studies focussed on aHUS have shown that mutations in CFH, complement factor I (CFI), membrane cofactor protein (MCP), thrombomodulin (THBD), and in genes encoding complement activators CFB and C3 as well as copy number variations in the CFHR gene cluster predispose patients to aHUS. Anti-factor H antibodies have also been shown in patients with aHUS. These antibodies bind to short consensus repeats, thus reducing the CFH activity.[27] Reduction in MCP expression is reported in >80% of cases with a mutation in this gene.[28] Genetic disorders are rarely related to CFI.[29] THBD mutations with hyperactivity have been found in only 3–5% of patients.[30] The role of non-complement related mechanisms and genetic variations as the deficiency of DGKE encoded by the DGKE gene in the aetiology of C3G and aHUS has been reported in recent studies.[7,24] It has been proposed that lack of DGKE causes enhanced signalling through arachidonic acid containing diacylglycerols and results in a prothrombotic phenotype, or may cause an enhanced activity of TRPC6 in the podocyte causing dysfunction of glomerular permselective properties.[24]

Despite the recent progress in understanding the pathophysiology of the ACPAG, there are few and inadequately validated clinical tools available for risk stratification, prediction of exacerbations, identification of autoimmune forms and selection of treatment options, and monitoring of drug response in aHUS and C3G.[31] However, Noris et al.[32] developed an *ex vivo* test evaluating the *ex vivo* serum induced C5b-9 endothelial deposits that might be a sensitive tool to monitor complement activation and eculizumab effectiveness.[32] Although STEC-HUS is characterised by full recovery in >80% of patients, patients with aHUS have a poorer prognosis. The majority of patients need dialysis at admission, and until very recently, half of all patients never recovered kidney function.[1,6] aHUS patients with end stage renal disease (ESRD) also have a 60% risk of disease recurrence in the allograft; this almost always (in 90% of cases) leads to graft loss of the affected kidney.[33] The risks of post-transplantation disease recurrence are best predicted in patients harbouring pathological mutations in known complement-associated genes. Gene discovery studies also showed that patients with mutations in DGKE carry a very low risk of post-transplantation disease recurrence.[6] Recently, the introduction of the anti-C5 monoclonal antibody eculizumab as a treatment has dramatically improved the prognosis for these patients.[34,35] Effectiveness of eculizumab was shown not only in a progressive group but also in a group of patients who required chronic plasma exchange.[35] In an open-label, uncontrolled trial, eculizumab was effective in >80% of patients in controlling haemolysis, improving renal function, and allowing the withdrawal of plasma therapy.[35] Undoubtedly, current treatment guidelines will prominently feature eculizumab treatment and suggest that treatment should be started early.

Other Complement Associated Glomerulopathies

Anti-Glomerular Basement Membrane Glomerulonephritis

Anti-GBM disease is a rare but life-threatening autoimmune disease, which clinically manifests rapidly progressive glomerulonephritis with or without pulmonary haemorrhage. In the renal biopsy of patients, linear deposition of Immunoglobin G (IgG) is often accompanied by C3 deposits, as well as a linear or granular staining pattern on the glomerular capillary wall, which indicates that complement activation is involved in the kidney injury.[36] Recently, the complement system has been shown to be activated via both the alternative and classical pathways in the kidneys of human anti-GBM disease.[37] The inflammatory response through C5a activation and/or the cell lysis effect of C5b-9 are enhanced in patients with anti-GBM glomerulonephritis.[36]

Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis

Several authors documented the involvement of complement in anti-neutrophil cytoplasmic antibody (ANCA) glomerulonephritis.[38,39] Chen et al.[38] documented C3 deposits in the glomeruli of

patients with high levels of proteinuria and poor renal function. C5-9, C3d, and CFB were also reported in biopsies from patients with myeloperoxidase (MPO)-ANCA-associated pauci immune glomerulonephritis. Xing et al.[39] observed that C4d was negative in biopsies of patients with MPO-ANCA glomerulonephritis. These studies suggest that this model of glomerulonephritis requires the activation of the alternative pathway, not the classical or the leptin pathways. Further studies in patients with active ANCA-associated vasculitis documented high levels of C3a, C5a, soluble C5b-9, and Bb.[40] Recently, other authors have shown that the C5a specific receptor (C5aR) expressed on neutrophils is involved in the pathogenesis of ANCA-induced glomerulonephritis.[41] Therefore, targeting the C5a-C5aR receptor interaction in such patients might represent a therapeutic strategy.[42] A clinical trial to evaluate the safety and efficacy of an inhibitor of the C5a receptor (CCX168) is ongoing and a short-term analysis reported promising results.[42]

C1q Nephropathy

C1q nephropathy is characterised by the C1q binding to poly-anionic substances (DNA, RNA, viral proteins) or to C1q receptors. Some authors suggested that C1q nephropathy is a subgroup of primary focal segmental glomerulosclerosis (FSGS). In histopathological evaluation, C1q nephropathy is characterised by the presence of noticeable C1q immune deposits in glomeruli with no evidence of systemic lupus erythematosus (SLE) or MPGN Type I.[43]

Focal Segmental Glomerulosclerosis

The pathogenesis of FSGS remains unclear, but IgM and C3 deposits are commonly observed in the affected glomeruli.[44] Mutations in CFH and C3 have been described in cases of FSGS,[45] and a murine model of IgG-initiated FSGS in decay accelerating factor-deficient mice[46] supports a role for complement dysregulation in some cases. Complement inhibition has not been carefully studied as a therapy for FSGS.

IgA Nephropathy

IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis. The disease is characterised by mesangial deposition of polymeric IgA1. A large number of studies established that increased levels of circulating galactose-deficient IgA1 in association with the production of unique anti-glycan antibodies leads to the formation of pathogenic IgA1 containing circulating immune complexes that are deposited within the mesangium, leading to activation of mesangial cells and thus glomerular damage. Complement components including C3, C4d, C5b-9, properdin, factor H, C4BP, and mannose binding lectin (MBL) are deposited within the glomerulus in IgAN, which has been known for some time.[47,48] The alternative pathway has a primary role in 75% of cases.[49] These biopsies show colocalisation of polymeric IgA1, C3, and the MAC in the absence of other immunoglobulins, C1q, C4, or MBL. In 25% of biopsy specimens, the presence of glomerular IgA1 and C3 is associated with MBL and MBL-associated serine protease 1 (MASP-1) deposition. MBL binds to the abnormally galactosylated region of the IgA1 through its carbohydrate binding domain, resulting in complement catabolism through the lectin binding pathway. The presence of MBL and MASP-1 is associated with disease severity and poor histological prognostic features.[50]

Table 2: Key aspects of complement-associated glomerulopathies.

The kidney is a susceptible organ to abnormal regulation of complement
Major complement dysregulation related renal pathologies are: • aHUS (endothelial complement activation) • C3Gs (C3 glomerulonephritis, dense deposit disease, CFHR5 nephropathy)
Eculizumab is the first complement inhibitor and is effective in aHUS, although resistance can occur
Ex vivo serum-induced endothelial C5b-9 deposits are a sensitive tool to monitor complement activation and eculizumab effectiveness
Mycophenolate mofetil + steroid treatment can be an option for C3G treatment
There are ongoing studies evaluating the new complement inhibitors (TCR1, anti-C5a, lampalizumab, compstatin [APL-1 and APL-2])

CFHR5: complement factor H related 5; aHUS: atypical haemolytic uraemic syndrome; TCR1: T cell receptor 1; C3G: C3 glomerulopathy.

A recent genome-wide association study identified a major susceptibility locus for IgAN within the gene encoding CFH.[51] A common deletion in the regulators of complement activation cluster at 1q32, which incorporates the genes (CFHR3 and CFHR1) encoding factor H-related protein 3 and 1, has been shown to protect against IgAN,[51] possibly by reducing the ability of CFHR proteins to inhibit the regulatory function of CFH.[52] It thus seems possible that complement inhibition might be beneficial in the treatment of IgAN and in particular the crescentic form with rapidly progressive glomerulonephritis, which has a poor prognosis. The efficiency of eculizumab in a patient with Henoch–Schönlein purpura and crescentic IgAN has been recently reported.[53]

Lupus Nephritis

Proliferative lupus nephritis is classically associated with the 'full house' immunology of glomerular antibody and complement deposition in combination with peripheral consumption of the classical complement proteins. Deficiencies in the early components of the classical pathway (C1q, C1r, C1s, C2, and C4) predispose to the development of SLE.[54] While heritable mutations of C1q leading to deficiency are rare, deficiency due to excessive complement activation because of interaction with immune complexes is common, and low C1q levels are associated with active disease.[55] C1q antibodies are present in one-third of patients with SLE and are associated with lupus nephritis, sensitivity, and a specificity of >90%.[56] The generation of these autoantibodies may be precipitated by the existence of C1q on apoptotic debris that is not removed in a timely fashion. The alternative and the lectin pathways also appear to play a role in the progression of glomerular damage.[57] Patients presenting with the glomerular deposition of properdin, a positive regulator of the alternative pathway, and patients with MBL/L-ficolin, showed increased urinary protein excretion.[57] Taken together, glomerular deposition of C1q in the context of immune complexes, complement activation, and functional Fc gamma receptors appear to be necessary to cause renal damage in lupus nephritis.

Membranous Nephropathy

Membranous nephropathy (MN) is a common cause of nephrotic syndrome in Caucasians and is characterised by the immune deposits between the lamina rara externa of the GBM and the podocyte. According to the latest studies, M-type phospholipase A2 receptor (PLA2R) located on podocytes has been identified as the target antigen in idiopathic MN. The predominant anti-PLA2R IgG subclass activates the alternative or the MBL pathway.[58] This is confirmed by some studies documenting glomerular MBL and C4b deposition in MN.[58] In human secondary MN, C1q, C3, C4, CFB, MBL, and C5b-9 are typically present and co-deposited with IgG, suggesting that the lectin and alternative pathways could play the relevant role.[58] Terminal complement activation causes insertion of the MAC into the podocyte cell membrane. Podocyte apoptosis may result from direct mechanical injury following MAC insertion or be secondary to the toxic effects of injury related chemicals.[58] Detachment of podocytes from the GBM and failure of podocyte proliferation also contribute to the onset of proteinuria and are mediated by the complement cascade.[58]

Post-Infectious Glomerulonephritis

Post-infectious glomerulonephritis, classically after *Streptococcus pyogenes* infection, is characterised by proliferative glomerulonephritis and deposition of C3 with or without immunoglobulins.[59] Although the majority of patients achieve complete remission of the associated nephritic syndrome, some experience delayed resolution or chronic glomerulonephritis, resulting in ESRD. A recent study carried out on 11 patients at the Mayo Clinic found multiple underlying causes of alternative pathway dysregulation in these chronic patients, including mutations in CFH or CFHR5 and/or the presence of C3 nephritic factors.[59]

SUMMARY

The complement system causes kidney injury in a variety of different diseases, and clinical evaluation of the complement system is an important part of the diagnostic workup of patients with glomerulonephritis (Table 2). Complement inhibition is effective for treatment of aHUS, and complement inhibitors are likely to be tested in other complement pathway associated glomerulopathies in the future. Although understanding the role of the complement system in the pathogenesis of many kidney diseases has improved, there is not a simple algorithm for identifying which patients should be treated with complement inhibitors or for how long complement inhibition should be continued.

REFERENCES

1. Noris M, Remuzzi G. Glomerular Diseases Dependent on Complement Activation, Including Atypical Hemolytic Uremic Syndrome, Membranoproliferative Glomerulonephritis, and C3 Glomerulopathy: Core Curriculum 2015. Am J Kidney Dis. 2015;66(2):359-75.

2. Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis--a new look at an old entity. N Engl J Med. 2012;366(12):1119-31.

3. Ricklin D et al. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010;11(9):785-97.

4. Boels MG et al. The endothelial glycocalyx as a potential modifier of the hemolytic uremic syndrome. Eur J Intern Med. 2013;24(6):503-9.

5. Brown KM et al. Mechanisms of disease: the complement system in renal injury – new ways of looking at an old foe. Nat Clin Pract Nephrol. 2007;3(5):277-86.

6. Caprioli J et al. International Registry of Recurrent and Familial HUS/TTP. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. Blood. 2006;108(4):1267-79.

7. Ozaltin F et al. DGKE variants cause a glomerular microangiopathy that mimics membranoproliferative GN. J Am Soc Nephrol. 2013;24(3):377-84.

8. Sethi S et al. Membranoproliferative glomerulonephritis and C3 glomerulopathy: resolving the confusion. Kidney Int. 2012;81(5):434-41.

9. Fakhouri F et al. C3 glomerulopathy: a new classification. Nat Rev Nephrol. 2010;6(8):494-9.

10. D'Agati VD, Bomback AS. C3 glomerulopathy: what's in a name? Kidney Int. 2012;82(4):379-81.

11. Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis: pathogenetic heterogeneity and proposal for a new classification. Semin Nephrol. 2011;31(4):341-8.

12. Sethi S et al. C3 glomerulonephritis: clinicopathological findings, complement abnormalities, glomerular proteomic profile, treatment, and follow-up. Kidney Int. 2012;82(4):465-73.

13. Gale DP et al. Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. Lancet. 2010;376(9743):794-801.

14. Malik TH et al. A hybrid CFHR3-1 gene causes familial C3 glomerulopathy. J Am Soc Nephrol. 2012;23(7):1155-60.

15. Bomback AS et al. Eculizumab for dense deposit disease and C3 glomerulonephritis. Clin J Am Soc Nephrol. 2012;7(5):748-56.

16. Le Quintrec M et al. Eculizumab for treatment of rapidly progressive C3 glomerulopathy. Am J Kidney Dis. 2015;65:484-9.

17. Columbia University, Alexion Pharmaceuticals. Eculizumab Therapy for Dense Deposit Disease and C3 Nephropathy. NCT01221181. https://clinicaltrials.gov/ct2/show/NCT01221181. Last accessed: 8 December 2015.

18. Mario Negri Institute for Pharmacological Research, Alexion Pharmaceuticals. Eculizumab in Primary MPGN (EAGLE). NCT02093533. https://clinicaltrials.gov/ct2/show/NCT02093533. Last accessed: 8 December 2015.

19. McCaughan JA et al. Recurrent dense deposit disease after renal transplantation: an emerging role for complementary therapies. Am J Transplant. 2012;12:1046-51.

20. Zhang Y et al. Soluble CR1 therapy improves complement regulation in C3 glomerulopathy. J Am Soc Nephrol. 2013;24(11):1820-9.

21. Rabasco C et al. Effectiveness of mycophenolate mofetil in C3 glomerulonephritis. Kidney Int. 2015;doi:10.1038/ki.2015.227.

22. Noris M et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol. 2010;5:1844-59.

23. Sellier-Leclerc AL et al; French Society of Pediatric Nephrology. Differential impact of complement mutations on clinical characteristics in atypical hemolytic uremic syndrome. J Am Soc Nephrol. 2007;18(8):2392-400.

24. Lemaire M et al. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. Nat Genet. 2013;45(5):531-6.

25. Westland R et al. Phenotypic expansion of DGKE-associated diseases. J Am Soc Nephrol. 2014;25(7):1408-14.

26. Dragon-Durey MA et al. Heterozygous and homozygous factor h deficiencies associated with hemolytic uremic syndrome or membranoproliferative glomerulonephritis: report and genetic analysis of 16 cases. J Am Soc Nephrol. 2004;15(3):787-95.

27. Skerka C et al. Autoantibodies in haemolytic uraemic syndrome (HUS). Thromb Haemost. 2009;101(2):227-32.

28. Provaznikova D et al. Manifestation of atypical hemolytic uremic syndrome caused by novel mutations in MCP. Pediatr Nephrol. 2012;27(1):73-81.

29. Bienaime F et al. Mutations in components of complement influence the outcome of Factor I-associated atypical hemolytic uremic syndrome. Kidney Int. 2010;77(4):339-49.

30. Delvaeye M et al. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361(4):345-57.

31. Caliskan Y et al. Predicting progression of C3 glomerulopathy: histological and clinical markers. Abstract SP353. 51st ERA-EDTA Congress, Amsterdam, Netherlands, 31 May-3 June, 2014.

32. Noris M et al. Dynamics of complement activation in aHUS and how to monitor eculizumab therapy. Blood. 2014;124(11):1715-26.

33. Bresin E et al. Outcome of renal transplantation in patients with non-Shiga toxin-associated hemolytic uremic syndrome: prognostic significance of genetic background. Clin J Am Soc Nephrol. 2006;1(1):88-99.

34. Licht C et al. Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. Kidney Int. 2015;87(5):1061-73.

35. Legendre CM et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 2013;368(23):2169-81.

36. Ma R et al. Complement Activation Contributes to the Injury and Outcome of Kidney in Human Anti-glomerular Basement Membrane Disease. J Clin Immunol. 2013;33(1):172-8.

37. Rui M et al. The Alternative Pathway of Complement Activation May Be Involved in the Renal Damage of Human Anti-Glomerular Basement Membrane Disease. PLoS One. 2014;9(3):e91250.

38. Chen M et al. Complement deposition in renal histopathology of patients with ANCA-associated pauci-immune glomerulonephritis. Nephrol Dial Transplant. 2009;24(4):1247-52.

39. Xing GQ et al. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. J Clin Immunol. 2009;29(3):282-91.

40. Gou SJ et al. Circulating complement activation in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. Kidney Int. 2013;83(1):129-37.

41. Schreiber A et al. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. J Am Soc Nephrol. 2009;20(2):289-98.

42. Xiao H et al. C5a receptor (CD88) blockade protects against MPO-ANCA GN. J Am Soc Nephrol. 2014;25(2):225-31.

43. Mii A et al. Current status and issues of C1q nephropathy. Clin Exp Nephrol. 2009;13(4):263-74.

44. Strassheim D et al. IgM contributes to glomerular injury in FSGS. J Am Soc Nephrol. 2013;24(3):393-406.

45. Sethi S et al. Secondary focal and segmental glomerulosclerosis associated with single-nucleotide polymorphisms in the genes encoding complement factor H and C3. Am J Kidney Dis. 2012;60(2): 316-21.

46. Bao L et al. Focal and segmental glomerulosclerosis induced in mice lacking decay-accelerating factor in T cells. J Clin Invest. 2009;119(5):1264-74.

47. Floege J et al. New insights into the pathogenesis of IgA nephropathy. Semin Immunopathol. 2014;36(4):431-42.

48. Espinosa M et al. Association of C4d deposition with clinical outcomes in IgA nephropathy. Clin J Am Soc Nephrol. 2014;9(5):897-904.

49. Lai KN. Pathogenesis of IgA nephropathy. Nat Rev Nephrol. 2012;8(5): 275-83.

50. Roos A et al. Human IgA activates the complement system via the mannan-binding lectin pathway. J Immunol. 2001;167(5):2861-8.

51. Gharavi AG et al. Genome-wide association study identifies susceptibility loci for IgA nephropathy. Nat Genet. 2011;43(4):321-7.

52. Zhu L et al. Variants in complement factor H and complement factor H-related protein genes, CFHR3 and CFHR1, effect complement activation in IgA nephropathy. J Am Soc Nephrol. 2015;26(5):1195–204.

53. Ring T et al. Use of eculizumab in crescentic IgA nephropathy: proof of principle and conundrum? Clin Kidney J. 2015;8(5):489-91.

54. Cook HT, Botto M. Mechanisms of Disease: the complement system and the pathogenesis of systemic lupus erythematosus. Nat Clin Pract Rheumatol. 2006;2(6):330-7.

55. Botto M, Walport MJ. C1q, autoimmunity and apoptosis. Immunobiology. 2002;205(4-5):395-406.

56. Seelen MA et al. Diagnostic and prognostic significance of anti-C1q antibodies in systemic lupus erythematosus. Curr Opin Nephrol Hypertens. 2003;12(6):619-24.

57. Sato N et al. Significance of glomerular activation of the alternative pathway and lectin pathway in lupus nephritis. Lupus. 2011;20(13):1378-86.

58. Ma H et al. The role of complement in membranous nephropathy. Semin Nephrol. 2013;33(6):531-42.

59. Sethi S et al. Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement. Kidney Int. 2013;83(2): 293-9.

HORMONAL THERAPIES FOR PATIENTS WITH ADVANCED PROSTATE CANCER

***Cora N. Sternberg,[1] Bertrand Tombal[2]**

1. Department of Medical Oncology, San Camillo and Forlanini Hospitals, Rome, Italy
2. Institut de Recherche Clinique (IREC), Cliniques universitaires Saint-Luc, Brussels, Belgium
**Correspondence to cnsternberg@corasternberg.com*

Disclosure: The authors have declared no conflicts of interest.
Support: The publication of this article was funded by Sandoz. The views and opinions expressed are those of the authors and not necessarily of Sandoz.
Received: 19.02.15 **Accepted:** 27.04.15
Citation: EMJ. 2016;1[1]:39-51.

ABSTRACT

Prostate cancer (PCa) is the second most common cancer in men, comprising 15% of new cancer cases. While most cases are diagnosed at an early stage and can be managed conservatively or by local treatment alone, up to 30% of patients will receive androgen deprivation therapy (ADT). Indeed, high-risk localised and locally advanced PCa require either surgery or ADT in combination with radiation as a local strategy. On the other hand, metastatic patients are treated upfront with ADT, eventually combined with docetaxel, as suggested by recent studies.

ADT has been in use for more than 60 years and during this time it has undergone considerable evolution. Gonadotropin-releasing hormone (GnRH) agonists have supplanted surgical castration and oestrogens, and are now challenged by GnRH antagonists. ADT induces profound but often short-lasting responses. In a low serum testosterone environment, the androgen receptor (AR) pathway may be reactivated either by overexpression, by mutation of the AR itself, or by adrenal or intracrine production of androgens. These mechanisms underlie the development of the majority of castration-resistant prostate cancers (CRPCs). In addition to AR adaptation, several AR-independent mechanisms may also underlie progression of these cancers on ADT.

A new generation of AR-pathway inhibitors have succeeded first-generation anti-androgens and steroids, and are proven to extend survival in patients with metastatic CRPC. This review aims to summarise the current standard of care and available hormonal strategies in advanced PCa and future therapeutic perspectives that could change treatment paradigms in the coming years.

Keywords: Prostate cancer (PCa), androgen deprivation therapy (ADT), anti-androgens (AAs), novel anti-androgen therapies, advanced prostate cancer.

INTRODUCTION

Prostate cancer (PCa) is the second most common cancer in men, comprising 15% of new cancer cases worldwide. This amounts to an overall 5-year prevalence of 1.3 million in Europe and 3.8 million worldwide.[1,2] The 5-year relative survival rate in Europe has significantly improved over the last two decades, possibly due to increased use of prostate-specific antigen (PSA) testing, which has led to higher rates of early detection and access to healthcare resources.[3] While most cases are diagnosed very early in the disease and are either treated with local treatment (i.e. prostatectomy or radiation therapy) or managed conservatively, 7–10% of patients[4,5] present with metastatic PCa at diagnosis. Moreover, up to 40% of high-risk localised and locally advanced cases will develop PSA recurrence and metastasis over the course of the disease,[6] which will require the use of androgen deprivation therapy (ADT) alone or as part of a multimodality treatment.

This review aims to summarise the current standard of care (SoC) and available strategies in these clinical settings, as well as future therapeutic perspectives that could change treatment paradigms in the coming years.

HISTORICAL EVOLUTION OF ANDROGEN DEPRIVATION THERAPY

Mechanisms of Action and General Principles of Androgen Deprivation Therapy

In 1941, Huggins and Hodges[7] established the role of androgens, particularly testosterone, in the growth and functional processes of the prostate cell (both normal and cancerous).[5] Most androgen production occurs in the testes, and testosterone secretion is regulated by the hypothalamic-pituitary-gonadal axis. The hypothalamus produces the hypothalamic gonadotropin-releasing hormone (GnRH). This hormone stimulates the production of the luteinising hormone (LH) and the follicle-stimulating hormone (FSH) by the pituitary gland, which subsequently triggers testosterone production by testicular cells.

When androgenic stimulation is removed, both normal and cancerous prostate cells undergo apoptosis. This is why ADT has become the mainstay of systemic treatment for PCa.[8] ADT can be achieved by surgical orchiectomy or by down-regulating the production of LH and FSH with GnRH agonists (GnRHa) or antagonists. In addition, intracellular androgen synthesis can be blocked by CYP17A inhibitors, and the androgen receptor (AR) may be directly inhibited by anti-androgens (AAs).[9]

When used alone in patients with locally advanced or metastatic PCa, ADT only modestly improves survival and should therefore be considered as a palliative treatment. Extensive clinical data have established its clinical outcomes, namely the normalisation of serum PSA (associated with symptom alleviation) and tumour response in approximately 90% of patients.[10]

ADT also has a beneficial impact on quality of life (QoL), bone pain control, and complication rates of PCa.[5]

ANDROGEN DEPRIVATION THERAPY MODALITIES

Oestrogens

Prior to the development of GnRHa/antagonists, oestrogens such as diethylstilbestrol were used due to their role in GnRH secretion and androgen inactivation in order to suppress serum testosterone levels. However, this treatment modality was abandoned following studies suggesting that the effects of the treatment were equivalent to orchiectomy, but with an increased risk of heart disease and stroke.[11,12] The PATCH PR09 UK trial recently investigated whether the use of oestrogen patches could avoid the long-term complications associated with GnRHa and the thromboembolic complications associated with oral oestrogens.[13] A Phase III study is currently ongoing (Clinicaltrials.gov NCT00303784) which aims to recruit 2,150 patients.[14]

Surgical and Medical Castration

Today, ADT is achieved through surgical (bilateral orchiectomy) or medical (GnRHa or antagonists) castration. The level of serum testosterone necessary for an effective castration has long been a matter of debate.[15]

Historically, the FDA has requested that medical castration therapies lower testosterone to <0.7 nM (50 ng/dL). However, it has been suggested more recently that a lower level would be optimal. Recently, Klotz et al.[16] reviewed the results of the PR-7 study that randomly assigned patients experiencing biochemical failure after radiation therapy, or surgery plus radiation therapy to continuous or intermittent ADT.

It is worth noting that patients with first-year nadir testosterone consistently >50 ng/dL had a significantly higher risk of dying due to disease (0.7–1.7 nmol/L: hazard ratio [HR], 2.08; 95% confidence interval [CI], 1.28–3.38; >1.7 nmol/L: HR, 2.93; 95% CI, 0.70–12.30) and progressing to castration resistance (0.7–1.7 nmol/L: HR, 1.62; 95% CI, 1.20–2.18; ≥1.7 nmol/L: HR, 1.90; 95% CI, 0.77–4.70). Maximum testosterone ≥1.7 nmol/L predicted for a higher risk of dying as a result of disease (p=0.02).[16]

Bilateral Orchiectomy

Bilateral orchiectomy is an inexpensive, quick, and definitive procedure. It is irreversible and thus not applicable for (neo)adjuvant strategies and intermittent ADT. Orchiectomy has been mostly abandoned in high-income countries in favour of GnRHa as, when given the choice, more than two-thirds of men prefer an injection. In 1992, Cassileth et al.[17] asked 147 men with advanced PCa what treatment they would choose for ADT: 115 selected treatment with goserelin acetate, while only 32 chose orchiectomy.[17]

MEDICAL CASTRATION WITH GONADOTROPIN-RELEASING HORMONE AGONISTS/ANTAGONISTS

Gonadotropin-Releasing Hormone Agonists

GnRHa are long-acting synthetic GnRH analogues that have been extensively used for more than 30 years, and they are currently the main forms of ADT.[18] The current therapeutic armamentarium comprises leuprorelin (leuprolide acetate), goserelin, triptorelin, buserelin, and histrelin. The options currently available mainly include monthly, tri-monthly, 6-monthly, or yearly depot preparations of intramuscular or subcutaneous injections containing long-lasting formulations.[19-21]

As such compounds are agonists, they first stimulate the pituitary secretion of LH and FSH before down-regulating them. This causes a transient rise in the secretion of testosterone. In most patients, the testosterone surge will result in a transient increase in PSA.[22] Eventually however, in patients with a high-burden metastatic or locally advanced disease, it can potentially result in increased bone pain or urinary symptoms, acute urinary retention, and even spinal cord compression.[23]

Castration is usually achieved within 2–4 weeks.[24,25] The amplitude and duration of the testosterone surge varies according to the baseline testosterone level.[26] Pre-emptive and concomitant administration of an AA, usually a non-steroidal AA (NSAA), is thus recommended for the first 4–6 weeks of treatment with a GnRHa.

However, it should be kept in mind that the AA does not suppress the testosterone surge and only partially prevents its consequences. In one of the first leuprolide trials, flare prevention with flutamide treatment demonstrated no change or worsening of pain in 73–77% of patients, performance status in 88–90%, and alkaline phosphatase in 65%.[27]

Gonadotropin-Releasing Hormone Antagonists

In contrast to agonists, GnRH antagonists directly block the GnRH receptor without inducing an initial testosterone surge, resulting in an immediate suppression of testosterone. Degarelix is the only commercially available GnRH antagonist and is currently only available as a monthly subcutaneous injection. In the registration trial of degarelix 240/80 mg, 96% of the patients achieved a testosterone level <50 ng/dL by Day 3.[25]

In addition to the rapid onset of castration, GnRH antagonists may confer several advantages including a longer PSA progression-free survival (PFS), a more rapid effect on local symptoms, and a reduced rate of urinary infection and musculoskeletal side effects,[22,28-30] but their definitive superiority over the luteinising hormone-releasing hormone (LHRH) analogues remains to be proven.[24] The main specific side effect is 'a somewhat painful injection (moderate or mild)' reported by 40% of patients, mainly after the first injection.

ANTI-ANDROGENS

First-Generation Anti-Androgens

Cyproterone acetate (CPA) is a synthetic steroidal AA that competes with androgens at the AR level and also suppresses androgen biosynthesis, thus preventing the initial testosterone surge induced by GnRHa.[31] This is why in the past it was the preferred AA for flare prevention in Europe. Steroidal AAs are associated with loss of libido, erectile dysfunction, gynaecomastia, and cardiotoxicity associated with a risk of deep venous and arterial thromboses.

NSAAs, including flutamide, nilutamide, and bicalutamide, competitively bind to ARs. When used alone, they increase serum testosterone through a feedback mechanism. This is the reason why they are not frequently used alone. A recent Cochrane Systematic Review based on studies including 3,060 patients receiving NSAA monotherapy for advanced PCa concluded that this option was less effective than ADT in terms of overall survival (OS), clinical progression, treatment failure, and treatment discontinuation due to adverse events.

However, this review included trials with several NSAAs at various doses, including low doses that were never registered.[32] Indeed, bicalutamide, at the higher dose of 150 mg, has been extensively compared to castration in patients with locally advanced T3/T4 non-metastatic disease (M0) or metastatic disease (M1), and is registered in Europe for the treatment of patients with non-metastatic disease.[33,34]

The definitive analysis for M0 patients was performed after a median follow-up of 6.3 years.[33] In that setting, there was no difference between bicalutamide 150 mg and castration in OS (HR, 1.05; p=0.70) or time to progression (HR, 1.20; p=0.11). In contrast, there was a statistically significant

benefit in the bicalutamide monotherapy group with respect to sexual interest (p=0.029) and physical capacity (p=0.046). Bicalutamide 150 mg was well tolerated, with breast pain and gynaecomastia being the most frequent side effects. Further studies confirmed that bicalutamide 150 mg induces fewer bothersome side effects than LHRH agonists, does not decrease bone mineral density, and has less impact on lipid metabolism.[35,36]

With the development of a new generation of more potent NSAAs, there is however renewed enthusiasm for the use of these drugs as monotherapies. Tombal et al.[37] investigated the efficacy of enzalutamide monotherapy in 67 patients with advanced PCa and reported a PSA decline of ≥80% in 92.5% of the patients, with mild-to-moderate toxicity. However, NSAAs are most frequently used to prevent GnRHa-induced flare or are combined over the duration of treatment to achieve complete or maximal androgen blockade (CAB or MAB, respectively).[9]

Several meta-analyses have shown that CAB provides a significant, but limited survival advantage (2–3%) when compared with GnRHa monotherapy.[38] The Prostate Cancer Trialists' Collaborative Group (PCTCG) meta-analysis demonstrated that CAB increases 5-year OS by 1.8% (p=0.11) compared with GnRHa alone, depending on the class of AAs used. CAB with nilutamide and flutamide decreases the risk of death over castration alone by 8%, which translates into a 2.9% increase in the 5-year OS. In contrast, MAB with CPA significantly increases the risk of death by 13%, therefore reducing the 5-year OS by 2.8%.

NSAAs increase the rate of several side effects versus castration alone: diarrhoea (10% versus 2%), gastrointestinal pain (7% versus 2%), and non-specific ophthalmologic events (29% versus 5%). It is important to note that none of the meta-analyses performed so far have incorporated studies with bicalutamide 50 mg, which is the most frequently used AA due to its daily dosage and low frequency of gastrointestinal and ophthalmologic adverse effects.

Second-Generation Anti-Androgens

There are additional compounds that have been developed to target the AR pathway. These mainly aim to address current treatment gaps for progressive disease following first-line therapy of castration-resistant prostate cancers (CRPC). For example, abiraterone acetate, an androgen synthesis inhibitor; and enzalutamide, an androgen signalling pathway antagonist, are two major new agents that offer additional improvement in OS for metastatic CRPC (mCRPC) patients.[39-46]

CONTEMPORARY INDICATIONS OF ANDROGEN DEPRIVATION THERAPY IN ADVANCED PROSTATE CANCER

Although hormone therapy (HT) is the mainstay of systemic therapy in PCa, its indications are still poorly recognised by many urologists outside the context of a symptomatic patient with metastatic PCa. Indeed, the answers to a simple question on the evidence provided in the literature are not unanimous regarding the appropriate timing and duration of ADT.

Metastatic Patients

Patients who initially present with disseminated disease should receive immediate ADT for surgical castration, GnRHa and a short course of AA, or GnRH antagonists. In the recently published early results of the STAMPEDE (Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy) trial, 8,000 newly diagnosed PCa patients received ADT (control arm) or experimental therapy. Nine-hundred-and-seventeen M1 PCa patients were included in the control arm and the results were published at a median follow-up of 20 months.[47] The median OS was 42 months. However, the extremely broad interquartile range (IQR) of 22.7–90.7 months suggested a very heterogeneous response to ADT.

The initial response to ADT is fairly short but very heterogeneous, with a median IQR failure-free survival of 11.2 (range: 5.1–28.8) months. This means that men diagnosed with metastatic PCa will spend three-quarters of their life in the CRPC state, receiving multiple lines of therapies.

Primary Androgen Deprivation Therapy in Non-Metastatic Patients

Many asymptomatic men receive primary ADT for localised PCa (T1-2, N0 [No regional lymph node involvement], M0) to avoid or postpone radical therapy. In 2005, Shahinian et al.[48] assessed 100,274 PCa patients from the SEER registry and reported a consistent increase in GnRHa use by year for all ages, stages, and grades from 1991–1999. Even in men ≥80 years with localised stage and low-to-moderate grade tumours, primary GnRHa use

increased over the study period. The Early Prostate Cancer trial, which included 8,113 men with M0 PCa and aimed to assess the efficacy of early treatment with bicalutamide, clearly demonstrated that there is no long-term benefit to the use of this treatment.

After a median follow-up of 7.4 years, there was a clear trend (HR, 1.16; p=0.07) towards shortened survival in patients with localised disease treated with immediate HT.[49] More recently, Potosky et al.[50] conducted a retrospective cohort study on 15,170 men diagnosed with localised PCa between 1995 and 2008 who were treated with primary ADT. Primary ADT was associated with neither a risk of all-cause mortality nor PCa-specific mortality, except among the subgroup of men with a high-risk of PCa progression.

Similarly, ADT has become the SoC for most men with an isolated rise in PSA after radical treatment. Interestingly, there is no evidence to support such immediate treatment. Instead, it has been suggested that this treatment line may only benefit a minority of patients while hurting a majority by exposing them to long-term side effects.

Two large series have retrospectively investigated the potential benefit of an immediate treatment. Moul et al.[51] reviewed a database of 4,967 patients treated by radical prostatectomy (RP), 1,352 of whom had a PSA recurrence. In the overall cohort, early ADT did not have an impact on clinical metastases. Early ADT was associated with delayed clinical metastasis only in patients with a Gleason score of >7 or a PSA doubling time of ≤12 months. Race, age at RP, and PSA at diagnosis had no effect on metastasis-free survival (p>0.05). This was recently confirmed by Garcia-Albeniz et al.[52] in a retrospective review of 2,096 patients treated with RP or radiotherapy. The adjusted mortality HR for immediate versus deferred ADT was 0.91 (95% CI, 0.52–1.60), which translated into a similar 5-year OS (difference between groups: -2.0%; 95% CI, -10.0–5.9%).

The same paradigm also applies to locally advanced PCa for which radical treatment is denied. The EORTC trial 30891 clearly suggests that ADT can be safely postponed in many patients with locally advanced PCa (T1-2, N+, M0, or T3-4, Nx, M0) who are not eligible for radical treatment. This trial, which randomised 985 patients to receive immediate ADT versus deferred ADT at symptomatic disease progression, reported a modest increase in OS in the case of immediate ADT (HR, 1.25; p>0.1) as a result of fewer non-PCa related deaths. Notably, the time from randomisation to CRPC did not differ significantly. More importantly, the median time to start deferred treatment was 7 years, and 26% of patients in the delayed ADT group died without ever receiving treatment.[53] Additional analysis of this EORTC trial suggest that only men with PSA level >50 ng/mL or with PSA doubling time <12 months are at risk of progression.[54]

Taken together, these data suggest that in many patients with asymptomatic locally advanced PCa, HT can be delayed, thus avoiding the adverse effects associated with long-term treatment. Careful selection of patients based on age, PSA kinetics, imaging, and Gleason score could aid in the identification of patients who would most benefit from HT.

Androgen Deprivation Therapy as Adjuvant to Local Therapies

In contrast to its limited benefits as a primary therapy, ADT has gained a major role in the adjuvant setting, in addition to radical therapies. Here it has been shown to significantly increase OS, especially in conjunction with radiotherapy. The only randomised trial showing a substantial advantage for immediate ADT after surgery is the ECOG trial, which compared immediate versus deferred ADT in patients with positive lymph nodes who underwent RP and pelvic lymph node dissection.[55] At follow-up (median of 11.9 years), immediate ADT significantly improved OS (HR, 1.84; p=0.04) and PCa-specific survival (HR, 4.09; p=0.0004). However, the patients in this study had higher tumour burden (e.g. seminal vesicle involvement, positive surgical margins, Gleason score of 8–10) than most contemporary patients, and recent RP series suggest that not all patients with a positive lymph node dissection require immediate HT.[56]

The benefit of ADT in combination with external beam radiation therapy (EBRT) has been extensively studied. In the EORTC 22863 trial, it was shown that 3 years of treatment with an adjuvant LHRH agonist in EBRT patients with locally advanced PCa, decreased the risk of death by 49% versus EBRT alone.[57] In the RTOG 85-31 trial, lifelong administration of an LHRH agonist decreased the risk of death by 23% versus EBRT alone.[58] The optimal duration of adjuvant ADT for

locally advanced PCa is still unclear. The EORTC 22961 trial compared 6 months with 3 years of (neo)adjuvant ADT in 1,113 patients with locally advanced PCa treated with EBRT. A slight OS benefit in favour of long-term ADT was demonstrated.[59] In the trial, 5-year overall mortality for short-term and long-term suppression was 19.0% and 15.2%, respectively; the observed HR was 1.42. The benefit of extending the duration of ADT beyond 6 months should therefore be discussed with the patient as regards to the long-term toxicity of ADT.

A shorter adjuvant HT treatment is also beneficial for patients with high-risk localised PCa (i.e. Gleason score >7 or PSA >20 ng/mL or Stage T2c) who are primarily treated with EBRT. In a trial conducted by D'Amico et al.,[60] 206 patients were randomised to receive EBRT alone or combined with HT administered over 6 months. After a median follow-up of 4.5 years, the ADT/EBRT combination was associated with a longer time to PSA recurrence (HR, 0.22; p<0.001), PCa-specific mortality (HR, 0.23; p=0.005), and all-cause mortality (HR, 0.30; p<0.001).

Intermittent Androgen Deprivation Therapy

The rationale for using intermittent androgen deprivation (IAD) therapy has arisen from studies conducted in an animal model of Shionogi mammary carcinoma in the early 1990s. These studies suggest that IAD prolongs the duration of androgen dependence.[61] This generated the hypothesis that IAD would delay the onset of CRPC and its associated complications, which are both debilitating and deadly.

Several trials have investigated the role of IAD, including four large Phase III randomised trials, from which core evidence has been produced. The South European Uroncological Group (SEUG) trial enrolled 766 patients with locally advanced or metastatic PCa.[62] FinnProstate Study VII enrolled 852 locally advanced or metastatic patients.[63] The NCIC Clinical Trials Group enrolled 1,386 patients with a PSA level >3 ng/mL more than 1 year after primary or salvage radiotherapy for localised PCa.[64] Finally, The SWOG trial 9346, randomised 3,040 metastatic PCa patients.

Several meta-analyses and systemic reviews have been conducted, and generally discussed the equivalence of IAD versus continuous ADT. In a systematic review by Niraula et al.,[65] nine studies with 5,508 patients met criteria for inclusion. There were no significant differences in time-to-event outcomes between the groups in the studies. The pooled HR for OS was 1.02 (95% CI, 0.94–1.11) for IAD compared with CAD, and the HR for PFS was 0.96 (95% CI, 0.76–1.20). More PCa-related deaths with IAD were balanced by more deaths not related to PCa with CAD. Superiority of IAD for sexual function, physical activity, and general wellbeing was observed in some trials. Median cost savings with IAD were estimated to be 48%.

However, some important facts should be mentioned about IAD. Firstly, the Phase III trials were based on a conditional randomisation methodology, thus only including patients who had experienced a major PSA decrease. Secondly, most of the trials reported an increase in PCa-related deaths with IAD that is to be balanced by more deaths not related to PCa with continuous ADT.[65] Thirdly, although some trials report a benefit in QoL, it is important to mention that none of these trials used a placebo control, and compared men in whom treatment was suspended: men left with the disappointment of having to prolong ADT. This is important, considering that the recovery of normal testosterone is unpredictable and usually slow when agonists are suspended.[66]

Finally, the role of ADT in M1 PCa patients is still controversial, considering the results of SWOG 9346.[67] The study was designed to show that IAD was not inferior to CAD in terms of OS post-randomisation. In total, 3,040 metastatic PCa patients were recruited, but after 7 months of CAB, only 1,535 patients had achieved a PSA of ≤4.0 ng/mL and were randomised. The median and 10-year OS rates from randomisation were 5.8 years and 29% for CAD, and 5.1 years and 23% for IAD. Therefore the studies failed to prove that IAD was non-inferior to CAD (HR, 1.09; 95% CI, 0.95–1.24). Patients were stratified by disease extent using a definition of minimal disease (spine, pelvis bone metastases, and/or lymph nodes) versus extensive disease (>4 bone metastases with at least 1 beyond pelvis and vertebral column, and/or visceral disease [lung or liver]). The median OS of patients with extensive disease patients was 4.9 years for IAD and 4.4 years for CAD; in patients with minimal disease, median OS was 5.4 years for IAD and 6.9 years for CAD.

THE ROLE OF HORMONE THERAPY IN THE TREATMENT OF CASTRATION-RESISTANT PROSTATE CANCER

After initial response to ADT therapy, most patients with advanced or metastatic PCa will eventually progress to CRPC, defined by several rises in PSA or by clinical or radiological progression of disease (based on RECIST criteria).[68]

In the last 10 years, it has been demonstrated that most patients progressed in a low testosterone environment through reactivation of AR pathways. Several mechanisms have been involved, including two that can be addressed with modern drugs. Taxane-based chemotherapy with docetaxel plus prednisone was established as the SoC for first-line therapy in CRPC[69,70] after two Phase III trials demonstrated the benefits of docetaxel and prednisone on OS, 3-year survival, and PSA response rates, compared with mitoxantrone and prednisone.[71,72] This has been the SoC since 2004, but the scenario has changed with the arrival of novel HTs into the clinic.

Switching Gonadotropin-Releasing Hormone Agonists at the Time of Castration-Resistant Prostate Cancer Progression

The late reactivation of the AR-underlying CRPC may arise from GnRHa losing their efficacy over time, with the consequence that testosterone increases above the castration level, a phenomenon known as testosterone breakthrough.[73] In a retrospective study by Morote et al.,[74] PSA PFS was 88 and 137 months, respectively, in patients with or without testosterone breakthroughs >32 ng/dL ($p<0.03$).

Lawrentschuk et al.[75] investigated the benefit of re-challenging 39 CRPC patients with a different GNRHa. Sixty-nine percent of the patients experienced a PSA decrease, and the median PSA decrease was 69.3% in patients switching from leuprolide to goserelin, and 6.4% in patients switching from goserelin to leuprolide.[75] Practically, this stresses the importance of measuring each patient's testosterone levels in case of CRPC progression and eventually adapting therapy to optimise testosterone control.

Role of First-Generation Hormonal Therapies in the Management of Castration-Resistant Prostate Cancer Progression

In the absence of curative second-line treatment, most physicians have historically prescribed various AAs such as bicalutamide, flutamide, or nilutamide; adrenal synthesis inhibitors such as ketoconazole or aminoglutethimide; oestrogens and derivatives, or steroids including prednisone, prednisolone, hydrocortisone, or dexamethasone.

These drugs have mostly been tested in small Phase II trials with PSA response and PFS as the main endpoints. These trials were extensively reviewed by Tombal in 2012.[76] Overall, a PSA response decrease of >50% was observed in 25–65% of patients for durations of 3–6 months. With the exception of low-dose bicalutamide, none of these agents have been compared with modern AR pathway inhibitors such as abiraterone and enzalutamide. The TERRAIN trial compared bicalutamide 50 mg with enzalutamide 160 mg in 375 asymptomatic or mildly symptomatic mCRPC patients prior to chemotherapy.[77] Median PFS was 15.7 months in the enzalutamide arm compared with 5.8 months in the bicalutamide arm (HR, 0.44; 95% CI, 0.34–0.57; $p<0.0001$). Serious adverse events were experienced by 31.1% and 23.3% of patients treated with enzalutamide or bicalutamide, respectively.[77]

The remaining role of these agents in the modern era was discussed during the recent St Gallen Advanced Prostate Cancer Consensus Conference (APCCC) in 2015.[78] A majority (52%) of the panellists felt that these older agents are not appropriate treatment options for a patient who is not considered a candidate for chemotherapy, where abiraterone and enzalutamide are available and reimbursed. Nevertheless, 32% of the panellists would still recommend them in a minority of select patients and 16% suggested that they would recommend these agents in a majority of patients. However, all panel members considered it appropriate to use these first-generation AAs, if abiraterone and enzalutamide are unavailable.

MODERN ANDROGEN RECEPTOR PATHWAY INHIBITORS

Abiraterone

Abiraterone acetate is a CYP17A (α-hydroxylase) enzyme inhibitor and thus an androgen biosynthesis inhibitor. In CRPC patients, it primarily acts to inhibit the synthesis of androgens at the adrenal level and in PCa cells that upregulate CYP14A. Through a feedback mechanism, it increases adrenocorticotropic hormone and generates a mineralocorticoid excess, potentially

causing side effects such as hypertension, hypokalaemia, and lower-limb oedema. When administered with prednisone 5 mg twice daily, it shows an excellent tolerability profile. Abiraterone is also converted to a more active compound D4A with dual function: a steroidogenic enzyme blocker and a potent AR antagonist, and thus may have more than one mechanism of action.[79]

Abiraterone/prednisone has demonstrated improved OS in both docetaxel-naïve and docetaxel-treated patients in key Phase III trials.[42,45,80,81] In the first Phase III study in 1,195 docetaxel-treated mCRPC patients (COU-AA-301),[42,43,80] OS with abiraterone and prednisone was improved over placebo and prednisone (median 15.8 months versus 11.2 months; HR, 0.74; 95% CI, 0.64–0.86). Statistically significant outcomes were also reported for time to PSA progression and PFS.

Similar results were observed in asymptomatic or mildly symptomatic chemotherapy-naïve patients with no visceral metastases, particularly in a Phase III trial of 1,088 CRPC patients who were randomised to either abiraterone plus prednisone or placebo plus prednisone (COU-AA-302).[44,81] In the study, Abiraterone and prednisone doubled the time to radiological progression-free survival (rPFS) compared with placebo and prednisone (HR,0.52; 95% CI, 0.45-0.61), p<0.0001).[44] After a median follow-up of 49.2 months, OS was significantly higher in the abiraterone arm (median 34.7 months versus 30.3 months; HR, 0.81; 95% CI, 0.70–0.93).[39,45] Abiraterone treatment effect was more pronounced when adjusting for the 44% of placebo prednisone patients who subsequently received abiraterone (HR=0.74). After a median follow-up of 49.2 months, OS was significantly higher in the abiraterone arm (median 34.7 months versus 30.3 months; HR, 0.81; 95% CI, 0.70-0.93).[39,45]

In these patients, clinically significant endpoints in the COU-AA-302 trial, and early use of abiraterone and prednisone in asymptomatic or mildly symptomatic patients, are the delay in the time to chemotherapy by 8.4 months, median time to progression of worse pain intensity by 7.3 months, and median time to functional status deterioration (FACT-P total score) by 4.4 months.

Enzalutamide

Enzalutamide is an AR-signalling inhibitor that targets multiple steps in the AR signalling pathway.[82] It blocks androgen binding to AR, prevents nuclear translocation of AR, and impairs AR binding to DNA, thus preventing modulation of gene expression.[82,83] Enzalutamide is an AR antagonist with higher AR affinity than bicalutamide in cells overexpressing the AR.[82]

The AFFIRM trial randomised 1,199 patients post-docetaxel between enzalutamide and placebo.[41,46] After a median follow-up of 14.4 months, improved outcomes were observed in the enzalutamide group versus placebo, both in terms of median survival (18.4 months versus 13.6 months) and PSA response, as well as PFS and QoL.

In the Phase III PREVAIL study,[40] which aimed to evaluate the efficacy and safety of enzalutamide in 1,717 mCRPC patients who were asymptomatic or mildly symptomatic and chemotherapy-naïve, median OS (HR=0.71; p<0.0001) was significantly higher in the enzalutamide arm compared with placebo. Enzalutamide reduced the risk of radiographic progression by 81% (HR, 0.19; 95% CI, 0.15–0.23; p<0.001). This trial differed from the COU-AA-302 trial in that there was no prednisone in either the active or placebo arm, and patients with visceral metastases (lung or liver metastases) were permitted into the study. The trial led to the extension of the indication in chemotherapy-naïve patients by the European Medicines Agency (EMA) in October 2014.[84]

Similar to COU-AA-302, the PREVAIL trial was highly remarkable for its secondary endpoints. Indeed, enzalutamide delayed the time to chemotherapy by 17.2 months, median time until decline in relation to the FACT-P global score by 5.7 months, and time to pain progression (by FACT-P) by 5.5 months.[85]

FUTURE OUTLOOK ON ANDROGEN DEPRIVATION THERAPY

Early Combination of Androgen Deprivation Therapy with Docetaxel

ADT may be given with upfront docetaxel chemotherapy in patients presenting with metastatic hormone-sensitive disease, particularly those with high volume disease (HVD), defined as: presence of visceral metastases or four bone lesions, where at least one of them is outside the axial skeleton.

In the CHAARTED trial, 790 men with such criteria (65% of whom had HVD) received ADT plus six cycles of docetaxel without prednisone as a

first-line treatment compared with ADT alone.[86,87] After a median follow-up of 29 months, OS was significantly improved over ADT alone (58 versus 44 months, respectively). Most notable were the results observed in patients from the HVD subgroup. A median difference in OS of 17 months (49 versus 32 months) was observed.

This study differs from what was found in the French GETUG-15 trial,[86] which primarily contained a lower-risk patient group (only 21% in high-risk Glass group). Clinical PFS and biochemical PFS were significantly improved but no OS difference was seen with the addition of up to nine cycles of docetaxel chemotherapy.

At ASCO GU 2015, updated results of the GETUG-15 trial were presented with a longer follow-up of about 80 months. These results showed that the median OS was 46.5 months in the ADT arm and 60.9 months in the ADT with docetaxel arm (HR, 0.9; 95% CI, 0.7–1.2). In a retrospective analysis, which used the same definition of HVD as the CHAARTED trial discussed below, the subgroup of patients with HVD showed a median OS of 35.1 months in the ADT alone arm, compared with 39 months in the ADT plus chemotherapy arm (HR, 0.8; 95% CI, 0.6–1.2). The outcomes in HVD patients were similar to those in the CHAARTED trial, however the trial showed a non-significant improvement in OS with ADT with docetaxel of about 4 months.[88,89]

Additional data have come from the ongoing STAMPEDE trial, which is also evaluating the role of ADT plus docetaxel therapy in patients with locally advanced and metastatic disease.[90] The first OS results were presented at the ASCO meeting in June 2015.[47,91] Data from 2,962 hormone-naïve men from four of the study's nine arms revealed that after a median follow-up of 42 months, median OS was 77 months in the SoC plus docetaxel arm versus 67 months in the SoC arm alone. This translated into a 24% reduction in the risk of death associated with docetaxel chemotherapy and ADT (HR, 0.76; 95% CI, 0.63-0.91). The results are most remarkable in metastatic patients as opposed to the M0 population. In the metastatic PCa patient subpopulation, there was a 22-month difference in OS (65 versus 43 months) between both arms, respectively.

Vale et al.[92] have conducted a systematic review of these three trials and have shown that the addition of docetaxel to SoC improves 4-year survival by 9% (95% CI, 5-14; HR, 0.77; 95% CI, 0.68-0.87; p<0.0001).[92]

Neoadjuvant Therapy and Rising Prostate-Specific Antigen: Combinations with Novel Anti-Androgens

Few clinical data are currently available to determine whether additional clinical benefit can be obtained by combining GnRHa with second-generation AAs in the neoadjuvant setting. In a Phase II randomised neoadjuvant study, 58 patients with high-risk PCa received intense ADT with leuprolide plus abiraterone. The combination seemed more effective than leuprolide alone, as intratumoural androgen levels were significantly lower in the combination group for a higher total pathological response (34% versus 15%).[93]

A Phase II study is currently recruiting patients to evaluate 'enzalutamide plus leuprolide' versus 'enzalutamide plus leuprolide, abiraterone, and prednisone' as neoadjuvant therapy for high-risk PCa patients undergoing prostatectomy.[94] A Phase III, randomised efficacy and safety study will soon begin recruitment to evaluate 'enzalutamide plus leuprolide, enzalutamide monotherapy', and 'placebo plus leuprolide' in men with high-risk non-metastatic PCa progressing after definitive therapy.[95] Other novel agents such as ARN-509 are also being evaluated in combination with abiraterone.[96]

Adverse Events and Quality of Life of Patients Undergoing Androgen Deprivation Therapy

ADT remains the cornerstone of advanced PCa treatment, and is now used as a neoadjuvant or adjuvant therapy with radiotherapy in earlier stages. Adverse events and reduced QoL in men undergoing ADT must be considered before widening selection criteria for ADT use in PCa patients, especially since many PCa patients are aged 65 years and above, and may present with several comorbidities. Numerous adverse events have been reported with ADT: hot flushes, loss of libido, sexual dysfunction, gynaecomastia, decrease in bone mineral density, increase in fat mass associated with loss of lean muscle mass, metabolic syndrome, diabetes, and increased risk of myocardial infarction and cardiovascular disease in general.[97-101]

Short and long-term ADT can be associated with impaired QoL, including decreased short-term mental, cognitive, and emotional well-being, as well

as physical symptoms. Patients often self-report memory loss, depressive symptoms, insomnia or sleep disturbances, difficulty in concentrating, and nervousness.[102-105] The physical impact of ADT comprises bone density changes and fractures, loss of muscle strength, and fatigue. A third cluster of symptoms comprises relationship and affective symptoms, often triggered by gynaecomastia and functional changes in sexuality and sexual organs.[106-109] Amongst the measures that can alleviate the side effects of ADT, supervised resistance training exercise is expected to play a major role. Several trials have examined whether various exercise strategies can counteract the metabolic effects of ADT.[110]

Cost-Effectiveness of Androgen Deprivation Therapy in Advanced Prostate Cancer

In a meta-analysis published in 2000, the cost-effectiveness of ADT was evaluated in advanced PCa. Orchiectomy was established as the most cost-effective ADT modality, while CAB was the least economically effective option.[111] While the combination of ADT with docetaxel or novel AAs may generate higher costs, some GnRHa have generic formulations that are already on the market, which could help to improve the cost-efficacy of the management of advanced PCa, lower overall costs, and increase patient access to therapy.

CONCLUSION

ADT in advanced PCa has gone through considerable evolution, but still remains a cornerstone of the therapeutic armamentarium in the treatment of PCa. Its contemporary role in advanced PCa, particularly with second-generation AAs, has emerged as a therapeutic modality in select settings for both castration-sensitive and castration-resistant advanced PCa. ADT in combination with novel hormonal agents is now being explored in the neoadjuvant setting, and chemotherapy in combination with ADT must be seriously considered in selected patients after the results of recent randomised trials. Upcoming clinical data will help to further refine risk stratification and optimal strategies in advanced PCa, particularly in light of benefit-to-risk ratios, QoL, and pharmacoeconomic considerations.

Acknowledgements

Medical writing assistance was provided by Dr Caroline Charles (Scilink Medical Writing, Biarritz, France).

REFERENCES

1. Ferlay J et al; International Agency for Research on Cancer. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. 2013. Available at: http://globocan.iarc.fr. Last accessed: 12 January 2016.

2. Arnold M et al. Recent trends in incidence of five common cancers in 26 European countries since 1988: Analysis of the European Cancer Observatory. Eur J Cancer. 2015;51(9):1164-87.

3. De Angelis R et al. Cancer survival in Europe 1999-2007 by country and age: results of EUROCARE--5-a population-based study. Lancet Oncol. 2014;15(1): 23-34.

4. Pokorny MR, Scott DJ. Do Maori and Pacific Islander men present with more advanced prostate cancer than European New Zealand men? An analysis of 486 men undergoing biopsy in Auckland. BJU international. 2011;107 Suppl 3:27-32.

5. Yap TA et al. The changing therapeutic landscape of castration-resistant prostate cancer. Nat Rev Clin Oncol. 2011;8(10): 597-610.

6. Beltran H et al. New therapies for castration-resistant prostate cancer: efficacy and safety. Eur Urol. 2011;60(2):279-90.

7. Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. CA Cancer J Clin. 1972;22(4):232-40.

8. Sharifi N et al. Androgen deprivation therapy for prostate cancer. JAMA. 2005;294(2):238-44.

9. Loblaw DA et al; American Society of Clinical Oncology. Initial hormonal management of androgen-sensitive metastatic, recurrent, or progressive prostate cancer: 2006 update of an American Society of Clinical Oncology practice guideline. J Clin Oncol. 2007;25(12):1596-605.

10. Dai B et al. Kinetics of testosterone recovery in clinically localized prostate cancer patients treated with radical prostatectomy and subsequent short-term adjuvant androgen deprivation therapy. Asian J Androl. 2013;15(4): 466-70.

11. Byar DP. Proceedings: The Veterans Administration Cooperative Urological Research Group's studies of cancer of the prostate. Cancer.1973;32(5):1126-30.

12. Byar DP, Corle DK. Hormone therapy for prostate cancer: results of the Veterans Administration Cooperative Urological Research Group studies. NCI Monogr. 1988;(7):165-70.

13. Langley RE et al. Cardiovascular outcomes in patients with locally advanced and metastatic prostate cancer treated with luteinising-hormone-releasing-hormone agonists or transdermal oestrogen: the randomised, phase 2 MRC PATCH trial (PR09). Lancet Oncol. 2013;14(4):306-16.

14. National Cancer Institute; Imperial College London. Hormone Therapy in Treating Patients With Locally Advanced or Metastatic Prostate Cancer.

NCT00303784. https://clinicaltrials.gov/ct2/show/NCT00303784.

15. Djavan B et al. Testosterone in prostate cancer: the Bethesda consensus. BJU Int. 2012;110(3):344-52.

16. Klotz L et al. Nadir testosterone within first year of androgen-deprivation therapy (ADT) predicts for time to castration-resistant progression: a secondary analysis of the PR-7 trial of intermittent versus continuous ADT. J Clin Oncol. 2015;33(10):1151-6.

17. Cassileth BR et al. Quality of life and psychosocial status in stage D prostate cancer. Zoladex Prostate Cancer Study Group. Qual Life Res. 1992;1(5):323-9.

18. Tolis G et al. Tumor growth inhibition in patients with prostatic carcinoma treated with luteinizing hormone-releasing hormone agonists. Proc Natl Acad Sci U S A. 1982;79(5):1658-62.

19. Fowler JE et al. Evaluation of an implant that delivers leuprolide for 1 year for the palliative treatment of prostate cancer. Urology. 2000;55(5):639-42.

20. Fowler JE et al. Safety and efficacy of an implantable leuprolide delivery system in patients with advanced prostate cancer. J Urol. 2000;164(3 Pt 1):730-4.

21. Geiges G et al. Clinical development of two innovative pharmaceutical forms of leuprorelin acetate. Ther Adv Urol. 2013;5(1):3-10.

22. Tombal B et al. Additional analysis of the secondary end point of biochemical recurrence rate in a phase 3 trial (CS21) comparing degarelix 80 mg versus leuprolide in prostate cancer patients segmented by baseline characteristics. Eur Urol. 2010;57(5):836-42.

23. Waxman J et al. Importance of early tumour exacerbation in patients treated with long acting analogues of gonadotrophin releasing hormone for advanced prostatic cancer. Br Med J. 1985;291(6506):1387-8.

24. Mottet N et al. EAU Guidelines on Prostate Cancer 2015. 2015. Accessible at: http://uroweb.org/guideline/prostate-cancer/. Last accessed: 30 November 2015.

25. Van Poppel H et al. Degarelix: a novel gonadotropin-releasing hormone (GnRH) receptor blocker--results from a 1-yr, multicentre, randomised, phase 2 dosage-finding study in the treatment of prostate cancer. Eur Urol. 2008;54(4):805-13.

26. Damber JE et al. The effect of baseline testosterone on the efficacy of degarelix and leuprolide: further insights from a 12-month, comparative, phase III study in prostate cancer patients. Urology. 2012;80(1):174-80.

27. Crawford ED et al. A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. N Engl J Med. 1989;321(7):419-24.

28. Klotz L et al. Disease control outcomes from analysis of pooled individual patient data from five comparative randomised clinical trials of degarelix versus luteinising hormone-releasing hormone agonists. Eur Urol. 2014;66(6):1101-8.

29. Albertsen PC et al. Cardiovascular morbidity associated with gonadotropin releasing hormone agonists and an antagonist. Eur Urol. 2014;65(3):565-73.

30. Axcrona K et al. Androgen deprivation therapy for volume reduction, lower urinary tract symptom relief and quality of life improvement in patients with prostate cancer: degarelix vs goserelin plus bicalutamide. BJU Int. 2012;110(11):1721-8.

31. Sugiono M et al. Bicalutamide vs cyproterone acetate in preventing flare with LHRH analogue therapy for prostate cancer--a pilot study. Prostate Cancer Prostatic Dis. 2005;8(1):91-4.

32. Kunath F et al. Non-steroidal antiandrogen monotherapy compared with luteinising hormone-releasing hormone agonists or surgical castration monotherapy for advanced prostate cancer. Cochrane Database Syst Rev. 2014;6:CD009266.

33. Iversen P et al. Bicalutamide monotherapy compared with castration in patients with nonmetastatic locally advanced prostate cancer: 6.3 years of followup. J Urol. 2000;164(5):1579-82.

34. Iversen P et al. Casodex (bicalutamide) 150-mg monotherapy compared with castration in patients with previously untreated nonmetastatic prostate cancer: results from two multicenter randomized trials at a median follow-up of 4 years. Urology. 1998;51(3):389-96.

35. Smith MR et al. Cross-sectional study of bone turnover during bicalutamide monotherapy for prostate cancer. Urology. 2003;61(1):127-31.

36. Smith MR et al. Bicalutamide monotherapy versus leuprolide monotherapy for prostate cancer: effects on bone mineral density and body composition. J Clin Onc. 2004;22(13):2546-53.

37. Tombal B et al. Enzalutamide monotherapy in hormone-naive prostate cancer: primary analysis of an open-label, single-arm, phase 2 study. Lancet Oncol. 2014;15(6):592-600.

38. Maximum androgen blockade in advanced prostate cancer: an overview of the randomised trials. Prostate Cancer Trialists' Collaborative Group. Lancet. 2000;355(9214):1491-8.

39. Ryan CJ et al. Final overall survival (OS) analysis of COU-AA-302, a randomized phase 3 study of abiraterone acetate (AA) in metastatic castration-resistant prostate cancer (mCRPC) patients (pts) without prior chemotherapy. Abstract 753O. 2014 European Society of Medical Oncology (ESMO) meeting, Madrid, Spain, 26-30 September 2014.

40. Beer TM, Tombal B. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med. 2014;371(18):1755-6.

41. Fizazi K et al. Effect of enzalutamide on time to first skeletal-related event, pain, and quality of life in men with castration-resistant prostate cancer: results from the randomised, phase 3 AFFIRM trial. Lancet Oncol. 2014;15(10):1147-56.

42. Fizazi K et al. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 2012;13(10):983-92.

43. Logothetis CJ et al. Effect of abiraterone acetate and prednisone compared with placebo and prednisone on pain control and skeletal-related events in patients with metastatic castration-resistant prostate cancer: exploratory analysis of data from the COU-AA-301 randomised trial. Lancet Oncol. 2012;13(12):1210-7.

44. Rathkopf DE et al. Updated Interim Efficacy Analysis and Long-term Safety of Abiraterone Acetate in Metastatic Castration-resistant Prostate Cancer Patients Without Prior Chemotherapy (COU-AA-302). Eur Urol. 2014;66(5):815-25.

45. Ryan CJ et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 2015;16(2):152-60.

46. Scher HI et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012;367(13):1187-97.

47. James ND et al. Survival with Newly Diagnosed Metastatic Prostate Cancer in the "Docetaxel Era": Data from 917 Patients in the Control Arm of the STAMPEDE Trial (MRC PR08, CRUK/06/019). Eur Urol. 2015;67(6):1028-38.

48. Shahinian VB et al. Increasing use of gonadotropin-releasing hormone agonists for the treatment of localized prostate carcinoma. Cancer. 2005;103(8):1615-24.

49. McLeod DG et al. Bicalutamide 150 mg plus standard care vs standard care alone for early prostate cancer. BJU Int. 2006;97(2):247-54.

50. Potosky AL et al. Effectiveness of primary androgen-deprivation therapy for clinically localized prostate cancer. J Clin Oncol. 2014;32(13):1324-30.

51. Moul JW et al. Early versus delayed

hormonal therapy for prostate specific antigen only recurrence of prostate cancer after radical prostatectomy. J Urol. 2004;171(3):1141-7.

52. Garcia-Albeniz X et al. Immediate versus deferred initiation of androgen deprivation therapy in prostate cancer patients with PSA-only relapse. An observational follow-up study. Eur J Cancer. 2015;51(7):817-24.

53. Studer UE et al. Differences in time to disease progression do not predict for cancer-specific survival in patients receiving immediate or deferred androgen-deprivation therapy for prostate cancer: final results of EORTC randomized trial 30891 with 12 years of follow-up. Eur Urol. 2014;66(5):829-38.

54. Studer UE et al. Using PSA to guide timing of androgen deprivation in patients with T0-4 N0-2 M0 prostate cancer not suitable for local curative treatment (EORTC 30891). Eur Urol. 2008;53(5): 941-9.

55. Messing EM et al. Immediate versus deferred androgen deprivation treatment in patients with node-positive prostate cancer after radical prostatectomy and pelvic lymphadenectomy. Lancet Oncol. 2006;7(6):472-9.

56. Briganti A et al. Two positive nodes represent a significant cut-off value for cancer specific survival in patients with node positive prostate cancer. A new proposal based on a two-institution experience on 703 consecutive N+ patients treated with radical prostatectomy, extended pelvic lymph node dissection and adjuvant therapy. Eur Urol. 2009;55(2):261-70.

57. Bolla M et al. Long-term results with immediate androgen suppression and external irradiation in patients with locally advanced prostate cancer (an EORTC study): a phase III randomised trial. Lancet. 2002;360(9327):103-6.

58. Pilepich MV et al. Androgen suppression adjuvant to definitive radiotherapy in prostate carcinoma--long-term results of phase III RTOG 85-31. Int J Radiat Oncol Biol Phys. 2005;61(5): 1285-90.

59. Bolla M et al. Duration of androgen suppression in the treatment of prostate cancer. N Engl J Med. 2009;360(24): 2516-27.

60. D'Amico AV et al. Six-month androgen suppression plus radiation therapy compared with radiation therapy alone for men with prostate cancer and a rapidly increasing pretreatment prostate-specific antigen level. J Clin Oncol. 2006;24(25):4190-5.

61. Akakura K et al. Effects of intermittent androgen suppression on androgen-dependent tumors. Apoptosis and serum prostate-specific antigen. Cancer. 1993;71(9):2782-90.

62. Calais da Silva FE et al. Intermittent androgen deprivation for locally advanced and metastatic prostate cancer: results from a randomised phase 3 study of the South European Uroncological Group. Eur Urol. 2009;55(6):1269-77.

63. Salonen AJ et al. The FinnProstate Study VII: intermittent versus continuous androgen deprivation in patients with advanced prostate cancer. J Urol. 2012;187(6):2074-81.

64. Crook JM et al. Intermittent androgen suppression for rising PSA level after radiotherapy. N Engl J Med. 2012;367(10):895-903.

65. Niraula S et al. Treatment of prostate cancer with intermittent versus continuous androgen deprivation: a systematic review of randomized trials. J Clin Oncol. 2013;31(16):2029-36.

66. Kaku H et al. Time course of serum testosterone and luteinizing hormone levels after cessation of long-term luteinizing hormone-releasing hormone agonist treatment in patients with prostate cancer. Prostate. 2006;66(4):439-44.

67. Hussain M et al. Intermittent versus continuous androgen deprivation in prostate cancer. N Engl J Med. 2013;368(14):1314-25.

68. Fitzpatrick JM et al. Optimal management of metastatic castration-resistant prostate cancer: highlights from a European Expert Consensus Panel. Eur J Cancer. 2014;50(9):1617-27.

69. Heidenreich A et al; European Association of Urology. EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer. Eur Urol. 2014;65(2):467-79.

70. Basch E et al. Systemic therapy in men with metastatic castration-resistant prostate cancer: American Society of Clinical Oncology and Cancer Care Ontario clinical practice guideline. J Clin Oncol. 2014;32(30):3436-48.

71. Petrylak DP et al. Evaluation of prostate-specific antigen declines for surrogacy in patients treated on SWOG 99-16. J Natl Cancer Inst. 2006;98(8): 516-21.

72. Tannock IF et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med. 2004;351(15):1502-12.

73. Tombal B, Berges R. Optimal control of testosterone: a clinical case-based approach of modern androgen-deprivation therapy. Eur Urol Suppl. 2008;7(1):15-21.

74. Morote J et al. Redefining clinically significant castration levels in patients with prostate cancer receiving continuous androgen deprivation therapy. J Urol. 2007;178(4 Pt 1):1290-5.

75. Lawrentschuk N et al. Efficacy of a second line luteinizing hormone-releasing hormone agonist after advanced prostate cancer biochemical recurrence. J Urol. 2011;185(3):848-54.

76. Tombal B. Non-metastatic CRPC and asymptomatic metastatic CRPC: which treatment for which patient? Ann Oncol. 2012;23 Suppl 10:x251-8.

77. Baskin-Bey ES et al. TERRAIN: A randomized, double-blind, phase II study comparing MDV3100 with bicalutamide (Bic) in men with metastatic castrate-resistant prostate cancer (CRPC). Abstract TPS4698^. ASCO Annual Meeting, Chicago, IL, USA, 1–5 June 2012.

78. Gillessen S et al. Management of patients with advanced prostate cancer: recommendations of the St Gallen Advanced Prostate Cancer Consensus Conference (APCCC) 2015. Ann Oncol. 2015;26(8):1589-604.

79. Li Z et al. Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. Nature. 2015;523(7560):347-51.

80. de Bono JS et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011;364(21): 1995-2005.

81. Ryan CJ et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med. 2013;368(2):138-48.

82. Tran C et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science. 2009;324(5928):787-90.

83. Hu R et al. Molecular processes leading to aberrant androgen receptor signaling and castration resistance in prostate cancer. Expert Rev Endocrinol Metab. 2010;5(5):753-64.

84. European Medicines Agency (EMA). Meeting highlights from the Committee for Medicinal Products for Human Use (CHMP) 20–23 October 2014. 2014. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2014/10/news_detail_002191.jsp&mid=WC0b01ac058004d5c1. Last Accessed: 17 January 2014.

85. Loriot Y et al. Effect of enzalutamide on health-related quality of life, pain, and skeletal-related events in asymptomatic and minimally symptomatic, chemotherapy-naive patients with metastatic castration-resistant prostate cancer (PREVAIL): results from a randomised, phase 3 trial. Lancet Oncol. 2015;16(5):509-21.

86. Gravis G et al. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. Lancet Oncol. 2013;14(2):149-58.

87. Sweeney CJ et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med. 2015;373(8):737-46.

88. Gravis G et al. Androgen deprivation therapy plus docetaxel versus ADT alone for hormone-naive metastatic prostate cancer: Long-term analysis of the GETUG-AFU 15 phase III trial. Abstract 140. Genitourinary Cancers Symposium. Orlando, Florida, 26–28 February 2015.

89. Gravis G et al. Androgen Deprivation Therapy (ADT) Plus Docetaxel Versus ADT Alone in Metastatic Non castrate Prostate Cancer: Impact of Metastatic Burden and Long-term Survival Analysis of the Randomized Phase 3 GETUG-AFU15 Trial. Eur Urol. 2015. [Epub ahead of print].

90. Medical Research Council. STAMPEDE: Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy: A Multi-Stage Multi-Arm Randomised Controlled Trial. NCT00268476. http://www.clinicaltrials.gov/ct2/show/NCT00268476.

91. James ND et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. Lancet. 2015. [Epub ahead of print].

92. Vale CL et al. Addition of docetaxel or bisphosphonates to standard of care in men with localised or metastatic, hormone-sensitive prostate cancer: a systematic review and meta-analyses of aggregate data. Lancet Oncol. 2015. [Epub ahead of print].

93. Taplin ME et al. Intense androgen-deprivation therapy with abiraterone acetate plus leuprolide acetate in patients with localized high-risk prostate cancer: results of a randomized phase II neoadjuvant study. J Clin Oncol. 2014;32(33):3705-15.

94. Dana-Farber Cancer Institute; Medivation, Inc. Enzalutamide/Leuprolide +/- Abiraterone/Pred in Prostate. NCT02268175. https://clinicaltrials.gov/ct2/show/NCT02268175.

95. Medivation, Inc. Safety and Efficacy Study of Enzalutamide Plus Leuprolide in Patients With Nonmetastatic Prostate Cancer (EMBARK). NCT02319837. https://clinicaltrials.gov/ct2/show/NCT02319837.

96. Aragon Pharmaceuticals, Inc. A Study of JNJ-56021927 (ARN-509) and Abiraterone Acetate in Participants With Metastatic Castration-Resistant Prostate Cancer. NCT02123758. https://clinicaltrials.gov/ct2/show/NCT02123758.

97. Herr HW. A comparison of the quality of life of patients with metastatic prostate cancer who received or did not receive hormonal therapy. Cancer. 1993;71(3 Suppl):1143-50.

98. Alibhai SM et al. Long-term side effects of androgen deprivation therapy in men with non-metastatic prostate cancer: a systematic literature review. Crit Rev Oncol Hematol. 2006;60(3):201-15.

99. Saylor PJ, Smith MR. Metabolic complications of androgen deprivation therapy for prostate cancer. J Urol. 2009;181(5):1998-2006.

100. Keating NL et al. Diabetes and cardiovascular disease during androgen deprivation therapy: observational study of veterans with prostate cancer. J Natl Cancer Inst. 2010;102(1):39-46.

101. O'Farrell S et al. Risk and Timing of Cardiovascular Disease After Androgen-Deprivation Therapy in Men With Prostate Cancer. J Clin Oncol. 2015;33(11):1243-51.

102. Basaria S et al. Long-term effects of androgen deprivation therapy in prostate cancer patients. Clin Endocrinol (Oxf). 2002;56(6):779-86.

103. Diamond T et al. The effect of combined androgen blockade on bone turnover and bone mineral densities in men treated for prostate carcinoma: longitudinal evaluation and response to intermittent cyclic etidronate therapy. Cancer. 1998;83(8):1561-6.

104. Chen AC, Petrylak DP. Complications of androgen-deprivation therapy in men with prostate cancer. Curr Urol Rep. 2005;6(3):210-6.

105. Newton C et al. Memory complaints associated with the use of gonadotropin-releasing hormone agonists: a preliminary study. Fertil Steril. 1996;65(6):1253-5.

106. Harrington JM et al. Body image perceptions in men with prostate cancer. Oncol Nurs Forum. 2009;36(2):167-72.

107. Harrington JM, Badger TA. Body image and quality of life in men with prostate cancer. Cancer Nurs. 2009;32(2):E1-7.

108. Matthew AG et al. Sexual dysfunction after radical prostatectomy: prevalence, treatments, restricted use of treatments and distress. J Urol. 2005;174(6):2105-10.

109. Wassersug RJ. Mastering emasculation. J Clin Oncol. 2009;27(4):634-6.

110. Nguyen PL et al. Adverse effects of androgen deprivation therapy and strategies to mitigate them. Eur Urol. 2015;67(5):825-36.

111. Bayoumi AM et al. Cost-effectiveness of androgen suppression therapies in advanced prostate cancer. J Natl Cancer Inst. 2000;92(21):1731-9.

Exclusive Interviews from a variety of Therapeutic areas

(Click on video clip to view)

Prevention and Treatment of Chemotherapy-Induced Nausea and Vomiting

Overcoming Acquired Resistance to Targeted Therapies in Lung Cancer

An Interview with Prof. Dmitry Pushkar at EAU2015

Evaluating Genomic Tests for Breast Cancer Management

Nivolumab and Cabozantinib Versus Standard of Care in Renal Cell Carcinoma

Nivolumab Shows Highly Promising Activity in Advanced Liver Cancer in Early-Stage Trial

To view more of the latest videos, please click here

www.emjreviews.com

EUROPEAN UNION
Investing in Your Future
European Regional Development Fund 2007-13

MALE LOWER URINARY TRACT SYMPTOMS: IS THERE ANY BREAKING NEWS?

***George Kasyan**

Moscow State University of Medicine and Dentistry, Moscow, Russia
**Correspondence to g.kasyan@gmail.com*

Disclosure: The author has declared no conflicts of interest.
Received: 17.11.15 **Accepted:** 18.01.16
Citation: EMJ 2016;1[1]:54-57.

ABSTRACT

During recent decades, the lower urinary tract symptoms (LUTS) presented by patients have remained the same, but our understanding and treatment options have improved due to progressions in the field of functional urology. The role of nitric oxide in the ageing of the pelvic floor, bladder, prostate, and urethra is an important mechanism of LUTS. Phosphodiesterase-5 inhibitors are increasingly used in the treatment of these patients. Metabolic syndrome has been suggested as another important aetiological factor for both storage and voiding symptoms. Despite dramatic evolution in treatment strategies, non-adherence to pharmacological therapy is very high. The ineffectiveness and side effects are the primary reasons for the patients stopping treatment. The different aspects of combination therapy and the reported outcomes of patients will be discussed in this review.

Keywords: Lower urinary tract symptoms (LUTS), benign prostatic hyperplasia (BPH), overactive bladder (OAB), incontinence.

INTRODUCTION

The term lower urinary tract symptoms (LUTS) includes many medical problems in male urology and represents one of the most common clinical complaints in adult men. It can be divided into storage, voiding, and post-micturition symptoms. In the past, male LUTS was attributed to prostatic diseases and conditions; this was the case even for male storage symptoms. In 1997, Drs. Abrams and Wein introduced the term of overactive bladder (OAB) when they co-chaired a consensus conference.[1] Furthermore, increasing knowledge of the role of bladder dysfunction in LUTS pathophysiology has dramatically changed the landscape of the treatment options for both males and females. The LUTS presented by patients remain the same, but our understanding and treatment options have improved as we have progressed in the field of functional urology. LUTS are not necessarily the same group of diseases in each patient, but rather a complex of similar complaints that are created by one or many diseases (Figure 1). Moreover, many patients with LUTS present with more than one of the factors involved. The multifactorial aetiology of LUTS forces us to regard the whole urinary tract as a single functional unit. This review presents recent developments and updates in LUTS.

PELVIC FLOOR AGEING

The pathophysiology of LUTS in men is still far from being fully explored. Recent investigations in this field demonstrated the role of pelvic ischaemia, caused by RhoA/Rho-kinase pathway activation. Autonomic overactivity and increased afferent neural activity of the bladder are caused by highly complex and multifactorial factors. The role of nitric oxide in the relaxing of the smooth muscles of the bladder, prostate, and urethra is far from being completely understood. All of these important mechanisms of benign prostatic hyperplasia (BPH)/LUTS may be positively affected by phosphodiesterase-5 (PDE5) inhibitors.

Nitric oxide synthase (NOS) was recently introduced as another pathogenic enzyme that plays a role in bladder outlet obstruction (BOO) and LUTS in men. During the last century, PDE5 inhibitors have been increasingly used in the

treatment of male LUTS. A recent study of PDE5 distribution in human tissues demonstrated that PDE5 activity in the bladder, prostatic urethra, and prostate tissues is distributed unevenly, with less in the bladder neck, more in prostatic urethra, and even more in prostate tissue.[2] This selective distribution and activity of PDE5 in patients suffering from LUTS, and a decrease of the RhoA/Rho-kinase contractile mechanism in the bladder, could be the simplified rationale for the use of PDE5 inhibitors to diminish bladder dysfunction and outlet obstruction. The role of the bladder as a target of PDE5 inhibitors in LUTS is further explored by the functional improvement of urodynamic parameters in spinal cord injury patients after PDE5 inhibitor administration[3] and the effectiveness of PDE5 inhibitors on continence recovery after radical prostatectomy a prostate cancer, and therefore in men without a prostate.[4] Chronic ischaemia due to pelvic artery insufficiency that is caused by metabolic syndrome induces morphologic and functional changes in the bladder and prostate.[5] Additionally, modulation of autonomic nervous system overactivity and bladder/prostate afferent nerve activity by PDE5 inhibitors has also been suggested.[5] Although the exact mechanism of these medications still needs to be clarified, inhibition of PDE5 that increases the amount of NO in the smooth muscles has been shown to have an effect on several pathogenetic pathways contributing to LUTS.

METABOLIC SYNDROME AND LOWER URINARY TRACT SYMPTOMS

BPH and OAB may be aetiologically linked, not only in a cause-and-effect way but also by shared risk factors. As mentioned above, bladder ischaemia and metabolic syndrome have been suggested as aetiological factors for both storage and voiding disorders in LUTS. Epidemiologic studies suggest that metabolic syndrome components, such as increased waist circumference, are associated with the severity of LUTS as assessed by the international prostate symptom score (IPSS).[6] The proposed mechanism behind this observation is hyperinsulinaemia-associated increased sympathetic activity, leading to increases in smooth muscle contraction throughout male genitourinary tract structures, and subsequently LUTS. Other proposed mechanisms include impaired nitrergic innervation, increased Rho kinase activity, proinflammatory status, and changes in sex hormones. Mild-to-moderate bladder ischaemia occurring in metabolic syndrome damages the urothelium and intramural nerves, and induces detrusor overactivity (DO), resulting in the appearance of OAB. This was also proved in an animal study of rabbits with hyperlipidaemia and progressive atherosclerosis, where a reduction in bladder blood flow was associated with DO and urinary frequency.[7] Nevertheless, other studies refute the association of OAB and metabolic syndrome.[8]

The influence of testosterone and oestradiol on LUTS in men with BPH is another hot topic. In a recent, post-hoc analysis of 958 men with a history of LUTS of >6 months and an IPSS ≥13, patients were randomised to 5 mg tadalafil daily.[9] The aims of the investigation were to observe the characteristic testosterone levels in these patients, and to evaluate the cross-sectional relationship of 17β-oestradiol, testosterone, and LUTS before treatment. At the same time, a longitudinal association between baseline 17β-oestradiol, testosterone, and any improvement or worsening of LUTS after 12 months of tadalafil treatment was carefully analysed. According to the published data, the baseline testosterone was not significantly associated with IPSS score, whilst 17β-oestradiol was inversely correlated with IPSS. The PDE5 inhibitor tadalafil was more effective in patients with lower baseline levels of 17β-oestradiol. The increase in the IPSS level was more visible with total voiding scores than in storage domains. It was shown that lower levels of testosterone were not predictive of change in IPSS after tadalafil treatment. However, men with lower 17β-oestradiol levels demonstrated a better response to treatment.

COMBINATION THERAPY

The coexistence of storage and voiding symptoms in men poses a significant therapeutic challenge. There are quite a few agents to use either alone or in combination to target the prostate or the bladder. If the exact aetiologic factors and mechanisms for male LUTS were known, patient selection for certain treatments and, most importantly, treatment selection for individual patients would be much easier. Unfortunately, the pathophysiology of male LUTS remains to a certain degree an enigma, despite advances in our understanding of the mechanisms involved and a continuous effort to decode relevant molecular pathways.[10] For many years, BOO was aetiologically linked to all male LUTS and therefore reduction of the dynamic and mechanical components of BOO,

with alpha-blockers (AB) and 5-alpha-reductase inhibitors (5ARIs) respectively, was the primary therapeutic target.[11] Storage symptoms were either ignored or expected to improve once their cause, outlet obstruction, was relieved. This belief was based on evidence that urethral obstruction causes both morphological and functional changes in the bladder that are responsible for the storage symptoms. Failure to substantially relieve storage symptoms with prostate-targeting therapies led to an increasing recognition of the bladder as a major contributor to male LUTS. Regardless of whether DO was the consequence of obstruction or a distinct pathology, it had to be treated. The addition of antimuscarinics for residual storage symptoms was initially used reluctantly, due to fear of retention. Nevertheless, current knowledge suggests that antimuscarinics as an add-on treatment, as well as part of combination regimens for treatment initiation, do not substantially increase the risk of retention, at least for patients with moderate outlet obstruction and residual volumes <150–200 mL. For patients with more severe obstruction and larger residual storage symptoms, the risk is not adequately assessed, and caution is advised.

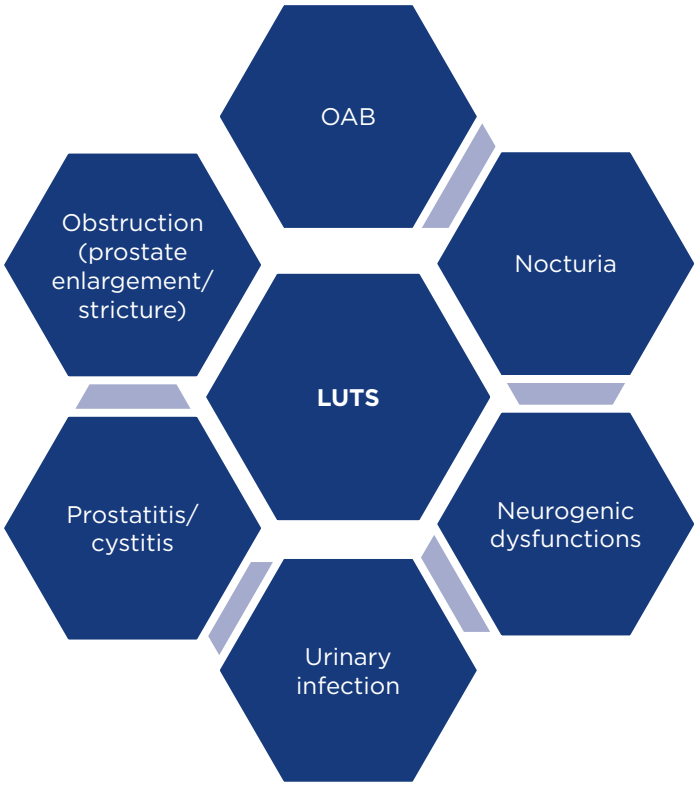


Figure 1: Main causes of male lower urinary tract symptoms (LUTS).
OAB: overactive bladder.

Traditionally, AB and antimuscarinics were the first-line medications for patients with LUTS.[12] Non-adherence to this pharmacological therapy is very high. The ineffectiveness and side effects are often cited as the primary reasons for patients stopping the treatments. Patient adherence to the medication and the clinical consequences of non-adherence were analysed in men with BPH-associated LUTS; the study was looking at the differences between drug classes whilst comparing mono versus combination therapy. A large retrospective cohort study using an administrative database of 1.5 million Italian men was carefully analysed. Patients >40 years of age, prescribed AB and 5ARIs, alone or in combination (AB/5ARI) for BPH-associated LUTS were investigated. The 1-year and long-term adherence were examined, together with the analysis of the rate of hospitalisation for BPH-related surgery.[13]

Patients exposed to at least 6 months of therapy had a 1-year overall adherence of 29% (AB: 35%; 5ARI: 18%; AB/5ARI: 9%). Patient adherence progressively decreases to 15%, 8%, and 3% for AB, 5ARI, and AB/5ARI, respectively, in the 5th year of follow-up. Patients on the combination therapy had a higher discontinuation rate than others. At the same time, AB/5ARI was associated with a reduced risk of hospitalisation for BPH-related surgery compared with AB. In general, the adherence to therapy for BPH-associated LUTS is low but varies depending on drug group. Patients under combination therapy have a higher treatment discontinuation rate for reasons that should be investigated further in the future. It is obvious that new strategies aiming to increase patient adherence to the prescribed treatment are necessary to prevent BPH progression.

QUALITY OF LIFE

Storage symptoms, the most common and bothersome subset of LUTS, predominantly affect the aged population with a worldwide distribution. Traditionally, nocturia is described as the most troublesome symptom in males with LUTS. A recent Japanese study[14] investigated the impact of storage symptoms on health-related quality of life (QoL) in male patients. Overall, 567 men who presented in a urology department completed the IPSS, incontinence-frequency score (IFS), and 36-Item Short-Form Health Survey (SF-36) questionnaires. Among 230 men with urological symptoms, it was shown that IPSS item scores of urgency, nocturia, and straining correlated with scores of the SF-36.

Incontinence was counted as the most influential factor that had a negative impact on general health perception, physical activities, vitality, social functioning, and mental health. Nocturia, straining, and urgency were significantly associated with a negative impact on QoL.

The relationship between LUTS, depression, and anxiety in men remains unclear. Pelvic inflammation is an independent risk factor for LUTS and depression according to up-to-date knowledge. An interplay between depression and LUTS accompanied by inflammation was investigated in a trial using patients randomly included from an urban community-dwelling cohort of men aged 35–80 years at recruitment (n=1195; sample response rate: 67.8%). Among 730 men who attended baseline (2002–5) and follow-up clinic visits (2007–10) without prostate or bladder cancer and the history of surgery, antipsychotic medication use, or neurodegenerative conditions were included in the present study. The incident of storage and voiding LUTS and the incidence of depression and anxiety were adjusted to serum inflammatory markers (high-sensitive C-reactive protein, tumour necrosis factor-alpha, interleukin-6, myeloperoxidase, soluble E-selectin). Multiple regression analysis was used to assess the moderating effect of all factors.[15]

Men with storage LUTS and anxiety at baseline had an increased likelihood of depression. Otherwise, men with depression and voiding LUTS were more likely to have anxiety at follow-up. Inflammation markers presented a significant moderating effect on the development of storage LUTS, depression, and anxiety. Finally, the authors conclude that there is a bidirectional relationship between storage LUTS and both depression and anxiety.

CONCLUSION

Ageing of the pelvis with ischaemia of the pelvic organs is being actively investigated. Within the frames of this concept, PDE5 inhibitors are becoming pathogenic treatment options for LUTS, as well as for conditions related to the bladder and prostate. Metabolic changes and hypogonadism are playing an important role in the symptom intensity and the efficacy of the medications. In spite of significant efforts, the adherence of the patients to the different LUTS treatments remain insufficient for all pharmacological groups and combinations. The QoL of the patients with LUTS is significantly diminished by nocturia, urgency, and straining, often causing depression and anxiety. Further investigations are ongoing into both the pathophysiology and pharmacogenetics of these complex patients.

REFERENCES

1. Abrams P, Wein AJ. Introduction: overactive bladder and its treatments. Urology. 2000;55(5, Supplement 1):1-2.

2. Andersson KE et al. Chronic Pelvic Ischemia: Contribution to the Pathogenesis of Lower Urinary Tract Symptoms (LUTS): A New Target for Pharmacological Treatment? Low Urin Tract Symptoms. 2015;7(1):1-8.

3. Gacci M et al. Vardenafil improves urodynamic parameters in men with spinal cord injury: results from a single dose, pilot study. J Urol. 2007;178(5):2040-3.

4. Patel HR et al. Effects of tadalafil treatment after bilateral nerve-sparing radical prostatectomy: quality of life, psychosocial outcomes, and treatment satisfaction results from a randomized, placebo-controlled phase IV study. BMC Urology. 2015;15:31.

5. Morelli A et al. Acute vardenafil administration improves bladder oxygenation in spontaneously hypertensive rats. J Sex Med. 2010;7(1): 107-20.

6. Lee RK et al. Central obesity as measured by waist circumference is predictive of severity of lower urinary tract symptoms. BJU Int. 2012;110(4): 540-5.

7. Yoshida M et al. The effects of chronic hyperlipidemia on bladder function in myocardial infarction-prone Watanabe heritable hyperlipidemic (WHHLMI) rabbits. Neurourol Urodyn. 2010;29(7):1350-4.

8. Behr-Roussel D et al. Vardenafil decreases bladder afferent nerve activity in unanesthetized, decerebrate, spinal cord-injured rats. Eur Urol. 2011;59(6): 272-9.

9. Egan KB et al. Do baseline estrogen and testosterone affect lower urinary tract symptoms (LUTS) prior to or after pharmacologic treatment with tadalafil? Andrology. 2015;3(6):1165-72.

10. Al-Zahrani AA, Gajewski JB. Association of symptoms with urodynamic findings in men with overactive bladder syndrome. BJU Int. 2012;110(11 Pt C):E891-5.

11. Kaplan SA et al. Combination therapy with doxazosin and finasteride for benign prostatic hyperplasia in patients with lower urinary tract symptoms and a baseline total prostate volume of 25 Ml or greater. J Urol. 2006;175(1):217-20.

12. Drake MJ et al. Long-term safety and efficacy of single-tablet combinations of solifenacin and tamsulosin oral controlled absorption system in men with storage and voiding lower urinary tract symptoms: results from the NEPTUNE Study and NEPTUNE II open-label extension. Eur Urol. 2015;67(2):262-70.

13 Cindolo L et al. Patient's adherence on pharmacological therapy for benign prostatic hyperplasia (BPH)-associated lower urinary tract symptoms (LUTS) is different: is combination therapy better than monotherapy? BMC Urol. 2015; 15(1):96.

14. Okada T et al. [The impact of lower urinary tract symptoms on generic health-related quality of life in male patients without co-morbidity]. Nihon Hinyokika Gakkai Zasshi. 2015;106(3):172-7.

15. Martin S et al. Lower Urinary Tract Symptoms, Depression, Anxiety and Systemic Inflammatory Factors in Men: A Population-Based Cohort Study. 2015; 10(10):e0137903.

URINARY INCONTINENCE, DEPRESSION, AND PSYCHOSOCIAL FACTORS - A REVIEW OF POPULATION STUDIES

***Jodie Avery, Nigel Stocks**

University of Adelaide, Adelaide, Australia
**Correspondence to jodie.avery@adelaide.edu.au*

Disclosure: The authors have declared no conflicts of interest.
Received: 29.10.15 **Accepted:** 04.01.15
Citation: EMJ. 2016;1[1]:58-67.

ABSTRACT

The psychological effects of urinary incontinence, such as psychological distress, depression, and anxiety are well recognised. Associations between incontinence, quality of life, and mental health have been demonstrated; however, research concerning incontinence and depression together, and the subsequent impact on health, quality of life, help-seeking, and other psychosocial factors, is limited. Examining associations between incontinence and psychosocial and mental health may provide an opportunity to address this health problem in a different way. A comprehensive review of the literature with regard to population studies in the area of urinary incontinence, psychosocial issues, and depression, as well as the interplay between these three concepts is presented, and the absence of research in this area is highlighted.

Keywords: Urinary incontinence, depression, psychosocial, population studies, epidemiology, review.

INTRODUCTION

The psychological effects of urinary incontinence, such as psychological distress, depression, and anxiety are well recognised[1] as Vigod et al.[2] have observed:

"Regardless of how the two disorders are related, the combined impact of urinary incontinence and major depression exceeds the impact of either condition alone [...] Leaving either of these disorders undiagnosed and thus untreated will clearly have significant impact on the health and quality of life of individual patients and the population as a whole."

A review of studies addressing the combined effects of depression with any chronic condition found that there were further associations with a number of other problems. These included an increase in the use and cost of medical resources, amplification of physical symptoms, additive effects in the area of functional impairment, decreased compliance with treatment and lifestyle changes, and also increased mortality.[3] However, research concerning the combined effect of incontinence and depression, and the subsequent impact on health, quality of life, help-seeking, and other psychosocial factors, is limited.[4-8]

Poor bladder control or leakage is termed 'urinary incontinence'.[9] The International Continence Society takes great care in its definition of urinary incontinence to include that incontinence is involuntary leakage in the context of type, frequency, severity, precipitating factors, social impact, effect on hygiene, and quality of life.[9]

In Australia, the prevalence urinary incontinence for women >18 years old has previously been reported at 25.8%.[10] South Australian data from 1998 report a prevalence of 20.3% for adults ≥16 years old.[11] The most current South Australian data, unpublished from the South Australian Health Omnibus Survey (SAHOS) in 2004, give an overall prevalence of urinary incontinence at 28.0%.[12]

Associations between incontinence, quality of life, and mental health have been demonstrated, but exploration into the implications of this connection with regard to psychosocial factors is necessary and there has recently been a call for research in this area.[1,5,13-17] Furthermore, little

research around psychosocial factors has considered men with incontinence and depression. Examining associations between incontinence and psychosocial and mental health may provide an opportunity to address this health problem in a different way. The treatment or management of symptoms can make a significant difference to the impact on individual quality of life and carers of people with incontinence, as well as the whole health system.[10,18,19]

There are a number of factors which contribute to the difficulty of studying incontinence and depression epidemiologically. These include the various definitions of the diagnosis for both incontinence and depression, different epidemiological methods used to estimate prevalence in the community, and the underreporting of these conditions due to stigma or perceptions of those experiencing the condition. However, this review intends to critically analyse these problems within each of the studies presented, so that a clearer picture of the conditions and outcomes studied may be obtained.

Methodologically, prevalence data considering an individuals' self-reporting of illness and disease are most accurately collected using community based population surveys,[20] and this review has sought to include these. Samples derived from clinical populations only consider those members of the community who realise or acknowledge they have a problem and seek medical assessment, excluding the majority of the community who, for various reasons, have not accessed such care. Cross-sectional studies, as reported in this review, are not able to infer causality or accurately define the chronology of events for the development of co-morbidities, depression, and psychosocial factors that can be associated with incontinence.

The aim of this review is to provide a comprehensive discussion of the literature with regard to incontinence related psychosocial issues and depression, as well as the interplay between these concepts. Key studies regarding urinary incontinence and depression will be discussed. Finally, the absence of research in this area will be highlighted.

METHODS

Search Strategy

A systematic search of the literature, using the keywords 'incontinence', 'AND', and 'depression' carried out between 2005–2014, identified articles written in English using PubMed. Articles were also mainly limited to those published in 1990, going back 25 years. Once articles were identified, individual reference lists derived from these were also hand-searched to discover additional articles. A number of automatic alerts were also used over the period to identify useful papers.

RESULTS: URINARY INCONTINENCE AND DEPRESSION

The association between incontinence and depression has been demonstrated in several studies.[1,5,13-17,21] Of those self-reporting urinary incontinence, 20.5% also scored for other symptoms or major depression on the Primary Care Evaluation of Mental Disorders-Patient Health Questionnaire (PRIME-MD PHQ) scale, and 1.6-times more people with urinary incontinence experienced depression than those without incontinence.[21] Clearly, if someone is incontinent, they may become depressed, particularly if it affects their quality of life. However, other explanations proposed for the relationship between urinary incontinence and depression include biochemical models, such as that in experimental animals, where lowering monoamines such as serotonin and noradrenaline in the central nervous system (CNS) leads to depression and increased urinary frequency and a hyperactive bladder.[22] Depression may not only be a result of persistent urinary incontinence, but individuals with altered monoamines in the CNS could manifest both depression and an overactive bladder.[23]

The prevalence of depression in those experiencing urinary incontinence has been consistent across many studies and is similar for both clinically based studies and population surveys internationally.[24,25] Some studies determine actual prevalence, some quote mean scores from depression scales, and others allude to a higher risk of depression in the incontinent population, when compared with the general population.[26,27]

Clinical studies include research where the sample is derived from a clinic, hospital, or practice where the respondents may already be receiving treatment for incontinence, or for other medical problems, such as in gynaecological or menopause clinics, or even general practice. Studies in this area have generally had a small sample size, and are not useful for determining the overall population prevalence of incontinence. However, diagnosis of

the specific type of urinary incontinence is usually medically verifiable in these situations, instead of relying on self-report. Various instruments have been used to determine depression in those with urinary and anal incontinence, from self-assessment to psychiatric evaluation. We have not addressed clinical studies in this review.

Population studies regarding incontinence and depression have, in general, examined a higher number of cases leading to greater statistical power, and have also identified a greater number of people who have not been diagnosed with, received treatment, or even sought help for either of these conditions. However, the majority of these studies have only examined women or the older population.

Some studies comment only on the statistical associations between incontinence and depression, and these have been included. Reviews of the literature for each area have also been commented upon. Population studies in urinary incontinence and depression concerning both men and women have been summarised in Table 1 and for women only in Table 2.

Population Studies: Men and Women

Telephone Interviews

Two computer assisted telephone interviewing (CATI) surveys from the USA have determined the prevalence of depression in respondents with urinary incontinence. One of the studies found that in those aged ≥40 years, 20.6% of respondents with urinary incontinence self-reported feeling depressed.[28] The other study, using a screening questionnaire for depression in older respondents aged ≥60 years, found that 43.0% of respondents with urinary incontinence had depression, and this occurred in 24.0% of men and 38.0% of women.[24]

To determine associations of depression in men and women with urinary incontinence, a number of population studies have used the Center for Epidemiologic Studies Depression Scale (CES-D), with a cut-off score of ≥16. One American study administering the CES-D via CATI to adults ≥53 years old found a statistically significant association between depression and urinary incontinence.[28] A third American study, this time including younger adults aged ≥18 years, using the CES-D in CATI interviews, had a similar statistically significant finding.[26]

Face to Face Interviews

A study from Korea using the CES-D looked at incontinence and depression with quality of life but did not look at the prevalence of these in combination, and did discover that lower urinary tract symptoms (LUTS) and depression were the principal predictors of quality of life in older adults.[29] A recent Australian study looked at the associations between incontinence, depression, and quality of life and observed urinary incontinence with comorbid depression in 4.3% of the overall population, aged ≥15 years. The interaction of the presence of incontinence and the presence of depression was significantly associated with the dimensions of physical functioning on the Short Form (SF-36).[21]

Mixed Methods Population Studies

Research utilising a combination of face to face and telephone interviews included a study of African Americans in the USA aged 52–68 years, which found a prevalence of depression in those with incontinence of 38.8%.[30] An Australian study of those aged ≥65 years showed that women with any incontinence had a higher negative affect, and that men with stress urinary incontinence also had a higher negative affect.[27]

Internet Panels

Increasingly, some of the more recent studies have made use of population panels where participants are able to answer questionnaires over the internet. One such study, known as the EpiLUTS from the US, UK, and Sweden that interviewed 30,000 men and women aged ≥40 years, found that men and women with multiple LUTS reported the lowest levels of urinary-specific quality of life and generic health, and had the highest rates of clinical anxiety and depression,[31] as well as observing that men with mixed urinary incontinence had the highest prevalence of depression (42.1%), and women with stress urinary incontinence plus other incontinence had a prevalence of depression of 34.9%.[32]

Population Studies: Women

Face to Face Interviews

International population studies considering women only include three studies from the USA that used the CES-D to determine the prevalence of depression in people with urinary incontinence.

Table 1: Urinary incontinence and depression studies – men and women.

Author	Country	Participants n/N Age (years)	Survey Setting	Incontinence definition/ instrument	Depression definition/ instrument	Prevalence/Results
Urinary – Population Studies – Women and Men – Telephone						
Dugan E et al. (2000)[24]	North Carolina, USA	230/668 >60	Community residents RCT of primary care practices: CATI survey	Self-report, past 3 months, severity	Screener for depression	UI 52.5% **D & UI 43.0%** D & UI (W) 38.0% D & UI (M) 24.0%
Fultz N et al. (2001)[8]	Michigan, USA	206/1,322 ≥40	CATI, population study	Self-report, past 6 months, severity	Self-report past week	UI 15.6% **D & UI 20.6%**
Stewart WF et al. (2003)[26]	Baltimore, USA	538/5,204 ≥18	CATI, population study	OAB, UI, self-report	SF-36 CES-D	OAB 16.5% OAB & UUI 6.1% OAB & D SS Higher CES-D scores
Fultz NH et al. (2005)[28]	Michigan, USA	?/4987 ≥53	CATI, population study	Self-report, last month	CES-D	UI (W) 21.0% UI (M) 6.0% SS associated with dep
Irwin DE et al. (2006)[25]	France, Germany, Italy, Spain, Sweden, UK	1,272/11,521 40–64	CATI cross-sectional population-based survey (Spain direct interviews)	OAB with UI (frequency, urgency, urge incontinence, or nocturia) Self report, past 12 months	Asked about the negative impact associated with OAB symptoms on emotional well-being	OAB with UI & D 39.8%
Urinary – Population Studies – Women and Men – Face to Face						
Bogner HR et al. (2004)[13]	Baltimore, USA	747 ≥50	As above analysis by race	Self-report, past 12 months	Psychological distress (PD) GHQ score ≥4 for caseness	UI 20.0% PD & UI 28.5% As above
Song et al. (2012)[29]	Jeju, Korea	171 61–94	Cross-sectional, face to face	Involuntary urine loss once per month or more frequent past 6 months	Korean CES-D	D = 18.6% UI = 22.2% (no combination – only looked at QoL)
Urinary – Population Studies – Women and Men – Mixed Methods						
Malstrom TK et al. (2010)[30]	Missouri, USA	853 African Americans 52–68	Cross-sectional in home and CATI	Self-report, past 12 months	CES-D	UI 12.1% **D & UI 38.8%**
Sims et al. (2011)[27]	Melbourne, Australia	796 ≥65	Cohort study, face to face/ CATI	Self-report: Ever accidently passed urine, urgency question	Psychogeriatric assessment scales	UUI 28.0% SUI 21.0% W UI Higher negative affect M SUI Higher negative affect
Urinary – Population Studies – Women and Men – Internet Panels						
Coyne et al. (2009)[31]	USA, UK, Sweden	30,000 ≥40 Mean M = 53.9 Mean W = 60.3	EpiLUTS Cross-sectional population study via internet panels	PPBC OAB-q SF	HADS	D M 29.8% D W 37.6% M and W with multiple LUTS reported the lowest levels of urinary-specific HRQL and generic health, and had the highest rates of clinical anxiety and depression

Table 1 continued.

Author	Country	Participants n/N Age (years)	Survey Setting	Incontinence definition/ instrument	Depression definition/ instrument	Prevalence/Results
Urinary – Population Studies – Women and Men – Internet Panels						
Coyne et al. (2012)[32]	USA, UK, Sweden	30,000 ≥40 Mean M =53.9 Mean W =60.3	EpiLUTS Secondary analysis of cross-sectional population study via internet panels	PPBC OAB-q SF	HADS	UI M 45.8% UI W 67.6% M: D highest with MUI (42.1%), D & UUI plus OUI (33.8%), D & SUI plus OUI (31.5%). W: D highest with SUI plus OUI (34.9%), D & MUI (34.7%)

UI: urinary incontinence; UUI: urge urinary incontinence; SUI: stress urinary incontinence; MUI: mixed urinary incontinence; OUI: overflow urinary incontinence; LUTS: lower urinary tract symptoms; EpiLUTS: Epidemiology of LTUS; OAB: overactive bladder; OAB-q SF; OAB questionnaire short form; D: depression; PD: psychological distress; W: women; M: men; SS: statistically significant; PPBC: patient perception of bladder condition; HADS: hospital anxiety and depression scale; GHQ: general health questionnaire; CES-D: Center for Epidemiologic Studies Depression Scale; RCT: randomised controlled trial; CATI: computer assisted telephone interviewing; QoL: quality of life; HRQL: health-related quality of life.
Prevalence of D and UI highlighted in **BOLD**.

The first of these studies interviewed women aged 50–69 years and found a prevalence of depression of 14.2% in those with mild urinary incontinence, and 22.3% in those with severe urinary incontinence.[33] The second study found a prevalence of depression of 24.0% for women aged ≥70 years, with urinary incontinence occurring less than weekly, and 35.6% in those who were incontinent more than once a week. In those with urge urinary incontinence, the prevalence of depression was 12.0%, and 9.0% in those with stress urinary incontinence.[34] A third study found the prevalence of incontinence with depression to be 11.0% and although major depression predicted onset of urinary incontinence in a population-based sample of at-risk, community-dwelling women, incontinence did not predict onset of depression.[35] A further study from the USA looked at depression symptoms in women aged 30–79 years and found that urinary incontinence was associated with depression symptoms.[36]

Telephone

A Canadian CATI study undertaken with 69,000 women aged ≥18 years found the prevalence of major depression in those with urinary incontinence to be 15.5%, which was significantly higher than the prevalence in women without incontinence.[2] Another CATI study concerning women aged >52 years who were veterans, found a prevalence of stress urinary incontinence and depression using the Composite International Diagnostic Interview (CIDI) to be 32.8%, and urge or mixed incontinence at 43.5%.[37]

Mailed Questionnaires

One of the only Australian population studies concerning urinary incontinence and depression considered women only. The study was part of the ongoing Women's Health Australia (WHA) project, where over 40,000 women between the ages of 18 and 75 years filled out postal questionnaires regarding their health, of which questions regarding incontinence, as well as the SF-36 were included.[38] Respondents with urinary incontinence had lower scores on the Mental Component Summary (MCS) of the SF-36 than those without incontinence, and the youngest group had a mean score of 40.7, where a score of ≤42 on the MCS indicates clinical depression.[39]

One American study using the Beck Depression Inventory (BDI) through a mailed questionnaire, to detect depression (score >13) in women aged 27–90 years with urinary incontinence, found a prevalence of 22.0%, where the incidence of depression in the general population using this instrument is 6.0%.[40] Another study using the CES-D in a mailed questionnaire, found statistically

significant higher scores for depression in women aged ≥60 years with urinary incontinence across time.[16]

Another American study, using the PRIME-MD PHQ in women aged 30–90 years, found a prevalence of major depression of 6.1% in women with urinary incontinence, with a rate of 3.7% in the general sample.[41,42]

A further study from Sweden examining women by mailed questionnaires that asked for self-reports of feeling 'down' and 'blue' to determine depression, observed that depression in women aged 50–64 years was statistically significantly associated with urinary incontinence.[43]

In the UK, a study showed that in women ≥40 years of age, the prevalence of urinary incontinence with depression using the Hospital Anxiety and Depression Scale (HADS)[44] was 38.0%.[45] Similarly in the Netherlands, women aged 20–70 years had a prevalence of incontinence with depression of 42.8%, but urinary incontinence was not found to be a risk factor for depression.[46] In a study of women aged 40–44 years in Norway using the HADS, the prevalence of incontinence with depression was 11.8%.[47] The Nurses' Health Study in the USA also examined incontinence with depression. Overall they found a prevalence of 28.9%,[48] and when split, more frequent urinary incontinence and greater severity were significantly associated with higher prevalence of high depressive symptoms in both black and white women.[49]

Internet Panels

One women's study was a twin study undertaken via the internet and this found the rate of incontinence with depression to be 11.8%.[50]

Reviews

Reviews of the literature regarding the association between urinary incontinence and depression have been completed. An earlier American review concentrated on articles from the 1980s (outside the scope of our review) that name psychological distress and depression as outcomes of urinary incontinence, and provided reasons why this may be the case.[9] Other more recent reviews of this topic have discussed the psychological impact of incontinence and the management of the associated psychological morbidity,[51] the psychosocial and societal burden of incontinence (particularly in the aged[52]), the cognitive barriers and safety-behaviours involved in the development and maintenance of emotional distress in patients with urinary incontinence,[53] and the quality of life in people with incontinence particularly looking at anxiety and depression.[54]

Table 2: Urinary incontinence and depression studies – women only.

Author	Country	Participants n/N Age (years)	Survey Setting	Incontinence definition/ instrument	Depression definition/ instrument	Prevalence/Results
Urinary – Population Studies- Women - Face to Face						
Nygaard I et al. (2003)[33]	Iowa, USA	905/5,701 50–69	Population based face to face	Self-report	CES-D ≥16 CIDI	UI 16% **D & UI^{mild} 14.2%** **D & UI^{sev} 22.3%**
Jackson RA et al. (2004)[34]	Pennsylvania & Tennessee, USA	1,558 70–79	Health ABC Longitudinal cohort study	Self-report, Frequency (d, weekly, <weekly) Used ≥ weekly	CES-D >15	D & UI <weekly 24.0% D & UI ≥weekly 35.6% D & UUI 2.0% D & SUI 9.0%
Melville JL et al. (2009)[35]	Michigan, USA	5,820 Mean 59.3	HRS Longitudinal cohort interviews	Self-report past year	CES-D CIDI-SF	**D & UI 11.0%** **D & UI 18.0%** Major depression predicted onset of UI in a pop-based sample of at-risk, community-dwelling women. UI did not predict depression
Maserejian NN et al. (2014)[36]	Boston, USA	3,201 30–79	Boston Area Community Health Survey, obs cohort	Self-report and treatment status, monthly, weekly.		UI at baseline, persistence was associated with depression symptoms (monthly UI) OR=2.39

Table 2 continued.

Author	Country	Participants n/N Age (years)	Survey Setting	Incontinence definition/ instrument	Depression definition/ instrument	Prevalence/Results
Urinary – Population Studies- Women - Telephone						
Vigod SN et al. (2006)[2]	Canada	69,003 ≥18	Canadian Community Health Survey Population Study, CATI	Do you suffer from urinary incontinence?	CIDI-SF CCHS HUI III	UI 3.23% D 9.4% **D & UI($^{maj\ D}$) 15.5%** **Younger & increased risk (30%).**
Bradley et al. (2012)[37]	Iowa, USA	968 ≥52 Mean 38.7	Secondary analysis of veterans, CATI	Self-report	CIDI-SF	SUI 18.9% MUI 16.2% UUI 3.5% D & SUI 32.8% UUI/MUI & D 43.5%
Urinary – Population Studies- Women - Mailed						
Chiverto PA et al. (1996)[40]	New York, USA	125 27–90	Community residents, mailed questionnaire	Self-report: Uncontrolled urine loss Excessive day toileting frequency	BDI ≥13	**D & UI 22.0%**
Chiarelli P et al. (1999)[38]	Newcastle, Australia, Australia wide study	?/41,724 18–75	Australian Longitudinal Study on Women's Health: cohort study, mailed	Self-report	SF-36	UI by age group 12.8%, 36.1%, 35.0% Lower scores on MCS and PCS
Heidrich SM et al. (2004)[16]	Wisconsin, USA	26/103 >60	Longitudinal study, community dwelling, mailed questionnaire	Self-report	Bradburn Affect Balance Scale CES-D ≥15	UI 25.0% **D & UI** **Over time SSH D scores**
Melville JL et al.[41,42]	Washington, USA	1,458/3,438 30–90	Population based mailed generic questionnaire	Leakage at least monthly	PRIME-MD HQ	UI 45.0% **D & UI 6.1%**
Melville JL et al.[41,42]	Washington, USA	1,458/3,536 30–90	Population based mailed generic questionnaire	Leakage at least monthly Major Depression	PRIME-MD HQ	UI 45.0% **D & UI($^{maj\ D}$) 6.1%**
Moghaddas et al. (2005)[43]	Lund, Sweden	2,145/6,642 50–64	Population based mailed generic questionnaire and lab exam	Self-report occurrence of UI plus severity	Generic questionnaire: self-report feeling down and blue	UI 31.0% D 52.0% **D & UI SSH**
Perry S et al. (2006)[45]	Leicester & Rutland, UK	12,568 ≥40	Leicestershire MRC Incontinence Study, long postal survey	Self-report	HADS	UI 15.3% D 20.3% **D & UI 38%** UUI & D 37.6%
Van der Vaart et al. (2007)[46]	Utrecht, Netherlands	2,042 20–70	Population based, mailed questionnaires	UDI	CES-D	UI 51.1% **D & UI 42.8%** (UI not a risk factor for D)
Felde G et al. (2012)[47]	Hordaland, Norway	5,321 40–44	HUSK population study, Mailed questionnaire	Self-report	HADS	UI 26.2% D 10.8% **D & UI 11.8%** D & UUI 11.7%
Matthews et al. (2013)[48]	Boston, USA	64,396	NHSMQ	Self-report	History of D dx or anti-D medication use or a score >5 on Geriatric Dep Scale	UI 37.8% **D & UI 28.9%**

Table 2 continued.

Author	Country	Participants n/N Age (years)	Survey Setting	Incontinence definition/ instrument	Depression definition/ instrument	Prevalence/Results
Urinary – Population Studies- Women - Mailed						
Townsend MK et al. (2014)[49]	Boston, USA	72,000 58–83 Mean 70	NHSMQ	Self-report	CES-D	UI black 30.9% D black 13.7% UI white 46.7% D white 16.3%
Urinary – Population Studies- Women - Internet Panels						
Tettamanti et al. (2014) (Tettamanti et al. [2013])[50]	Stockholm, Sweden	42,852	STAGE Twin study, web based	Self-report	CES-D CIDI-SF	UI 7.0% D 23.8% **D & UI 11.8%**

UI: urinary incontinence; UUI: urge urinary incontinence; SUI: stress urinary incontinence; MUI: mixed urinary incontinence; D: depression; dx: diagnosis; W: women; M: men; SS: statistically significant; SSH: statistically significantly higher; HRS: Health and Retirement Study; NHSMQ: Nurses' Health Study Mailed questionnaire; d: daily; OR: overall response; CATI: computer assisted telephone interviewing; CIDI: composite international diagnostic interview; CES-D: Center for Epidemiologic Studies Depression Scale; MCS: mental component summary; PCS: physical component summary; HADS: Hospital Anxiety and Depression Scale; PRIME-MD HQ: Primary Care Evaluation of Mental Disorders-Patient Health Questionnaire; CCHS: congenital central hypoventilation syndrome; HUSK: Hordaland Health Study; UDI: urinary distress inventory; BDI: Beck Depression Index; HUI: Health Utilities Index. Prevalence of D and UI highlighted in **BOLD**.

CONCLUSION: GAPS IN THE RESEARCH

There is a paucity of research identifying associations between incontinence, psychosocial factors, and depression, with only two recent studies.[17,21] A number of studies had the potential to examine this relationship, but did not do so. One study found no significant relationship, and another examined an observational relationship, with no particular conclusions. The studies outlined above have been undertaken in both men and women, together and separately, using different methodologies and instruments in different country, cultural, and age groups. The majority of these studies also concentrate on older age groups with few considering the whole adult population.

Whenever possible, the prevalence of comorbid depression in the presence of urinary incontinence has been stated, and this has been reported here at anywhere between 6.0–43.0%. The gold standard methodologies, such as face to face population surveys, report a prevalence of 15.0–30.0% for women, depending on which scale was used, the age group, and the year. The studies described usually report a significantly higher rate of depression amongst those with incontinence. Comparing the rates of depression in those with urinary incontinence with those of the overall population (ranging from 5.0–15.0% as discussed previously); we can estimate that the burden of depression in those with urinary incontinence is greater. Studies that include males report lower prevalence rates for urinary incontinence than for females; however, studies show conflicting results as to whether men may be more likely to experience depression when they have incontinence when compared with women. In order to determine that this is the case within different populations, we must undertake research that explores the difference in the prevalence of depression in those with and without urinary incontinence for both sexes using quality population studies and validated instruments, amongst all age groups.

Primarily, the studies reviewed here identified the presence of depression in conjunction with incontinence. They did not explore the burden on society or the healthcare costs associated with incontinence and comorbid depression. The psychosocial factors that are associated with incontinence may be adversely impacted upon by depression. Incontinent people experiencing

comorbid depression may be less likely to seek help for their incontinence, their quality of life may be lower, they may be more socially isolated, their perception of symptom severity may be greater, and their use of health services may be less when compared to individuals with incontinence who are not depressed.[1,6,13-16] For the 15–30% of those with incontinence who also suffer from depression, an opportunity to reduce the burden of incontinence is presented, as treating depression may be more successful than treating incontinence.[8,23,55,56] However, psychosocial barriers to treatment such as reduced help-seeking present a dilemma.

In both those with only incontinence and those with only depression, only 30–60% of these groups seek help. Incontinence costs at least A$200 million[57] and depression, >A$20 billion,[58] representing a great economic burden on the community. It is unclear what impact the combination of these conditions has on society.

Both urinary incontinence and depression have an impact on quality of life.[11,59] However, this review has shown that little recent research has considered the association between incontinence and psychosocial factors, such as help-seeking and quality of life, with depression. We have, however, recently found that when incontinence and depression are combined, they have a greater effect on psychosocial factors than when these conditions stand alone,[21] and further research in this area is warranted, particularly into interventions that are able to manage the symptoms of either condition.

REFERENCES

1. Heymen S. Psychological and cognitive variables affecting treatment outcomes for urinary and fecal incontinence. Gastroenterology. 2004;126(1 Suppl 1):S146-51.

2. Vigod SN, Stewart DE. Major depression in female urinary incontinence. Psychosomatics. 2006;47(2):147-51.

3. Katon W. The Effect of Major Depression on Chronic Medical Illness. Semin Clin Neuropsychiatry. 1998;3(2):82-6.

4. Shaw C. A review of the psychosocial predictors of help-seeking behaviour and impact on quality of life in people with urinary incontinence. J Clin Nursing. 2001;10(1):15-24.

5. Gallagher MS. Urogenital distress and the psychosocial impact of urinary incontinence on elderly women. Rehabil Nurs. 1998;23(4):192-7.

6. Lagro-Janssen AL et al. Urinary incontinence in women and the effects on their lives. Scand J Prim Health Care. 1992;10(3):211-6.

7. Fultz NH, Herzog AR. Self-reported social and emotional impact of urinary incontinence. J Am Geriatr Soc. 2001;49(7):892-9.

8. Herzog AR et al. Urinary incontinence: medical and psychosocial aspects. Ann Rev Gerontol Geriatr. 1989;9:74-119.

9. Abrams P et al. The Standardization of Terminology of Lower Urinary Tract Function: Report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn. 2002;21(2):167-78.

10. Doran CM et al. Economic costs of urinary incontinence in community-dwelling Australian women. Med J Aust. 2001;174(9):456-8.

11. Avery J et al; Population Research and Outcomes Studies Unit, South Australian Department of Health. Incontinence in South Australia- Prevalence, Risks and Priorities. Available at: https://health.adelaide.edu.au/pros/docs/reports/Report2004_incontinence_SA.pdf. Last Accessed: 2 November 2015.

12. Avery J. South Australian Health Omnibus Survey Incontinence in South Australia. Adelaide: PROS University of Adelaide. Available at: https://health.adelaide.edu.au/pros/docs/Incontinence_HOS_04.pdf. Last accessed: 9 November 2015.

13. Bogner HR. Urinary Incontinence and Psychological Distress in Community-Dwelling Older African Americans and Whites. J Am Geriatr Soc. 2004;52(11):1870-4.

14. Avery J et al. Physical activity: The impact of incontinence on health-related quality of life in a South Australian population sample. Austr N Z J Public Health. 2004;28(2):173-9.

15. Costa P, Mottet N. Assessing the impact of urinary incontinence in a female population. Eur Urol. 1997;32(Suppl 2):25-7.

16. Heidrich SM, Wells TJ. Effects of urinary incontinence: psychological well-being and distress in older community-dwelling women. J Gerontol Nurs. 2004;30(5):47-54.

17. Rozensky RH et al. A quality of life survey of individuals with urinary incontinence who visit a self-help website: implications for those seeking healthcare information. J Clin Psychol Med Settings. 2013;20(3):275-83.

18. Marcell D et al. Treatment options alleviate female urge incontinence. Nurse Pract. 2003;28(2):48-54.

19. Burgio KL. Behavioral treatment options for urinary incontinence. Gastroenterology 2004;126(1 Suppl 1):S82-9.

20. Bowling A. Just one question: If one question works, why ask several? J Epidemiol Community Health. 2005;59(5):342-5.

21. Avery J et al. Identifying the quality of life effects of urinary incontinence with depression in an Australian population. BMC Urol. 2013;13(11).

22. Steers WD, Lee KS. Depression and incontinence. World J Urol. 2001;19(5):351-7.

23. Stach-Lempinen B et al. Severe depression determines quality of life in urinary incontinent women. Neurourol Urodyn. 2003;22(6):563-8.

24. Dugan E et al. The association of depressive symptoms and urinary incontinence among older adults. J Am Geriatr Soc. 2000;48(4):413-6.

25. Irwin DE et al. Impact of overactive bladder symptoms on employment, social interactions and emotional well-being in six European countries. BJU Int. 2006;97(1):96-100.

26. Stewart WF et al. Prevalence and burden of overactive bladder in the United States. World J Urol. 2003;20(6):327-36.

27. Sims J et al. Urinary incontinence in a community sample of older adults: prevalence and impact on quality of life. Disabil Rehabil. 2011;33(15-16):1389-98.

28. Fultz NH et al. The impact of own and spouse's urinary incontinence on depressive symptoms. Soc Sci Med. 2005;60(11):2537-48.

29. Song HJ et al. Impact of lower urinary

tract symptoms and depression on health-related quality of life in older adults. Int Neurourol J. 2012;16(3):132-8.

30. Malmstrom TK et al. Urinary and fecal incontinence and quality of life in African Americans. J Am Geriatr Soc. 2010;58(10):1941-5.

31. Coyne KS et al. The burden of lower urinary tract symptoms: evaluating the effect of LUTS on health-related quality of life, anxiety and depression: EpiLUTS. BJU Int. 2009;103 Suppl 3:4-11.

32. Coyne KS et al. Urinary incontinence and its relationship to mental health and health-related quality of life in men and women in Sweden, the United Kingdom, and the United States. Eur Urol. 2012;61(1):88-95.

33. Nygaard I et al. Urinary incontinence and depression in middle-aged United States women. Obstet Gynecol. 2003;101(1):149-56.

34. Jackson RA et al. Urinary incontinence in elderly women: findings from the Health, Aging, and Body Composition Study. Obstet Gynecol. 2004;104(2): 301-7.

35. Melville JL et al. Major depression and urinary incontinence in women: temporal associations in an epidemiologic sample. Am J Obstet Gynecol. 2009;201(5): 490 e1-7.

36. Maserejian NN et al. Treatment status and risk factors for incidence and persistence of urinary incontinence in women. Int Urogynecol J. 2014;25(6): 775-82.

37. Bradley CS et al. Urinary incontinence, depression and posttraumatic stress disorder in women veterans. Am J Obstet Gynecol. 2012;206(6):502 e1-8.

38. Chiarelli P et al. Leaking urine: prevalence and associated factors in Australian women. Neurourol Urodyn. 1999;18(6):567-77.

39. Ware J et al. SF-36 Physical and Mental Health Summary Scales : A user's manual. Boston, MA: The Health Institute, New England Medical Centre, 1994.

40. Chiverton PA et al. Psychological factors associated with urinary incontinence. Clin Nurse Spec. 1996;10(5):229-33.

41. Melville JL et al. Urinary Incontinence in US Women: A Population-Based Study. Arch Int Med. 2005;165(5):537-542.

42. Melville JL et al. Incontinence severity and major depression in incontinent women. Obstet Gynecol. 2005;106(3):585-92.

43. Moghaddas F et al. Prevalence of urinary incontinence in relation to self-reported depression, intake of serotonergic antidepressants, and hormone therapy in middle-aged women: a report from the Women's Health in the Lund Area study. Menopause. 2005;12(3):318-24.

44. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67(6):361-70.

45. Perry S et al. An investigation of the relationship between anxiety and depression and urge incontinence in women: development of a psychological model. Br J Health Psychol. 2006;11(Pt 3):463-82.

46. van der Vaart CH et al. Association between urogenital symptoms and depression in community-dwelling women aged 20 to 70 years. Urology 2007;69(4):691-6.

47. Felde G et al. Anxiety and depression associated with incontinence in middle-aged women: a large Norwegian cross-sectional study. Int Urogynecol J. 2012;23(3):299-306.

48. Matthews CA et al. Risk factors for urinary, fecal, or dual incontinence in the Nurses' Health Study. Obstet Gynecol. 2013;122(3):539-45.

49. Townsend MK et al. Urinary incontinence and prevalence of high depressive symptoms in older black versus white women. Int Urogynecol J. 2014;25(6):823-9.

50. Tettamanti G et al. Depression, neuroticism, and urinary incontinence in premenopausal women: a nationwide twin study. Twin Res Hum Genet. 2013;16(5):977-84.

51. Yip SK, Cardozo L. Psychological morbidity and female urinary incontinence. Best Pract Res Clin Obstet Gynaecol. 2007;21(2):321-9.

52. Farage MA et al. Psychosocial and societal burden of incontinence in the aged population: a review. Arch Gynecol Obstet 2008;277(4):285-90.

53. Molinuevo B, Batista-Miranda JE. Under the tip of the iceberg: psychological factors in incontinence. Neurourol Urodyn. 2012;31(5):669-71.

54. Davis S, Wailoo A. A review of the psychometric performance of the EQ-5D in people with urinary incontinence. Health Qual Life Outcomes 2013;11:20.

55. Dugan E et al. The quality of life of older adults with urinary incontinence: determining generic and condition-specific predictors. Qual Life Res. 1998;7(4):337-44.

56. Zorn BH et al. Urinary incontinence and depression. Journal of Urology 1999;162(1):82-4.

57. Deloitte Access Economics. The economic impact of incontinence in Australia. Available at: http://www.continence.org.au/resources.php/01tG0000002pcRqIAI/deloitte-access-economics-report-the-economic-impact-of-incontinence-in-australia. Last Accessed: 2 November 2015.

58. Council of Australian Governments (COAG). National Action Plan on Mental Health 2006 – 2011.2006. Available at https://www.coag.gov.au/sites/default/files/AHMC_COAG_mental_health.pdf Last Accessed 2nd November 2015.

59. Goldney RD et al. Major depression and its associated morbidity and quality of life in a random, representative Australian community sample. Aust N Z J Psychiatry. 2000;34(6):1022-9.

THE ROLE OF BODY FLUIDS IN THE HORIZONTAL TRANSMISSION OF HEPATITIS B VIRUS VIA HOUSEHOLD/CLOSE CONTACT

***Haruki Komatsu,[1] Ayano Inui,[2] Tomoo Fujisawa[2]**

1. Department of Pediatrics, Toho University Sakura Medical Center, Chiba, Japan
2. Department of Pediatric Hepatology and Gastroenterology, Eastern Yokohama Hospital, Kanagawa, Japan
**Correspondence to haruki-komatsu@chive.ocn.ne.jp*

Disclosure: The authors have declared no conflicts of interest.
Received: 27.11.15 **Accepted:** 20.01.16
Citation: EMJ. 2016;1[1]:68-75.

ABSTRACT

Hepatitis B virus (HBV) infection commonly occurs through horizontal transmission via household/close contact. Although the body fluids of patients infected with HBV are likely to play a significant role in horizontal transmission, the precise mechanism remains unclear. In the 1970s, the infectivity of body fluids including saliva, urine, and faeces was assessed for the presence of hepatitis B surface antigen (HBsAg). Over the last decade, the HBV DNA in the body fluids of chronically infected patients was quantified using real-time polymerase chain reaction. Chimpanzee, gibbon, and chimeric mice with human livers have also been used to investigate the infectivity of body fluids. HBsAg levels, HBV DNA levels, and animal experiments have indicated that saliva and tears are able to transmit HBV. Urine and faeces do not lead to horizontal transmission. The infectivity of the remaining body fluids remains controversial. Horizontal transmission is related to both virus and host factors; thus, evaluations of HBsAg and HBV DNA levels provide insufficient data to determine the infectivity of body fluids. Universal hepatitis B vaccination has been implemented worldwide (with the exception of Northern Europe); an understanding of the role that body fluids play in horizontal transmission will contribute to the eradication of HBV.

Keywords: Hepatitis B virus (HBV), infection, transmission, hepatitis B surface antigen (HBsAg), HBV DNA, animal models.

INTRODUCTION

There are three major modes of hepatitis B virus (HBV) infection that are currently considered: 1) perinatal (mother-to-child) transmission, 2) sexual transmission, and 3) unsafe injections.[1] In high-endemic areas, perinatal transmission is the most common mode. In low-endemic areas, unprotected sexual behaviour and unsafe injections are the predominant transmission routes. In addition to these modes of transmission, horizontal transmission through household/close contact also plays a crucial role in spreading HBV in high-endemic areas.[2]

Historically, horizontal transmission through household/close contact contributed to the understanding of HBV pathogenesis. Experience obtained during epidemics following disasters and wars led to the establishment of two types of hepatitis in 1947: 'infective hepatitis' (i.e. hepatitis A) and 'serum hepatitis' (i.e. hepatitis B).[3] The transmission sources of infective hepatitis were hypothesised to be food and blood, whereas serum hepatitis was thought to spread from person to person parenterally via infected blood, blood products, or the use of contaminated syringes or needles. In 1963, Blumberg et al. discovered an 'Australia antigen' (i.e. hepatitis B surface antigen [HBsAg]) and published an article about it in 1965.[4,5] Early on in the discovery process, these authors erroneously thought that this antigen was associated with leukaemia.[5] However, clinical and experimental findings revealed that the Australia antigen was related to viral hepatitis.[6,7]

Horizontal transmission through household contact in a mental health institution suggested that the Australia antigen was linked to an infectious agent.[6] Moreover, horizontal transmission through household contact provided researchers with the opportunity to determine the pathogenicity of hepatitis B. In 1953, viral hepatitis was spread through horizontal transmission at Willowbrook State School, a residential institution that cared for children with mental disabilities in New York, USA.[8] In 1967, Krugman et al. successfully identified the clinical features of hepatitis A and hepatitis B at this institution;[8,9] however, this experiment later presented ethical issues.[10,11]

The mechanism of horizontal transmission through household/close contact remains unclear. The exposure of abraded skin, cuts, minor open wounds, or mucosal surfaces to blood or body fluids containing HBV from the afflicted may lead to HBV infection. Although hepatitis B vaccination is part of the routine childhood immunisation programme in nearly all countries, investigations of the relationship between body fluids and HBV infection remain extremely important for HBV infection control. This review focusses on horizontal transmission via body fluids, summarises the past and present data regarding body fluids, and discusses the role that body fluids play in the development of HBV infection.

HEPATITIS B SURFACE ANTIGEN IN BODY FLUIDS

Early epidemiological investigations suggested that HBV infection occurred parenterally;[3] thus, suggesting that contact with blood and blood products presented a risk of HBV infection. Krugman et al. demonstrated that HBV was transmitted through the oral administration of serum from a child infected with HBV;[8,9] however, the development of HBV infection through oral administration has not been confirmed. Moreover, non-parenteral transmission was reported in the early 1970s.[12,13] To clarify the mechanism of non-parenteral HBV transmission, researchers began to study the infectivity of body fluids from people infected with HBV. At that time, the presence of HBsAg in body fluids was considered to represent a surrogate marker of infectivity. Table 1 shows the frequency of HBsAg detection in body fluid studies conducted in the 1970s. Saliva, urine, faeces, nasal washings, tears, semen, vaginal secretions, bile, sweat, pleural fluid, amniotic fluid, and breast milk were evaluated for the presence of HBsAg.[14-23] Of these body fluids, saliva showed a high frequency of HBsAg, ranging from 34–86%.[14,15,17,19] Therefore, saliva was considered to accurately reflect antigenaemia and act as a potential infectious source.[24,25] As saliva is easily collected, it has been used to diagnose HBV infection in epidemiological studies.[26,27] The frequency of HBsAg in urine ranges from 2–33%.[15,17,18] Compared with saliva, these frequencies are low. Due to the fact that the frequency of HBsAg in urine varied across studies, this fluid was not used for diagnosis. Based on the frequency of HBsAg, urine appeared to be less infectious than saliva.[25]

As with hepatitis A, an oral-faecal route was strongly suspected in the spread of HBV. An early study detected HBsAg in the faeces of patients infected with HBV.[28] However, subsequent studies showed conflicting results regarding the detection rate of faecal HBsAg.[15,17,18] Villarejos et al.[15] and Irwin et al.[17] did not find any patients with chronic HBV HBsAg-positive for faecal HBsAg, whereas Tiku et al.[18] showed that 10% of patients with chronic hepatitis B were positive for faecal HBsAg. In the late 1980s, faeces were considered unimportant for HBV transmission from an epidemiological standpoint.[25]

Other studies evaluated nasal washings, tears, pleural fluids, and sweat. Although the frequency of HBsAg varied across studies, it was detected in all of these fluids. Contact with nasal droplets, tears, and sweat is common in daily life. As the nasal cavity is connected to the oral cavity, saliva might influence the frequency of nasal washing. Sweat and tears displayed high frequencies of HBsAg (100%[20] and 56%,[22] respectively). While the infectivity of sweat is extremely important in daily contact, sweat and tears were considered to be unimportant infectious agents in HBV infection.[25] Semen, vaginal secretions, and amniotic fluids were positive for HBsAg,[14,19,23] and these fluids might represent the sources of sexual transmission and mother-to-child transmission. HBsAg was detected in the breast milk of mothers with HBV;[23] however, breast feeding did not represent an additional risk of mother-to-child transmission.[29] HBsAg quantification was lacking in these studies. Patients with acute or chronic infections were not differentiated. Moreover, the hepatitis B envelope antigen (HBeAg) serostatus was unknown. Presumably, patients with varying viral loads of HBV in the blood were enrolled.

Table 1: HBsAg-positive rate of body fluids in patients with hepatitis B infection.

Year	Author	Status of serum	Body fluid	Detection rate of HBsAg in body fluids %		HBsAg test
1974	Heathcote et al.[14]	HBsAg-positive (Acute and chronic infection)	Saliva	75	(18/24)	Radioimmunoassay
			Semen	52	(10/19)	
1974	Villarejos et al.[15]	HBsAg-positive (Acute and chronic infection)	Faeces	0	(0/120)	Radioimmunoassay
			Urine	2	(3/130)	
			Saliva	81	(75/93)	
1975	Pizza et al.[16]	HBsAg-positive (acute infection)	Bile	80	(4/5)	Immunodiffusion and immunoelectroosmophoresis
1975	Irwin et al.[17]	Chronic infection	Faeces	0	(0/30)	Radioimmunoassay
			Urine	16	(7/43)	
			Saliva	34	(14/41)	
1976	Tiku et al.[18]	HBsAg-positive (chronic infection)	Nasal washings	26	(14/53)	Radioimmunoassay
			Urine	33	(19/56)	
			Faeces	10	(3/30)	
1976	Parker et al.[19]	HBsAg-positive (acute infection)	Saliva	86	(6/7)	Radioimmunoassay
			Vaginal secretions	78	(7/9)	
1977	Telatar et al.[20]	HBsAg-positive (hepatitis and cirrhosis)	Sweat	100	(30/30)	Radioimmunoassay
1977	De Flora et al.[21]	HBsAg-positive	Pleural fluid	100	(1/1)	Radioimmunoassay
1978	Darrell et al.[22]	HBsAg-positive	Tears	56	(10/18)	Radioimmunoassay
1978	Lee et al.[23]	HBsAg-positive	Amniotic fluid	33	(17/52)	Radioimmunoassay
			Breast milk	71	(45/63)	

HBsAg: hepatitis B serum antigen.

Table 2: HBV DNA level of body fluids in patients with chronic HBV infection.

Year	Author	No. of subjects	HBeAg/Ab status or HBV DNA level in serum	Body fluid	Detection rate of HBV DNA in body fluids %		HBV DNA level in body fluids	
2004	van der Eijk et al.[30]	27	HBeAg (positive: n=15, negative: n=12)	Saliva	85	(23/27)	2.27 ×10^4	copies/mL (median)
			HBV DNA 2.1 × 10^5 genome equivalents/mL (median)					
2005	van der Eijk et al.[31]	150	HBeAg (positive: n=65, negative: n=82, unknown=3)	Saliva	47	(69/147*)	3.2	Log_{10} copies/mL (mean)
			HBV DNA 5.8 log copies/mL (mean)	Urine	32	(47/147*)	2.6	Log_{10} copies/mL (mean)
2006	Kidd-Ljunggren et al.[32]	10	HBeAg positive: n=5, HBeAb positive: n=5 HBV DNA levels were >10^5 copies/mL in all subjects	Saliva	80	(8/10)	1×10^2 –5.3×10^5	copies/mL
				Nasopharyngeal fluid	60	(6/10)	1×10^2 –7.5×10^6	copies/mL
				Tears	57	(4/7)	3×10^2 –1.4×10^4	copies/mL

Table 2 continued.

Year	Author	No. of subjects	HBeAg/Ab status or HBV DNA level in serum	Body fluid	Detection rate of HBV DNA in body fluids %		HBV DNA level in body fluids	
2010	Heiberg et al.[33]	25	HBeAg positive, HBV DNA 41.9×10^6 IU/mL (mean)	Saliva	NA	NA	33.9×10^3	IU/mL (mean)
		18	HBeAg negative, HBV DNA 880 IU/mL (mean)		0	NA	No detection	IU/mL (mean)
2012	Komatsu et al.[34]	47	HBeAg (positive: n=39, negative: n=8) HBV DNA >9 log copies/mL (median), HBV DNA was detected in all subjects	Urine	74	(14/19)	4.3	Log_{10} copies/mL (mean)
				Saliva	87	(33/38)	5.9	Log_{10} copies/mL (mean)
				Tears	100	(11/11)	6.2	Log_{10} copies/mL (mean)
				Sweat	100	(9/9)	5.2	Log_{10} copies/mL (mean)
2015	Komatsu et al.[35]	50	HBeAg (positive: n=37, negative: n=13) HBV DNA >9 log copies/mL: n=24, 6–9 log copies/mL: n=13 patients >2.1 to <6 log copies/mL: n=13	Faeces	74	(37/50)	5.6	Log_{10} copies/mL (mean)
2015	Fei et al.[36]	94	HBeAg positive, HBV DNA 8.1 log IU/mL (median)	Semen	68	NA	3.2	Log_{10} IU/mL (median)
		57	HBeAg negative, HBV DNA 3.2 log IU/mL (median)	Semen	2	NA	0	Log_{10} IU/mL (median)

NA: no data available; HBV: hepatitis B virus; HBeAG: hepatitis B envelope antigen; HBeAb: hepatitis B envelope antibody.

Radioimmunoassays were used to detect HBsAg in these studies. If advanced technologies such as enzyme immunoassays and chemiluminescent immunoassays are used to measure HBsAg,[30] then the details of HBsAg in body fluids might become clearer. These limitations may also have led to inconsistencies in the detection rate of HBsAg in body fluids.

HEPATITIS B VIRUS DNA IN BODY FLUIDS

Since the early 2000s, the level of HBV DNA in body fluids had been quantified using real-time polymerase chain reaction in Northern Europe and Japan, where the HBV vaccine was not introduced into routine child immunisation programs. Saliva, which is likely to be infectious, as well as urine, faeces, and tears, which are not likely to be infectious based on epidemiological data,[25] were evaluated in several studies. The HBV DNA levels of the body fluids of patients with chronic HBV infection are shown in Table 2.[31-37] The levels of HBV DNA in serum and HBeAg serostatus were also evaluated in these studies, and the detection rate of HBV DNA in saliva was higher than that in urine. A study from the Netherlands reported detection rates for HBV DNA in saliva and urine of 47% and 32%, respectively.[32] Similarly, a study from Japan reported HBV DNA detection rates in saliva and urine of 87% and 74%, respectively.[35] Moreover, these studies showed that the level of HBV DNA in saliva was higher than that in urine (saliva: 3.2 log_{10} copies/mL and urine 2.6 log_{10} copies/mL in a study from the Netherlands;[32] saliva: 5.9 log_{10} copies/mL and urine 4.3 log_{10} copies/mL in a study from Japan[35]). These findings suggest that saliva is more important than urine with regard to horizontal transmission. In addition, tears, sweat, faeces, and semen showed high HBV DNA detection rates (tears: 57%[32] and 100%;[35] sweat: 100%;[35] faeces: 74%;[36] semen: 68% [HBeAg positive]).[37]

Table 3: Infectivity of serum and body fluids with various routes of in human and animal experiments.

Year	Author	Model	Donor, HBeAg serostatus	Serum or body fluids	Route of inoculation				
					Oral	SC or IM	Intravaginal	Intravenous	Corneal surface
1967	Krugman et al.[8]	Human	Unknown	Serum	Yes	Yes (IM)	-	-	-
1977	Bancroft et al.[44]	Gibbon	Positive and negative mixed	Saliva	No	Yes (SC)	-	-	-
1977	Alter et al.[45]	Chimpanzee	Positive	Saliva	-	-	-	Yes	-
				Semen	-	-	-	Yes	-
1980	Scott et al.[46]	Gibbon	Positive	Saliva	No	Yes (SC)	-	-	-
				Semen		Yes (SC)	Yes	-	-
1981	Bond et al.[47]	Chimpanzee	Positive	Dried and stored plasm	-	-	-	Yes	-
1982	Bond et al.[48]	Chimpanzee	Unknown	Serum	-	-	-	-	Yes
2012	Komatsu et al.[34]	Chimeric mouse	Positive	Tear	-	-	-	Yes	-
2015	Komatsu et al.[35]	Chimeric mouse	Positive	Faeces	No	-	-	Yes	-

Yes: HBV infection is confirmed.
No: HBV infection is not confirmed.
IM: intramuscular; SC: subcutaneous; HBeAG: Hepatitis B envelope antigen; HBV: hepatitis B virus.

The levels of HBV DNA are between 2 $\log_{10}$ copies/mL and 6 $\log_{10}$ copies/mL in tears;[33,35] 5.2 $\log_{10}$ copies/mL in sweat;[35] 5.6 $\log_{10}$ copies/mL in faeces;[36] and 3.2 $\log_{10}$ copies/mL in semen.[37] Urine, tears, sweat, and faeces contain low or intermediate levels of HBV DNA. However, urine, tears, sweat, and faeces are not epidemiologically likely to transmit HBV.[25]

How Can We Explain These Findings?

The detection rate and the level of HBV DNA in body fluids were positively correlated with HBV DNA levels in the blood. The relationship between the level of HBV DNA in the saliva versus serum was described as follows: HBV DNA in saliva=1.01+0.56×($\log_{10}$ HBV DNA in serum),[31] HBV DNA in saliva=-6.63+0.92×($\log_{10}$ HBV DNA in serum),[34] and HBV DNA in saliva or tears=-3.23+1.06×($\log_{10}$ HBV DNA in serum).[35] According to these formulas, the level of HBV DNA in saliva is 10^3 to 10^6-fold lower than that in serum. This finding is consistent with that of a previous study that reported that the levels of HBV virus particles in saliva were 10^3 to 10^4-fold less than those simultaneously present in serum.[24] The most important discovery that needs to be made is the HBV DNA cut-off level for transmission. The answer to this issue might be found in the recommendations made to the management of healthcare workers (HCWs) infected with HBV. For instance, the U.S. Center for Disease Control and Prevention considers <5,000 copies/mL (<1,000 IU/mL) of HBV DNA in blood to be a safe value for exposure-prone procedures (EPPs).[38] The Society for Healthcare Epidemiology of America and the European Consensus group guidelines recommend a cut-off value of $<10^4$ copies/mL of HBV DNA in blood for HCWs to perform EPPs in patients with HBV.[39,40] The UK Department of Health guidelines determined that $<10^3$ copies/mL of HBV DNA in blood was the cut-off value to perform EPPs.[41] Although household/close contact conditions differ across EPPs, based on these recommendations, body fluids containing $\geq 10^5$ of HBV DNA have infection potential. Table 2 shows that the HBV DNA levels in tears exceeded 10^6 copies/mL. The HBV DNA levels in saliva, nasopharyngeal fluid, sweat, and faeces were $>10^5$ copies/mL. Taken together and based on HBV

DNA levels, tears, saliva (including nasopharyngeal fluid), sweat, and faeces appear to be infectious vehicles of HBV; however, all but saliva are likely to be epidemiologically unimportant with regard to HBV transmission.[25] Notably, several studies reported that the HBV DNA level in blood was not correlated with HBV infectivity in animal models.[42,43] Pre-acute-phase serum is 100-fold more infectious than late acute-phase serum.[42] Exposure to an HCW with a viral burden of 10^4 copies/mL in blood is associated with exposure to <1 virion.[39] However, details on HBV virions in body fluids remain unknown.

THE INFECTIVITY OF SERUM AND BODY FLUIDS IN HUMAN AND ANIMAL EXPERIMENTS

Animal experiments represent the gold standard for evaluating HBV infectivity. Primates such as chimpanzees and gibbons can be infected with HBV.[44-48] In addition, chimeric mice with human livers were recently developed and used for viral hepatitis experiments.[49] The infectivity of serum and body fluids in human and animal experiments are shown in Table 3.[8,35,36,44-48] These experiments have two objectives. The first is to evaluate the infectivity of serum and body fluids, and the other is to confirm the inoculation route. In 1967, Krugman proved that an intramuscular injection with serum induced HBV infection in humans. Moreover, HBV infection occurred through the oral inoculation of serum in humans.[8] Ten years after that experiment, saliva was shown to be an infectious agent via subcutaneous and intravenous inoculation.[44-46] Semen was also demonstrated to be an infectious agent via intravenous and intravaginal inoculation.[45,46] These findings suggested that intravenous, intramuscular, and subcutaneous inoculations were appropriate routes to determine the infectivity of body fluids. Interestingly, plasma that was dried and stored for 1 week was able to infect a chimpanzee through intravenous inoculation.[47] This study suggested that HBV has sufficient potential to survive as an infectious agent in humans. Semen, through intravaginal inoculation and serum corneal surface inoculation, also led to infection.[46,48] In contrast, two studies failed to demonstrate that saliva was infectious via oral inoculation in gibbons:[44,46] Krugman demonstrated HBV infection through oral inoculation with serum;[8] the difference in viral loads between serum and saliva might explain the failed oral inoculation. Thus, oral inoculation is not a potential route to evaluate body fluid infectivity in this context. Enzymes and bacteria in the gastrointestinal tract are likely to affect antigenicity and inactive HBV.[50-52] Tears were infectious using chimeric mice;[35] however, faeces (which contain low levels of HBsAg and intermediate levels of HBV DNA) were not infectious.[36] The body fluids currently shown to be infectious using animal experiments are saliva, semen, and tears.

CONCLUSIONS

As surrogate makers of infectivity, HBsAg and HBV DNA have been detected and quantified in body fluids. In addition, animal experiments have examined the infectivity of body fluids. Based on the HBsAg detection rate, HBV DNA level and animal experiments, saliva, and tears can transmit HBV through household/close contact. Urine and faeces do not appear to cause horizontal infection. The roles of the remaining body fluids remain controversial. The development of HBV infection through horizontal transmission is associated with multiple factors. Viral loads in body fluids, viral activity (intact or damaged virions) in body fluids, stage of infection (pre-acute phase, late-acute, or immunotolerant phase), the entry site of body fluids (widely open wounds, small skin cuts, abrasions, skin penetrations via needles, mucous membrane, or oral ingestion), and the individual's immune response can influence outcomes following contact with body fluids from people infected with HBV. Therefore, whether body fluids play a significant role in the horizontal transmission of HBV is difficult to determine via the evaluations of HBsAg and HBV DNA. Chimeric mice with human livers and fresh human hepatocytes isolated from chimeric mice might be useful in determining the infectivity of body fluids.[49,53] In the era of universal hepatitis B vaccine immunisation, less interest exists in the body fluids of people infected with HBV (with the exception of Northern Europeans). However, an investigation into the precise mechanism of horizontal transmission will contribute to the eradication of HBV.

REFERENCES

1. Trépo C et al. Hepatitis B virus infection. Lancet. 2014;384(9959):2053-63.

2. Dumpis U et al. Transmission of hepatitis B virus infection in Gambian families revealed by phylogenetic analysis. J Hepatol. 2001;35(1):99-104.

3. MacCallum FO. Early studies of viral hepatitis. Br Med Bull. 1972;28(2):105-8.

4. Alter H. Baruch Blumberg (1925-2011). Nature. 2011;473(7346):155.

5. Blumberg BS et al. A "new" antigen in leukemia sera. JAMA. 1965;191(7):541-6.

6. Sutnick AI et al. Anicteric hepatitis associated with Australia antigen. Occurrence in patients with Down's syndrome. JAMA. 1968;205(10):670-4.

7. London WT et al. Australia antigen and acute viral hepatitis. Ann Intern Med. 1969;70(1):55-9.

8. Krugman S et al. Infectious hepatitis. Evidence for two distinctive clinical, epidemiological, and immunological types of infection. JAMA. 1967;200(5):365-73.

9. Giles JP et al. Relation of Australia-SH antigen to the willowbrook MS-2 strain. N Engl J Med. 1969;281(3):119-22.

10. Goldby S. Experiments at the Willowbrook State School. Lancet. 1971;1(7702):749.

11. Krugman S. The Willowbrook hepatitis studies revisited: ethical aspects. Rev Infect Dis. 1986;8(1):157-62.

12. Hersh T et al. Nonparenteral transmission of viral hepatitis type B (Australia antigen-associated serum hepatitis). N Engl J Med. 1971;285(24): 1363-4.

13. Szmuness W et al. Familial clustering of hepatitis B infection. N Engl J Med. 1973;289(22):1162-6.

14. Heathcote J et al. Hepatitis-B antigen in saliva and semen. Lancet. 1974;1(7847): 71-3.

15. Villarejos VM et al. Role of saliva, urine and feces in the transmission of type B hepatitis. N Engl J Med. 1974;291(26): 1375-8.

16. Piazza M et al. Presence of HBsAg in the bile of subjects with acute HBsAg viral hepatitis. Pathol Microbiol (Basel). 1975;43(4):307-16.

17. Irwin GR et al. Hepatitis B antigen in saliva, urine, and stool. Infect Immun. 1975;11(1):142-5.

18. Tiku ML et al. Distribution and characteristics of hepatitis B surface antigen in body fluids of institutionalized children and adults. J Infect Dis. 1976;134(4):342-7.

19. Parker HW et al. Venereal transmission of hepatitis B virus. The possible role of vaginal secretions. Obstet Gynecol. 1976;48(4):410-2.

20. Telatar H et al. HB-Ag in sweat. Am J Gastroenterol. 1977;68(5):492-3.

21. De Flora S, Forci F. Hepatitis B surface antigen in pleural fluid. Lancet. 1977;1(8024):1269.

22. Darrell RW, Jacob GB. Hepatitis B surface antigen in human tears. Arch Ophthalmol. 1978;96(4):674-6.

23. Lee AK et al. Mechanisms of maternal-fetal transmission of hepatitis B virus. J Infect Dis. 1978;138(5):668-71.

24. Davis LG et al. Horizontal transmission of hepatitis B virus. Lancet. 1989;1(8643):889-93.

25. Zimmerman FH, Wormser GP. Exposure to hepatitis B: review of current concepts. Bull N Y Acad Med. 1989;65(7):741-56.

26. Quoilin S et al. A population-based prevalence study of hepatitis A, B and C virus using oral fluid in Flanders, Belgium. Eur J Epidemiol. 2007;22(3):195-202.

27. Sutton AJ et al. Modelling the force of infection for hepatitis B and hepatitis C in injecting drug users in England and Wales. BMC Infect Dis. 2006;6(93).

28. Grob PJ, Jemelka H. Faecal S.H. (Australia) antigen in acute hepatitis. Lancet. 1971;1(7692):206-8.

29. Petrova M, Kamburov V. Breastfeeding and chronic HBV infection: clinical and social implications. World J Gastroenterol. 2010;16(40):5042-6.

30. Deguchi M et al. Quantitation of hepatitis B surface antigen by an automated chemiluminescent microparticle immunoassay. J Virol Methods. 2004;115(2):217-22.

31. van der Eijk AA et al. Paired measurements of quantitative hepatitis B virus DNA in saliva and serum of chronic hepatitis B patients: implications for saliva as infectious agent. J Clin Virol. 2004;29(2):92-4.

32. van der Eijk AA et al. Paired, quantitative measurements of hepatitis B virus DNA in saliva, urine and serum of chronic hepatitis B patients. Eur J Gastroenterol Hepatol. 2005;17(11):1173-9.

33. Kidd-Ljunggren K et al. High levels of hepatitis B virus DNA in body fluids from chronic carriers. J Hosp Infect. 2006;64(4):352-7.

34. Heiberg IL et al. Hepatitis B virus DNA in saliva from children with chronic hepatitis B infection: implications for saliva as a potential mode of horizontal transmission. Pediatr Infect Dis J. 2010;29(5):465-7.

35. Komatsu H et al. Tears from children with chronic hepatitis B virus (HBV) infection are infectious vehicles of HBV transmission: experimental transmission of HBV by tears, using mice with chimeric human livers. J Infect Dis. 2012;206(4):478-85.

36. Komatsu H et al. Lack of infectivity of HBV in feces from patients with chronic hepatitis B virus infection, and infection using chimeric mice. BMC Res Notes. 2015;8(366).

37. Fei QJ et al. Can hepatitis B virus DNA in semen be predicted by serum levels of hepatitis B virus DNA, HBeAg, and HBsAg in chronically infected men from infertile couples? Andrology. 2015;3(3):506-11.

38. Centers for Disease Control and Prevention (CDC). Updated CDC recommendations for the management of hepatitis B virus-infected health-care providers and students. MMWR Recomm Rep. 2012;61(RR-3):1-12.

39. Henderson DK et al. SHEA guideline for management of healthcare workers who are infected with hepatitis B virus, hepatitis C virus, and/or human immunodeficiency virus. Infect Control Hosp Epidemiol. 2010;31(3):203-32.

40. Gunson RN et al. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in health care workers (HCWs): guidelines for prevention of transmission of HBV and HCV from HCW to patients. J Clin Virol. 2003;27(3):213-30.

41. UK Government Department of Health. Hepatitis B infected healthcare workers and antiviral therapy. Available at: http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/documents/digitalasset/dh_073133.pdf. 2007. Last accessed: 27 November 2015.

42. Tabuchi A et al. Titration of hepatitis B virus infectivity in the sera of pre-acute and late acute phases of HBV infection: transmission experiments to chimeric mice with human liver repopulated hepatocytes. J Med Virol. 2008;80(12):2064-8.

43. Asabe S et al. The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. J Virol. 2009;83(19):9652-62.

44. Bancroft WH et al. Transmission of hepatitis B virus to gibbons by exposure to human saliva containing hepatitis B surface antigen. J Infect Dis. 1977;135(1):79-85.

45. Alter HJ et al. Transmission of hepatitis B to chimpanzees by hepatitis B surface antigen-positive saliva and semen. Infect Immun. 1977;16(3):928-33.

46. Scott RM et al. Experimental transmission of hepatitis B virus by semen and saliva. J Infect Dis. 1980;142(1):67-71.

47. Bond WW et al. Survival of hepatitis B virus after drying and storage for one week. Lancet. 1981;1(8219):550-1.

48. Bond WW et al. Transmission of type B viral hepatitis via eye inoculation of a chimpanzee. J Clin Microbiol. 1982;15(3):533-4.

49. Chayama K et al. Animal model for study of human hepatitis viruses. J Gastroenterol Hepatol. 2011;26(1):13-8.

50. Mazzur S et al. Loss of immunologic reactivities of Australia antigen after incubation with bacteria. Proc Soc Exp Biol Med. 1973;142(1):327-32.

51. Moodie JW et al. The problem of the demonstration of hepatitis B antigen in faeces and bile. J Clin Pathol. 1974;27(9):693-7.

52. Weng LK et al. Interaction of hepatitis B surface antigen (Australia antigen) with membrane vesicles of Pseudomonas aeruginosa. Infect Immun. 1975;12(1): 180-6.

53. Ishida Y et al. Novel robust in vitro hepatitis B virus infection model using fresh human hepatocytes isolated from humanized mice. Am J Pathol. 2015;185(5):1275-85.

EMJ EUROPEAN MEDICAL JOURNAL

INTERACT WITH US ON SOCIAL MEDIA

EUROPEAN UNION
Investing in Your Future
European Regional Development Fund 2007-13

LATEST **NEWS** • **CONGRESS** UPDATES • JOIN **DISCUSSIONS** • **AWARENESS** CAMPAIGNS

UTILITY OF EXOSOMES IN THE DIAGNOSIS AND TREATMENT OF PANCREATIC ADENOCARCINOMA

Leo I. Amodu, Asaph Levy, Crystal Kyaw, Darshan Padmanabhan, Alexandra Osman, Mukesh Tiwari, Jeffrey Nicastro, Gene Coppa, Ernesto Molmenti, *Horacio L. Rodriguez Rilo

Center for Diseases of the Pancreas, Department of General Surgery, Hofstra North Shore-Long Island Jewish School of Medicine, Hempstead, New York, USA
**Correspondence to Hrilo@nshs.edu*

Disclosure: The authors have declared no conflicts of interest.
Received: 18.07.15 **Accepted:** 25.11.15
Citation: EMJ. 2016;1(1):77-85.

ABSTRACT

Pancreatic cancer is the most common lethal cancer, with annual incidence and mortality rates being approximately equal. This dismal prognosis can be attributed to late diagnosis making the cancers unresectable. These cancers respond poorly to chemotherapy and radiation, and surgical resection remains the most effective treatment available. Diagnostic tests that are sensitive, specific, and capable of early detection are urgently needed and would significantly impact upon pancreatic cancer treatment and outcomes. Exosomes, small membrane-bound vesicles which are fairly uniform in size (approximately 30–100 nm in diameter), contain messenger RNA, microRNA (miRNA), and proteins. They are ubiquitous and stable in most body fluids and exosomal miRNAs are also resistant to degradation by RNAses and DNAses. Expression profiles of serum exosomal miRNAs display sensitivity and specificity in the detection of pancreatic adenocarcinoma. Markers of pancreatic cancer-initiating cells are also expressed on serum exosomes. Exosomes exhibit key functions in addition to their distinct structural properties: they are involved in immune system modulation via the transfer of antigenic proteins, and through protease activity they modulate the extracellular environment prior to metastasis. Exosomes are being studied as potent gene delivery tools and dendritic cell exosomes are already used as cancer vaccines. This review focusses on the current state of exosomal research, particularly in relation to their applicability as diagnostic and therapeutic tools for patients with pancreatic adenocarcinoma.

Keywords: Adenocarcinoma, biomarkers, diagnosis, exosomes, pancreatic, vesicles.

INTRODUCTION

Pancreatic cancer is one of the most aggressive malignancies, resulting in poor prognosis for most patients.[1] It is considered the deadliest cancer and ranks fourth in cancer-related mortality.[2] The only effective treatment to date remains surgical resection. The poor prognosis for pancreatic adenocarcinoma (PaCa) is largely attributed to late detection and early metastasis. The 5-year survival rate for the earliest form of PaCa (Stage IA) is only 14%, and is a mere 1% for Stage IV.[3]

Pancreatic cancer research is now developing methods for early detection. Exosomes that are extracellular vesicles containing microRNAs (miRNAs), messenger RNA (mRNA), and proteins have recently been used as unique pancreatic cancer markers. miRNAs are non-coding RNAs that play a role in the regulation of post-transcriptional gene expression.[1] The miRNA expression profile of tumour-derived serum exosomes from PaCa patients differs significantly from those of healthy donors and patients with non-malignant disease.[2] Patients with cancer are thought to have exponentially higher numbers of circulating exosomes because they are secreted in large amounts during carcinogenesis.[4] In addition, exosomes released from tumour cells are readily detected in body fluids and have therefore emerged as potential non-invasive diagnostic tools capable of supporting earlier diagnosis due to their ubiquitous nature and structural stability.

STRUCTURE OF EXOSOMES

The classification of a particle as an exosome is based primarily on size, density, and membrane composition.[5] Exosomes are 30–100 nm vesicles of endocytic origin, and are secreted by a variety of cell types including cancer cells, mesenchymal cells, epithelial cells, haematopoietic cells, dendritic cells, mast cells, neurons, thrombocytes, and T cells.[5,6]

The first step in exosome biogenesis involves the inward budding of the plasma membrane to form early endosomes,[7] which then mature in the presence of several intracellular protein factors to become late endosomes, also known as multivesicular bodies (MVBs). These MVBs contain exosomes, which can be released from the cell or fused with intracellular lysosomes, leading to degradation of the contained exosomes.[7-9] More specifically, when releasing exosomes into the extracellular space, the MVBs fuse with the plasma membrane resulting in the release of exosomes by exocytosis.[7,10] Exosomes exhibit a lipid bilayer membrane carrying common exosomal marker proteins and cell type-specific markers,[11] which include tetraspanins such as CD9, CD63, CD81, and cytoplasmic proteins including actin, annexins, and Rab proteins.[11] The presence of Alix protein, tumour susceptibility gene 101, and tetraspanins reveals the MVB origin of exosomes,[5] which are packed with miRNA, mRNA, and proteins from the parent cell during formation. Kahlert et al.[6] demonstrated that pancreatic cancer cell-derived exosomes can contain fragments of double-stranded genomic DNA >10 kb in length. DNA samples from exosomes span all chromosomes, and mutations in the genes encoding K-ras and p53 can also be detected. In addition to the transfer of genes, Record et al.[8] describe the role of prostaglandin E2-rich, tumour-derived exosomes in tumour immune evasion and promotion of tumour growth by non-recognition.[8]

Exosomes are stable under varying conditions, allowing them to survive in many body fluids.[6] In the tumour microenvironment, released tumour-derived exosomes can transfer proteins and RNAs with oncogenic activity to recipient cells. Exosomes are found in several body fluids of cancer patients, such as synovial fluid, cerebrospinal fluid, bronchial lavage fluid, breast milk, serum, saliva, urine, ascites, and malignant effusions.[7,10,12-15] The ubiquitous nature of exosomes make them promising for potential early diagnosis of PaCa.

FUNCTIONS OF EXOSOMES

Exosomes facilitate intercellular communication and are involved in several physiological and pathological processes, including coagulation, inflammation, tumour progression, immune response regulation, antigen presentation, and transfer of nucleic acids, proteins, and infectious cargo such as prions and retroviruses.[16,17]

Exosomes are thought to play a key role in facilitating cell–cell communication in the tumour microenvironment,[6] and are implicated in angiogenesis and promotion of cell proliferation and survival.[5] More specifically, exosomes play several roles in the tumour microenvironment: 1) suppression of immune function by inducing apoptosis of activated cytotoxic T cells or by promoting differentiation of regulatory T cells, thus enabling tumour progression; 2) stimulation of angiogenesis and migration leading to metastasis.[11] Previous studies have demonstrated that exosomes are actively released into the peripheral circulation by cancerous cells,[18] and biomarker studies have shed light on the possibility of using exosomal protein and RNA profiles in cancer diagnosis.[19,20]

Tumour-derived exosomes can alter the molecular profile of their microenvironment and help to establish a metastatic niche to aid tumour growth and metastasis.[5] It has also been reported that exosomes utilise vascular endothelial growth factor and cytokine cargo to enhance recruitment of endothelial and haematopoietic precursor cells to enhance neoangiogenesis in the tumour.[5] It has been suggested that exosomes influence planar cell polarity and the extracellular matrix to allow tumour cell mobilisation.[5]

Proteomic analysis has detected extracellular proteases, particularly metalloproteinases, in exosomes[21] and these membrane proteases may alter the surface of the recipient cell through ectodomain shedding. These cleaved and soluble proteins may then act in an autocrine and/or paracrine fashion. Other cell membrane proteins studied for their role in metastasis have revealed mechanisms that are more clear. CD151 and Tspan8 are exosome-based tetraspanins that promote metastasis in several tumour systems.[14] CD151 supports migration via integrin trafficking and activation of Ras, Rac1, and Cdc42 recruitment.[14] It also regulates cell motility via protease activity that enhances both adhesion and matrix degradation. Tspan8 contributes further to motility by associating

with α6β4 (an integrin laminin receptor).[14] Interestingly, tumours in CD151/Tspan8 knockout animals have been shown to lose metastatic potential, underlining the necessity of these tetraspanins in metastatic growth.

Exosomes are known to trigger apoptosis in anti-tumour immune cells through Fas ligand and tumour necrosis factor pathways.[5] Other immunomodulatory effects of tumour-derived exosomes include disruption of immune cell differentiation, such as maturation of $CD14^{+}$ and $HLA\text{-}DR^{low/neg}$ monocyte precursors into dendritic cells, and effects on monocytes leading to an altered inflammatory cytokine profile, which results in impaired stimulation of T cells.[5]

ISOLATION OF EXOSOMES FROM HUMAN SPECIMENS

Exosomes have been isolated from several physiological fluids, including blood plasma/serum, breast milk, saliva, and urine.[22] Isolating exosomes from serum and saliva is likely to serve as the best screening method for early diagnosis of pancreatic cancer because obtaining these specimens requires less invasive methods. In 2013, Lau et al.[13] showed that pancreatic cancer-derived exosomes isolated from saliva could be detected and used as salivary biomarkers in a mouse model.

Several isolation methods have been described, including ultracentrifugation, ExoQuick™ precipitation, microfluidic device (ExoChip), and magnetic bead (DynaBeads®) isolation. Ultracentrifugation is considered the gold standard and is used largely in the research setting, although it is time-consuming, labour-intensive, and not ideal for clinical laboratories because it requires a large amount of starting material; exosome yields are typically low.[17,22]

Size-based isolation and exosome precipitation are additional methods. The first is completed using ultrafiltration, which is less time-consuming than other methods and does not require special equipment. It does not result in pure exosomes, however, but rather an exosome-rich sample because both cell culture media and body fluids contain a large number of nanoparticles within the same size range as exosomes. Conversely, exosome precipitation takes advantage of the differential solubility of exosomes in alternative solvents; ExoQuick, a proprietary reagent produced by System Biosciences (Mountain View, CA, USA) is an example. Rekker et al.[22] compared ultracentrifugation with ExoQuick and found that both methods are suitable for serum exosomal miRNA profiling. The miRNA profile was slightly affected by the method used, however, with the detection of two miRNAs (miR-92a and miR-486-5p) significantly influenced by the isolation method chosen. They also found that ExoQuick is more effective in precipitating exosomes from highly viscous biofluids, such as serum, regardless of origin.[22-24] Alvarez et al.[25] described similar results after comparing ultracentrifugation with precipitation-based exosomal isolation protocols using urine samples. These authors studied exosomal protein, miRNA, and mRNA levels and found that the highest exosome yield and RNA quantities were obtained by the ExoQuick precipitation-based method, while ultracentrifugation methods proved most suitable for protein analysis.

The ExoChip microfluidic device is an on-chip isolation, quantification, and characterisation method for circulating exosomes; it is functionalised with antibodies against CD63, an antigen commonly overexpressed in exosomes. Kanwar et al.[4] used the ExoChip method to isolate exosomes from the serum of both healthy and pancreatic cancer patients, and found this method to be suitable for diagnosis and screening of human cancer. The advantages of the ExoChip method include the rapidity and ability to simultaneously process a large number of samples.[4]

A more elaborate version of antibody-based exosome isolation are Dynabeads, magnetic beads that can be used to specifically isolate exosomes because they are coated in a specific antibody that is then used to isolate the molecule of interest. One disadvantage is that many washing steps may be required, potentially leading to unacceptable cell loss. In addition, unless a 'negative sorting' method is used, in which all biomolecules are removed using the beads, antibodies will remain attached to the cell if the exosomes are left in the supernatant.[26]

Taylor and Gercel-Taylor[18] have used antibodies against epithelial cell adhesion molecule to isolate tumour-derived exosomes as biomarkers of ovarian cancer using a modified magnetic activated cell sorting procedure. They compared current methods for exosome purification and found that exosomes isolated by ExoQuick precipitation produce exosomal RNA and protein in greater quantity and purity than chromatography, ultracentrifugation,

and DynaBeads. However, the authors cited a lack of specificity for the originating cell of the exosomes as a limitation of this method. Further analysis using techniques such as quantitative reverse transcription polymerase chain reaction (RT-PCR) profiling of miRNA, however, should adequately characterise these exosomes.

Considering the state of the current literature, it would seem that ExoQuick and ExoChip stand out as the preferred methods for exosome isolation in the clinical setting due to their specificity and ease of use.

USE OF EXOSOMES IN DIAGNOSIS OF PANCREATIC CANCER

Detection of exosomal biomarkers derived from pancreatic tumour cells in human serum that are sensitive and specific for PaCa would have a significant impact on patient outcomes. The ability of exosomal markers to predict tumour stage and degree of differentiation would also be beneficial. Very recently, Melo and colleagues[27] identified a cell surface proteoglycan, glypican-1 (GPC1), which is specifically enriched on cancer cell-derived exosomes. These exosomes, termed 'GPC1^{+}' exosomes, were monitored and isolated from the serum of patients and mice with pancreatic cancer using flow cytometry, and were detected with absolute sensitivity and specificity.[27] These exosomes were able to distinguish patients with early and late-stage PaCa from healthy individuals and patients with benign pancreatic disease. An additional finding was that the levels of GPC1^{+} exosomes correlated with tumour burden and with survival of pre and post-surgery patients.[27] It is noteworthy that GPC1^{+} exosomes were also detected in patients with breast cancer. The authors concluded that GPC1 is a pan-specific biomarker for cancer exosomes, and that many cancer cells overexpress GPC1, with the most abundant increases observed in PaCa.[27]

miRNAs, selectively concentrated into tumour-derived exosomes and differentially expressed, are suggested as potential markers for detection of early pancreatic cancer.[28,29] Zöller reported in 2013 that exosomal miRNA is derived from living cells, while free miRNA may mostly derive from dead cells, and as a result could change significantly during therapy or in late-stage PaCa.[28] miR-155 was reported to be a marker of early PaCa, while miR-196a correlated with disease progression. Evaluating a combination of conventional biomarkers, such as CA 19-9 and plasma miRNAs, in PaCa revealed that miR-155, miR-181a, miR-181b, and miR-196a differ significantly in patients with PaCa compared with healthy individuals.[28] In addition, only miR-16 and miR-196a allowed for discrimination from chronic pancreatitis.[28] In a study analysing serum exosomal miRNAs and their correlation with clinico-pathological features of PaCa patients, miR-17-5p, miR-21, miR-155, and miR-196a were selected for examination.[29] There were low expressions of exosomal miR-155 and miR-196a in serum samples from PaCa patients when U6 (a non-coding small nuclear RNA commonly used as an internal control in miRNA quantification by RT-PCR) was used as a control.[29]

The low expression of miR-155 and miR-196a reported in the study described above is in contrast to the results reported by Zöller.[28] Serum exosomal miR-17-5p was higher in PaCa patients than in non-PaCa patients and healthy participants. High levels of miR-17-5p also correlated with advanced stage and metastasis, which was inversely correlated with resectability.[29] MiR-21 has been reported to be strongly overexpressed in PaCa and contributes significantly to cell proliferation, invasion, and chemo-resistance in PaCa patients.[29] This study concluded that there was a high expression of serum exosomal miR-17-5p and miR-21 in PaCa patients compared with healthy participants and non-PaCa patients.[29] Madhavan and colleagues[2] demonstrated that a combined panel of proteins, markers of pancreatic cancer-initiating cells (PaCICs), and miRNA derived from PaCa-derived exosomes could serve as biomarkers for the diagnosis of PaCa. These proteins were described as markers of PaCICs by Wang et al.[30] in 2013. Diagnostic accuracy would be improved by the presence of markers specific for PaCICs because miRNAs could be abnormally expressed by other non-PaCa malignancies. These PaCICs are a small pool of cells with the capacity to self-renew and account for drug resistance, metastasis, and late recurrence after years of dormancy.[31] The study of tumour exosomes found that CD44v6, $\alpha 6\beta 4$, Tspan8, and CXCR4 were highly enriched in PaCICs and expressed in exosomes.[30] The combined evaluation of PaCIC protein markers and miRNAs expressed by serum exosomes of patients with PaCa displayed a sensitivity of 100%, and a specificity of 80% when compared with non-PaCa patients. The specificity rose to 93% when patients with other malignancies were excluded from the control group.[2] Evaluation of these serum

exosomal biomarkers as diagnostic tests for pancreatic cancer detection still requires large-scale, prospective clinical validation studies, and the optimal panel that provides the highest diagnostic accuracy remains to be determined. There are reports of altered miRNA expression in the tissue and pancreatic fluid of patients with premalignant lesions such as intraductal papillary mucinous neoplasms.[32,33] These panels of miRNAs have been found to differentiate between benign and premalignant, as well as high-risk from low-risk, cysts.[32,33]

While it has been reported that there is a correlation between tissue and serum miRNA levels, these findings have not been replicated in serum exosomes. Table 1 presents a list of studies evaluating exosomal biomarkers for the diagnosis of PaCa.[34,35]

Table 1: Studies and exosomal biomarkers used for the diagnosis of pancreatic cancer.

Study	Year	Specimen type and isolation method	Biomarkers	Results	Comments
Adamczyk et al.[34]	2011	Cell culture supernatant Ultrafiltration and ultracentrifugation	Soluble and exosomal forms of EGFR	EGFR is released as a full-length, intact receptor (170 kDa) and a 65 kDa processed form in exosomes released from pancreatic cancer cells.	EGFR is overexpressed in the majority of pancreatic cancers, and is regarded as a potential target for therapy.
Lau et al.[13]	2013	Saliva (mouse) Magnetic bead exosome extraction (Panc02 cell medium, serum, and saliva)	Apbb1ip, Aspn, BCO31781, Daf2, Foxp1, Gng2, Incenp	Suppression of exosome biogenesis in C56BL/6 mice resulted in lack of development of discriminatory biomarker in saliva.	Markers were also found to be significantly upregulated in the mouse pancreata.
Zöller M.[28]	2013	Serum Not applicable	miR-155, miR-196a, miR-181a, miR-181b, miR-196a, miR-16	No experiments conducted.	Free and exosomal microRNAs were examined.
Que et al.[29]	2013	Serum Filtration and ultracentrifugation	miR-155, miR-196a, miR-21, miR-17-5p	Low expression of exosomal miR-155 and miR-196a in serum samples of PaCa patients. miR-17-5p higher in PaCa patients compared with healthy participants and non-PaCa patients.	miR-17-5p reported to be the most specific for pancreatic cancer.
Kahlert et al.[6]	2014	Serum Filtration and ultracentrifugation	K-ras, p53	Exosomes from human serum samples, which span all chromosomes and contain DNA with mutated KRAS and TP53 genes, contain genomic DNA.	Exosomal double-stranded DNA was isolated from serum exosomes.
Madhavan et al.[2]	2015	Serum Ultracentrifugation	CD44v6, Tspan8, EpCAM, c-Met, CD104, miR-1246, miR-4644, miR-3976, miR-4306	miR-1246, miR-4644, miR-3976, miR-4306 were significantly upregulated in 83% of PaCa serum exosomes, but rarely in control groups. Most patients with PaCa (95%) reacted with a panel of anti-CD44v6, anti-Tspan8, anti-EpCAM, and anti-CD104.	Combined panel of PaCIC markers and microRNAs.

Table 1 continued.

Study	Year	Specimen type and isolation method	Biomarkers	Results	Comments
Melo et al.[27]	2015	Serum Filtration and ultracentrifugation	Glypican-1	Circulating GPC1+ exosomes were detected in the serum of pancreatic cancer patients with absolute sensitivity and specificity.	GPC1 is a cell surface proteoglycan specifically enriched on cancer cell-derived exosomes.
Klein-Scory et al.[35]	2015	Cell culture media Ultrafiltration and ultracentrifugation	NT5E/CD73	Membrane proteins, glycoproteins, small GTP-binding proteins, and a further heterogeneous group of proteins are enriched in vesicles. Proteins playing a role in carcinogenesis and modulators of the ECM are components of affinity-purified ECV.	Expressed in both exosomes and ectosomes.

ECM: extracellular matrix; ECV: extracellular vesicle; EGFR: epidermal growth factor receptor; GPC1: glypican-1; GTP: guanosine triphosphate; miR: microRNA; PaCa: pancreatic adenocarcinoma; PaCIC: pancreatic cancer-initiating cell.

Table 2: Studies showing therapeutic applications of exosomes in pancreatic cancer.

Study	Year	Mechanism	Outcome	Comments
Ohuchida et al.[36]	2011	High levels of expression of miR-142-5p and miR-204 were predictive of response to gemcitabine after resection for PaCa.	Increased survival in the gemcitabine treatment group.	Tissue levels of miRNAs were assessed. Yet to be correlated with exosomal miRNA.
Record et al.[37]	2011	Suppression of exosome secretion by tumour cells using the anti-hypertensive agent dimethyl amiloride.	Enhanced *in vivo* antitumor activity of cyclophosphamide.	None.
Aspe et al.[38]	2014	Delivery of exosomal survivin-T34A built from melanoma cell lines and plated on PaCa cell line.	Increased apoptotic cell death, and increased sensitivity to gemcitabine cytotoxicity.	Carried out *in vitro* using PaCa cell lines.
Mahmoodzadeh Hosseini H et al.[39]	2014	Delivery of exosomal staphylococcal enterotoxin B.	Induction of apoptosis in PaCa cells after 24 hours.	0.5 and 2.5 μg/100 μL of exosomal staphylococcal enterotoxin B significantly stimulated apoptosis after 24 hours.

PaCa: pancreatic adenocarcinoma.

USE OF EXOSOMES IN PANCREATIC CANCER TREATMENT

Due to their unique structure and transport functions, exosomes are regarded as potential therapeutic agents/vectors in cancer treatment. The use of exosomes in cancer immunotherapy has been previously studied (Table 2).[36-39] In a 2011 review by Record and colleagues,[37] exosomes were reported as a means of amplifying dendritic

cell-mediated cytotoxic T-cell responses. Exosomal immunotherapy was referred to as a type of cellular therapy, but exosomes were reported to be more convenient to handle and more stable compared with whole cells.[37] It was reported that reducing tumour exosome production using dimethyl amiloride (an anti-hypertensive agent) enhanced the *in vivo* antitumour efficacy of the chemotherapeutic agent cyclophosphamide.[37] Some studies have shown that delivering antigens *in vivo* through small secreted vesicles such as exosomes is more immunogenic than delivery of soluble antigens alone.[40,41] Ohuchida et al.[36] identified 24 miRNAs whose expression was altered in gemcitabine-resistant cells, and also found that patients with high miR-142-5p and miR-204 expression had significantly longer survival times than those with low miR-142-5p and miR-204 expression in the gemcitabine-treated group. Although the miRNA levels were determined in paraffin-embedded tissue, it is known that miRNAs in circulating exosomes are representative of those expressed in the tumour.[37] This highlights the potential use of tumour-derived serum exosomal biomarkers as predictors of response to chemotherapy, and demonstrates that therapeutic applications of exosomes go beyond their use in cancer immunotherapy. A 2013 study by Aspe et al.[38] isolated exosomes from a melanoma cell line and enhanced the cytotoxic effect of gemcitabine on pancreatic cancer cells *in vitro* through exosome-mediated delivery of survivin-T34A mutant protein. This suggests that exosomes may be used as a vector for therapeutic agents that treat or enhance the effects of other treatments for PaCa. Some reports show the induction of apoptosis in pancreatic cancer cell lines via the delivery of staphylococcal enterotoxin B in purified tumour-derived exosomes.[39] Our research indicates that the use of exosomes as a therapeutic tool in PaCa is still investigational, and their use is yet to make a significant impact on clinical practice in general. Table 3 lists ongoing clinical studies involving the use of exosomes for the treatment of gastrointestinal cancers.

CURRENT TRENDS IN EXOSOMAL RESEARCH

Research exploring potential therapeutic applications of synthetic nanovesicles is currently underway. The dominant area of research concerns liposomes, the building blocks of synthetic nanovesicles, which permit modification of specific features such as lipid type, electrical charge, size, distribution, and location of antigens.[42] Scientists are able to engineer nanovesicles to exhibit one or multiple features of exosomes. One way in which nanovesicles are being used to identify and treat PaCa is through creation of synthetic nanovesicles capable of specifically targeting cancer cells. The expression of matrix metalloproteinases in pancreatic cells can signal tumour growth,[43] and scientists can use this biomarker to identify and attack tumour cells via the use of gemcitabine delivered by nanovesicles.[44] Nanovesicles have been used as carriers of glucose in patients with diabetes and insulin deficiencies,[45] and have also been used as carriers of ibuprofen.[44] Nanovesicles offer powerful mechanisms for drug delivery because of their ability to target recipient cells.[46] Although there remains a lot yet to be understood about the design and applications of synthetic nanovesicles, there is a clear potential for therapeutic use that would permanently alter the scope and practice of pancreatic cancer treatment.

Table 3: Ongoing clinical studies involving exosomes in gastrointestinal cancers.

National Cancer Institute registration number	Title of study	Study design	Sponsor
NCT02393703	Interrogation of Exosome-mediated Intercellular Signaling in Patients With Pancreatic Cancer	Prospective observational (cohort)	Memorial Sloan Kettering Cancer Center, New York City, New York, USA
NCT01294072	Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	Interventional (Phase I)	James Graham Brown Cancer Center, Louisville, Kentucky, USA
NCT01779583	Circulating Exosomes As Potential Prognostic And Predictive Biomarkers In Advanced Gastric Cancer Patients ("EXO-PPP Study")	Prospective (case–control)	Hospital Miguel Servet, Zaragoza, Spain

CONCLUSION

Exosomes possess unique molecular characteristics that hold promise in the development of biomarkers for early detection of pancreatic cancer. Further studies are needed in order to validate an optimal set of markers that could provide the best diagnostic performance. The ability of exosomes to transport proteins and nucleic acids makes them suitable for use in cancer therapy. The use of exosomes in the treatment of PaCa requires validation in human patients and subsequent large-scale, prospective clinical studies. Research involving synthetic nanovesicles and their use in PaCa treatment is ongoing.

REFERENCES

1. Zhou M et al. Pancreatic cancer derived exosomes regulate the expression of TLR4 in dendritic cells via miR-203. Cell Immunol. 2014;292(1-2):65-9.

2. Madhavan B et al. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. Int J Cancer. 2015;136(11): 2616-27.

3. American Cancer Society. Pancreatic cancer survival by stage. Available at: http://www.cancer.org/cancer/pancreaticcancer/detailedguide/pancreatic-cancer-survival-rates. Last accessed: 7 December 2015.

4. Kanwar SS et al. Microfluidic device (ExoChip) for on-chip isolation, quantification and characterization of circulating exosomes. Lab Chip. 2014;14(11):1891-900.

5. Pant S et al. The multifaceted exosome: biogenesis, role in normal and aberrant cellular function, and frontiers for pharmacological and biomarker opportunities. Biochem Pharmacol. 2012;83(11):1484-94.

6. Kahlert C et al. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. J Biol Chem. 2014;289(7):3869-75.

7. Ludwig AK, Giebel B. Exosomes: small vesicles participating in intercellular communication. Int J Biochem Cell Biol. 2012;44(1):11-5.

8. Record M et al. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. Biochim Biophys Acta. 2014;1841(1):108-20.

9. Gold B et al. Do circulating tumor cells, exosomes, and circulating tumor nucleic acids have clinical utility? A report of the association for molecular pathology. J Mol Diagn. 2015;17(3):209-24.

10. Qin J, Xu Q. Functions and application of exosomes. Acta Pol Pharma. 2014;71(4):537-43.

11. Bang C, Thum T. Exosomes: new players in cell-cell communication. Int J Biochem Cell Biol. 2012;44(11):2060-4.

12. Peinado H et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat Med. 2012;18(6):883-91.

13. Lau C et al. Role of pancreatic cancer-derived exosomes in salivary biomarker development. J Biol Chem. 2013;288(37):26888-97.

14. Yue S et al. The tetraspanins CD151 and Tspan8 are essential exosome components for the crosstalk between cancer initiating cells and their surrounding. Oncotarget. 2015;6(4):2366-84.

15. Choi DS et al. Proteomic analysis of microvesicles derived from human colorectal cancer ascites. Proteomics. 2011;11(13):2745-51.

16. Cheow ES et al. Simultaneous Enrichment of Plasma Soluble and Extracellular Vesicular Glycoproteins Using Prolonged Ultracentrifugation-Electrostatic Repulsion-hydrophilic Interaction Chromatography (PUC-ERLIC) Approach. Mol Cell Proteomics. 2015;14(6):1657-71.

17. Zeringer E et al. Strategies for isolation of exosomes. Cold Spring Harb Protoc. 2015;2015(4):319-23.

18. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol. 2008;110(1):13-21.

19. Tanaka Y et al. Clinical impact of serum exosomal microRNA-21 as a clinical biomarker in human esophageal squamous cell carcinoma. Cancer. 2013;119(6):1159-67.

20. Liang B et al. Characterization and proteomic analysis of ovarian cancer-derived exosomes. J Proteomics. 2013;80:171-82.

21. Shimoda M, Khokha R. Proteolytic factors in exosomes. Proteomics. 2013;13(10-11):1624-36.

22. Rekker K et al. Comparison of serum exosome isolation methods for microRNA profiling. Clin Biochem. 2014;47(1-2): 135-8.

23. Taylor DD et al. Exosome isolation for proteomic analyses and RNA profiling. Methods Mol Biol. 2011;728:235-46.

24. Momen-Heravi F et al. Impact of biofluid viscosity on size and sedimentation efficiency of the isolated microvesicles. Front Physiol. 2012;3:162.

25. Alvarez ML et al. Comparison of protein, microRNA, and mRNA yields using different methods of urinary exosome isolation for the discovery of kidney disease biomarkers. Kidney Int. 2012;82(9):1024-32.

26. Clarke C, Davies S, "Immunomagnetic Cell Separation," Brooks SA, Schumacher U (eds.), Methods in Molecular Medicine, vol. 58: Metastasis Research Protocols, Vol. 2: Cell Behavior in Vitro and in Vivo (2001), Totowa, NJ: Humana Press Inc, pp.17-23.

27. Melo SA et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature. 2015;523(7559):177-82.

28. Zöller M. Pancreatic cancer diagnosis by free and exosomal microRNA. World J Gastrointest Pathophysiol. 2013;4(4): 74-90.

29. Que R et al. Analysis of serum exosomal microRNAs and clinicopathologic features of patients with pancreatic adenocarcinoma. World J Surg Oncol. 2013;11:219.

30. Wang H et al. Tspan8, CD44v6 and alpha6beta4 are biomarkers of migrating pancreatic cancer-initiating cells. Int J Cancer. 2013;133(2):416-26.

31. Quante M, Wang TC. Stem cells in gastroenterology and hepatology. Nat Rev Gastroenterol Hepatol. 2009;6(12): 724-37.

32. Matthaei H et al. MiRNA biomarkers in cyst fluid augment the diagnosis and management of pancreatic cysts. Clin Cancer Res. 2012;18(17):4713-24.

33. Henry JC et al. MicroRNA from pancreatic duct aspirate differentiates cystic lesions of the pancreas. Ann Surg Oncol. 2013;20 Suppl 3:S661-6.

34. Adamczyk KA et al. Characterization of soluble and exosomal forms of the EGFR released from pancreatic cancer cells. Life Sci. 2011;89(9-10):304-12.

35. Klein-Scory S et al. New insights in the composition of extracellular vesicles from pancreatic cancer cells: implications for biomarkers and functions. Proteome Sci. 2014;12(1):50.

36. Ohuchida K et al. MicroRNA expression as a predictive marker for gemcitabine response after surgical resection of pancreatic cancer. Ann Surg Oncol. 2011;18(8):2381-7.

37. Record M et al. Exosomes as intercellular signalosomes and pharmacological effectors. Biochem Pharmacol. 2011;81(10):1171-82.

38. Aspe JR et al. Enhancement of Gemcitabine sensitivity in pancreatic adenocarcinoma by novel exosome-mediated delivery of the Survivin-T34A mutant. J Extracell Vesicles. 2014;3.

39. Mahmoodzadeh Hosseini H et al. Exosome/staphylococcal enterotoxin B, an anti tumor compound against pancreatic cancer. J BUON. 2014;19(2):440-8.

40. Viaud S et al. Dendritic cell-derived exosomes for cancer immunotherapy: what's next? Cancer Res. 2010;70(4): 1281-5.

41. Zeelenberg IS et al. Targeting tumor antigens to secreted membrane vesicles in vivo induces efficient antitumor immune responses. Cancer Res. 2008;68(4): 1228-35.

42. Schwendener RA. Liposomes as vaccine delivery systems: a review of the recent advances. Ther Adv Vaccines. 2014;2(6):159-82.

43. Kulkarni P et al. Mmp-9 responsive PEG cleavable nanovesicles for efficient delivery of chemotherapeutics to pancreatic cancer. Mol Pharm. 2014;11(7): 2390-9.

44. Khare V et al. Targeted drug delivery systems for pancreatic cancer. J Biomed Nanotechnol. 2014;10(12):3462-82.

45. Tai W et al. Bio-inspired synthetic nanovesicles for glucose-responsive release of insulin. Biomacromolecules. 2014;15(10):3495-502.

46. Hood JL, Wickline SA. A systematic approach to exosome-based translational nanomedicine. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2012;4(4): 458-67.

HUMAN SPERM CRYOPRESERVATION

***Eva Mocé,[1] Andrés J. Fajardo,[1] James K. Graham[2]**

1. Centro de Tecnología Animal-Instituto Valenciano de Investigaciones Agrarias (CITA-IVIA), Segorbe (Castellón), Spain
2. Department of Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA
**Correspondence to moce_eva@gva.es*

Disclosure: E. Mocé is supported by MICINN (RYC-2010-06162) and co-financed by FEDER funds. A.J. Fajardo is supported by a scholarship from IVIA co-financed by European Social Funds. J.K. Graham has declared no conflicts of interest.
Received: 19.02.15 **Accepted:** 05.05.15
Citation: EMJ 2016;1[1]:86-91.

ABSTRACT

As the demand for assisted reproductive techniques in humans increases, so does the demand for the oocytes and sperm that are essential for these techniques. Human sperm banks play a key role in assisted human reproduction, as a reservoir of sperm from the semen of donors and as an option for genetic preservation for some patients. There are different techniques that can be used to store human sperm. This paper will provide an overview of the available techniques of human sperm preservation.

Keywords: Fertility preservation, vitrification, freezing–thawing, cryopreservation, sperm banks.

INTRODUCTION

Approximately 10-15% of couples worldwide who are trying to have children experience some form of infertility.[1] For these couples, problems with the sperm (entirely or in combination with female factors) account for 50% of the infertility problems.[2] These infertility issues are not only important in developed countries, but also in developing countries where the demand for assisted reproductive techniques (ART) as a treatment for infertility is increasing.[3] In addition, the demand for artificial insemination (AI) or ART is also increasing for non-traditional families, such as single women or homosexual couples.[4] To date, more than 5 million children worldwide have been born through ART (data that exclude those coming from AI),[5] and the number of children born using ART represented 1.73% of all children born in the year 2002 in the European countries that participated in the European registers.[6]

The limited lifespan of fresh sperm requires significant coordination in order to retrieve both sperm and oocytes at the same time,[7] which may be inconvenient or impossible in some cases. For this reason, research involved with extending the lifespan of the sperm, by cooling the cells, has been pursued. Generally speaking, the lower the temperature at which the sperm is stored, the longer the cell viability is maintained.[8] However, the fertilising ability of fresh sperm declines sharply after very few days of storage at 5°C.[9]

LONG-TERM STORAGE OF SPERM: AND SO IT BEGAN

Mantegazza observed in 1886 that human sperm survived cooling to −17°C for more than 4 days (reported by Curry[10]). However, 4 days does not extend the lifespan of the sperm sufficiently long for most purposes, and research was conducted to develop methods (extenders and protocols) for longer storage periods. Sixty years later, researchers discovered that glycerol was an effective cryoprotective agent (CPA) for freezing sperm.[11] Since then, glycerol has been the primary CPA used for freezing sperm from most animal species, including humans.[12] The discovery that glycerol was an effective CPA quickly resulted in the development of cryopreservation techniques that maintained not only sperm motility after freezing and thawing, but also fertilising ability; in 1953, Bunge and Sherman reported the first pregnancy resulting from cryopreserved human sperm, which had been frozen using glycerol and

stored on dry ice. However, it took another 20 years of research before the first commercial sperm banks were created in the USA (reviewed by Frankel[13]).

USES OF CRYOPRESERVED SPERM

Cryopreservation permits long-term storage of semen and, when cryopreserved correctly, the sperm maintains a state of metabolic arrest that prevents cellular ageing and retains the viability and fertilising ability of the sperm for an essentially unlimited period. In addition, cryopreservation can inhibit the transmission of several infectious diseases that can be transmitted through semen (such as HIV), leading to the requirement that semen from donors be cryopreserved. By using only frozen semen, possible disease carriers can be detected during a quarantine period,[14] and the risk of transmitting infectious agents is almost non-existent as long as the safety regulations are followed.[15]

Sperm cryobanks are currently used for several purposes.[16] Donor sperm can be used by women with or without a male partner, and cryobanking sperm for future use can be used for male patients undergoing procedures that would impair or curtail fertility (vasectomy, treatments with cytotoxic agents, or radiotherapy) or those who are exposed to sperm-damaging conditions in their professional career but would like to become fathers in the future. Cryobanks can also collect and store gametes needed in infertility cases in which the male partner cannot provide sufficient or suitable sperm for use in ART, or cases in which it is not possible to collect fresh semen on the day of the ART procedure. Finally, cryobanks are useful for storing samples from disease carriers (such as HIV) and minimising the risk of disease transmission to the female partner by storing and using only those samples with an undetectable infectious agent.

SPERM CRYOPRESERVATION

As previously stated, long-term storage of cells can be achieved if metabolism is arrested, and this can be achieved if the cells are stored at −196°C — the temperature of liquid nitrogen. At −196°C there is essentially no detectable biochemical activity[17] because there is not sufficient thermal energy for chemical reactions to occur and there is no liquid water, which is essential for metabolic processes.[18] However, living tissues and cells can be destroyed during the freezing and warming processes unless certain procedures are used. CPAs are added to the diluents for cryopreserving the sperm. They protect the sperm during the cryopreservation process and, as a result, the sperm cryosurvival rate is higher in their presence than in their absence.[17] There are two types of CPA differentiated by their ability to traverse the plasma membrane: permeating and non-permeating CPAs.

The freezing point of water is close to 0°C in the presence of nucleating particles, but in their absence it can be reduced to −42°C.[17] During the cryopreservation protocols, the solutions cool below their normal freezing points without changing their state from liquid to solid (ice). This phenomenon is known as supercooling and it is due to the need for nucleation to occur (the process by which a minimum crystal is formed) before an ice crystal can begin to grow. Thus, when the ice nucleus begins to grow, the solutes are excluded from the ice crystals and they concentrate in the unfrozen (liquid) fraction, and this lowers the freezing point of the remaining solution. Ice crystals continue growing until the solution at the interface has a freezing point equal to the temperature of the interface. Nucleation and ice crystal formation can be avoided if the temperature is reduced very fast in a process known as vitrification.[17] Currently, two techniques are effective for preserving cells: slow (equilibrium) freezing and vitrification.

SLOW-FREEZING PROTOCOLS

In these protocols, extracellular water solidifies into ice crystals and creates a two-phase state: ice crystals of pure water and an unfrozen fraction containing liquid water and all the salts, sugars, CPA, and cells of the original sample (for reviews see Chian[17] and Hammerstedt et al.[19]). The change in temperature alters the physical status of the sperm membrane as membrane lipids undergo a phase transition from the fluid to the gel state, which can damage the membrane.[19] In addition, the loss of water, in the form of ice, increases the osmolality of the solution in the unfrozen water fraction, which can also damage the cells.[19] Since sperm lack the ability to adapt to low temperatures (for a review see Parks[8]) we must provide the sperm with CPAs (such as glycerol and egg yolk) in order to help them survive these hypothermic conditions.

The beneficial components in egg yolk are the low-density lipoproteins that protect the sperm membrane during cryopreservation.[20] Glycerol acts as a permeating CPA and exerts its effects both

intracellularly and extracellularly. Extracellularly, glycerol lowers the salt concentration of the extracellular fluid and increases the percentage of unfrozen water fraction at any given temperature, minimising the osmotic effect.[21] Both egg yolk and glycerol are commonly added to the human sperm at room temperature, and the samples are equilibrated with the CPAs for several minutes (commonly at 35°C for 30 minutes[16]), after which the sperm are loaded into cryovials or straws. There is no consensus regarding an optimal freezing diluent or freezing protocol for human sperm. Therefore, practitioners can choose between different protocols depending on the equipment available to them and on the type of sample to be cryopreserved (for a review see Di Santo et al.[22]).

Regardless of the protocol chosen, some precautions should be taken in order to achieve optimal results. Firstly, although glycerol helps sperm survive the freezing process it also can have deleterious effects and, at high concentrations, can be cytotoxic.[23] However, this toxic effect is observed at concentrations higher than those used for freezing sperm (around 6% v/v). Secondly, glycerol exerts direct osmotic effects on cells. Glycerol crosses the sperm plasma membrane, but at a slower rate than water. Therefore, when glycerol is added or removed, the cells experience volume changes (shrinking and expansion, respectively) that can damage the cells if the volume changes surpass the osmotic tolerance limits of the sperm (for a review see Gao et al.[14]). To avoid this damage when adding the CPA, the freezing diluent is added to the sperm in several steps.[14] After adding CPA, the sperm are equilibrated with the freezing diluent for several minutes prior to actually freezing the sperm. During this period, the glycerol traverses the membrane, equilibrium is reached, and apparently protective membrane rearrangements occur, although this remains controversial.[14]

The temperature must also be carefully reduced, even in the presence of CPAs. For the avoidance of iatrogenic damage, the temperatures of the sperm, the diluents, and the instruments used to manipulate the samples must be similar. In addition, sperm can be damaged by decreasing the temperature from body temperature to near 0°C too quickly (for a review on the effects of hypothermia on sperm see Parks[8]). This damage, known as cold shock, correlates directly with temperature differential and cooling rate (for an extensive review see Watson[24]). The resistance of the sperm to this damage depends on the characteristics of the sperm membrane, lipid type, and cholesterol level, which are specific to different species. Therefore, resistance to cold shock is higher for sperm with ratios of polyunsaturated to saturated fatty acids in the membrane that are lower (≈1), and cholesterol to phospholipid molar ratios that are higher (≈1).[24] For human sperm, both molar ratios are close to 1, and for this reason human sperm are relatively cold-shock resistant. The implication for this is that the cooling/freezing rate for human sperm can be relatively fast, much faster than is used for sperm from many other species.

Freezing is performed in liquid nitrogen vapours. The cooling rates also have to be carefully chosen in order to avoid damaging the sperm. If sperm are cooled slowly then they will dehydrate and intracellular ice formation will be avoided, but they will be damaged by the high hyper-osmotic conditions created while the ice is growing. Fast cooling rates are also damaging because the water cannot leave the cells fast enough and it will supercool, which increases the probability of intracellular ice formation. The cooling rate also depends on the concentration of CPA.[25]

After freezing, the sperm are stored in liquid nitrogen where they remain essentially inert as long as they are kept at −196°C. However, before they can be used for AI or ART, they must go through a reverse process. To avoid cellular damage during thawing (either from recrystallisation of small crystals or from osmotic unbalance), the warming rate needs to be carefully chosen. The warming rate depends on the freezing rate, on the type of container used to load the sperm, and on the concentration of CPA.[19] As with freezing human sperm, several protocols for thawing human sperm have been described (usually the samples are thawed at either 37°C or at room temperature; for a review see Di Santo et al.[22]).

In addition, the freezing diluent is usually removed before using the sperm for ART. This step is of special concern because the sperm are now loaded with glycerol, and diluting the sperm in a diluent containing no glycerol will induce osmotic stress to the cells, which can swell to the point of being damaged. To alleviate this problem, the sperm should be diluted gradually using several steps with sufficient time for the sperm to equilibrate to each new osmotic condition.[14]

Slow-freezing protocols are still inefficient considering sperm cryosurvival and the functionality of the sperm surviving the process, as will be discussed later. In some patients, the percentage of sperm surviving the process can be too low to perform AI and these samples should be combined with other ART (such as *in vitro* fertilisation [IVF] or intracytoplasmic sperm injection [ICSI]). Nevertheless, slow-freezing protocols remain the most commonly used for the long-term storage of sperm.

SPERM VITRIFICATION

During vitrification, water solidifies as an amorphous glass-like structure, and not as ice.[17] Vitrification is achieved by cooling the solution at very high rates, and the process has some advantages over slow-freezing protocols. Thus, vitrification should cause little damage to the cells, minimal equipment is required, and little time is devoted to the process (the cells are plunged directly into liquid nitrogen). Indeed, if extremely high cooling rates are used, isotonic saline can be vitrified[25] and no CPAs are necessary, eliminating all the osmotic challenges that adding and removing CPAs involve.

Although it seems easy to implement, the reality is that effectively vitrifying sperm has been difficult. This is due to the difficulty in cooling the samples sufficiently fast enough for vitrification to occur. In order to vitrify many cell types (such as oocytes or embryos), high concentrations of CPAs are used (as high as 30–50%), and sample volumes are small.[25] However, sperm do not tolerate these high CPA concentrations and, for this reason, vitrification has not been considered a real possibility for conserving sperm. In 2002, the successful vitrification of human spermatozoa was reported by the direct plunging of small sperm volumes (20 µL) into liquid nitrogen without permeating CPAs.[26] Researchers demonstrated that the sperm maintained motility after warming and exhibited fertilising capacity. There are some problems with this type of conservation, including the risk of infectious agent transmission (the samples are in direct contact with the liquid nitrogen), difficulty in labelling the samples, and the small volumes used (insufficient for AI and for some ART). Thus, other investigations were conducted to resolve these issues (for an extensive review see Isachenko et al.[27]). In addition, special attention must be paid during the warming process because the warming velocity plays a crucial role in the sperm cryosurvival of the vitrification–devitrification process.[27]

Vitrification is less popular than slow-freezing procedures nowadays and research is still being conducted to optimise the technique. However, the sperm obtained with this method present similar or higher quality than the sperm frozen with slow-freezing protocols. In addition, vitrified sperm are also fertile by means of IVF/ICSI and intrauterine insemination, although reports are still scarce. Nevertheless, a few centres are using vitrification for long-term sperm storage with successful results,[28] and this number will probably increase in the following years because the technique is quite simple to perform.

OTHER TECHNIQUES FOR LONG-TERM STORAGE OF SPERM: FREEZE-DRIED SPERM

So far we have described techniques that require liquid nitrogen for storing the sperm. However, a continuous liquid nitrogen supply is necessary to maintain the samples, which makes this type of conservation costly. Therefore, other techniques that do not require liquid nitrogen are being developed for long-term sperm storage.[29] Lyophilising or freeze-drying sperm allows the sperm to be stored at suprazero temperatures. In this case, the samples are dehydrated by sublimation (for a review see Gil et al.[30]) and then stored at either 4°C or room temperature. Although the first attempts to lyophilise human sperm were made in the early 1950s,[31] no protocol to date has succeeded in maintaining motile or viable sperm. Nevertheless, the DNA of lyophilised sperm remains intact even though the other sperm structures are irreversibly damaged during the lyophilisation process,[32] and freeze-dried sperm used for ICSI have resulted in viable offspring for several animal species.[30] However, reports on the fertilising ability of lyophilised human sperm are lacking. Currently, lyophilisation must be considered as an experimental technique that should not be offered to patients because there is no information about the fertilising ability of this type of sperm in humans. Thus, this technique is still in the early stages but may become more promising as research continues to improve both the composition of the diluents used for lyophilisation and procedures for dehydration alternative to lyophilisation (for a review see Loi et al.[29]).

ARE THESE TECHNIQUES SAFE?

All of the techniques described above damage sperm more or less severely, since nearly half of the sperm are irreversibly damaged during cryopreservation.[33] On the other hand, the sperm from some individuals are not damaged as severely by the freezing process.[12] However, only 10% of human sperm donors provide sperm that can be effectively cryopreserved.[34] This would be a potential problem for preserving sperm samples from patients in fertility preservation programmes, whose sperm will not be suitable for the simple reproductive techniques (such as AI). In addition, the fertility of cryopreserved sperm is lower than that of fresh semen when used for AI,[35] which is similar to the fertility of fresh and frozen animal sperm.[12] This reduced fertility is due to sperm membrane damage that occurs during cryopreservation, which compromises membrane structure and function (for a review see Parks[8]). However, the fertility of frozen–thawed semen improves if high-quality insemination doses are used.[35] Therefore, whenever possible, high-quality sperm should be used for AI. However, when high-quality semen samples are not available, a number of ART have been developed and fertility can be achieved with cryopreserved sperm using IVF or ICSI.[35] Finally, the technique of cryopreservation is safe because there is no evidence of additional risks for birth defects or chromosomal abnormalities after using cryopreserved sperm.[5]

CONCLUSION

Several techniques are available for the long-term storage of human sperm. Among them, slow-freezing cryopreservation protocols are used most often. Cryopreserved sperm maintain their fertilising ability for an extended period, although the fertility of samples tends to be reduced compared with fresh semen, but only if AI is used. Therefore, the advantages of cryopreserved sperm surpass its disadvantages and cryopreservation is an important tool for the preservation of male gametes.

REFERENCES

1. Snijder CA et al. Occupational exposure to chemical substances and time to pregnancy: a systematic review. Hum Reprod Update. 2012;18(3):284-300.

2. Sharlip ID et al. Best practice policies for male infertility. Fertil Steril. 2002;77(5):873-82.

3. Vayena E et al. Assisted reproductive technologies in developing countries: are we caring yet? Fertil Steril. 2009;92(2):413-6.

4. Jouannet P. [Evolution of assisted reproductive technologies]. Bull Acad Natl Med. 2009;193(3):573-82.

5. Kennedy R; International Federation of Fertility Societies. Consensus on birth defects in children born following assisted reproduction. Available at: http://c.ymcdn.com/sites/www.iffs-reproduction.org/resource/resmgr/newsletters/iffs_spring_2_2013_lr.pdf. Last accessed: 27 July 2015.

6. Andersen AN et al; European IVF-monitoring programme (EIM) for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2002. Results generated from European registers by ESHRE. Hum Reprod. 2006;21(7):1680-97.

7. Miyaoka R, Esteves SC. Predictive factors for sperm retrieval and sperm injection outcomes in obstructive azoospermia: do etiology, retrieval techniques and gamete source play a role? Clinics. 2013;68 Suppl 1:111-9.

8. Parks JE. "Hypothermia and mammalian gametes," Karow AM, Critser JK (eds.), Reproductive Tissue Banking, Scientific Principles (1997), San Diego: Academic Press, pp. 229-61.

9. Vishwanath R, Shannon P. Do sperm cells age? A review of the physiological changes in sperm during storage at ambient temperature. Reprod Fertil Dev. 1997;9(3):321-31.

10. Curry, MR, "Cryopreservation of semen from domestic livestock," Day JG, McLellan MR (eds.), Methods in Molecular Biology, Vol. 38: Cryopreservation and Freeze-Drying Protocols (1995), New York: Humana Press Inc, pp. 189-97.

11. Polge C et al. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature. 1949;164(4172):666.

12. Holt WV. Basic aspects of frozen storage of semen. Anim Reprod Sci. 2000;62(1-3):3-22.

13. Frankel MS. Cryobanking of human sperm. J Med Ethics. 1975;1(1):36-8.

14. Gao D et al. "Fundamental Cryobiology of Mammalian Spermatozoa," Karow AM, Critser JK (eds.), Reproductive Tissue Banking, Scientific Principles (1997), San Diego: Academic Press, pp. 263-328.

15. Bielanski A, Vajta G. Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units. Hum Reprod. 2009;24(10):2457-67.

16. World Health Organization. WHO laboratory manual for the examination and processing of human semen, Fifth edition. Available at: http://www.ivf.at/Kinderwunsch/Upload/Behandlung/WHO%202010.pdf. Last accessed: 27 July 2015.

17. Chian, RC, "Cryobiology: an overview," Chian RC, Quinn P (eds.), Fertility cryopreservation (2010), Cambridge: Cambridge University Press, pp. 1-9.

18. Mazur P. Freezing of living cells: mechanisms and implications. Am J Physiol. 1984;247(3 Pt 1):C125-42.

19. Hammerstedt RH et al. Cryopreservation of mammalian sperm: what we ask them to survive. J Androl. 1990;11(1):73-88.

20. Manjunath P. New insights into the understanding of the mechanism of sperm protection by extender components. Anim Reprod. 2012;9(4):809-15.

21. Amann RP, Pickett BW. Principles of cryopreservation and a review of cryopreservation of stallion spermatozoa. Equine Vet Sci. 1987;7(3):145-73.

22. Di Santo M et al. Human sperm cryopreservation: update on techniques, effect on DNA integrity, and implications for ART. Adv Urol. 2012;2012:854837.

23. Mullen SF, Critser JK. "The Science of

Cryobiology," Woodruff TK, Snyder KA (eds.), Oncofertility: Fertility Preservations for Cancer Survivors (2007), New York: Springer Science + Business Media, LLC, pp. 83-109.

24. Watson PF. "The Effects of Cold Shock on Sperm Cell Membranes," Morris GJ, Clark A (eds.), Effects of Low Temperatures on Biological Membranes (1981), London: Academic Press, pp. 189-218.

25. Walters EM et al. "The History of Sperm Cryopreservation," Pacey AA, Tomlinson MJ (eds.) Sperm Banking: Theory and Practice (2009), Cambridge: Cambridge University Press, pp. 1-17.

26. Nawroth F et al. Vitrification of human spermatozoa without cryoprotectants. Cryo Letters. 2002;23(2):93-102.

27. Isachenko E et al. "Cryopreservation of Spermatozoa: Old Routine and New Perspectives," Donnez J, Kim SS (eds.), Principles and Practice of Fertility Preservation (2011), Cambridge: Cambridge University Press, pp. 176-98.

28. Isachenko V et al. Cryoprotectant-free vitrification of human spermatozoa in large (up to 0.5 mL) volume: a novel technology. Clin Lab. 2011;57(9-10): 643-50.

29. Loi P et al. Towards storage of cells and gametes in dry form. Trends Biotechnol. 2013;31(12):688-95.

30. Gil L et al. Current status of freeze-drying technology to preserve domestic animals sperm. Reprod Domest Anim. 2014;49(4):72-81.

31. Sherman JK. Freezing and freeze-drying of human spermatozoa. Fertil Steril. 1954;5(4):357-71.

32. Gianaroli L et al. DNA integrity is maintained after freeze-drying of human spermatozoa. Fertil Steril. 2012;97(5):1067-73.

33. Keel BA et al. Effects of cryopreservation on the motility characteristics of human spermatozoa. J Reprod Fert. 1987;81(1):213-20.

34. Watcher MAM. Ethical aspects of cryobiology: responsible applications in biomedicine and in clinical practice. Cryobiology. 2004;48(2):205-13.

35. Clarke GN et al. Artificial insemination and in-vitro fertilization using donor spermatozoa: a report on 15 years of experience. Hum Reprod. 1997:12(4): 722-6.

PRESCHOOL WHEEZING PHENOTYPES

***Andrew Bush,[1-3] Prasad Nagakumar[2,3]**

1. Department of Paediatrics, Imperial College London, London, UK
2. Department of Paediatric Respirology, National Heart and Lung Institute, Imperial College London, London, UK
3. Department of Paediatric Respiratory Medicine, Royal Brompton Hospital, London, UK
**Correspondence to a.bush@imperial.ac.uk*

Disclosure: A. Bush was supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London. P. Nagakumar has declared no conflicts of interest.
Received: 16.02.15 **Accepted:** 30.07.15
Citation: EMJ 2016;1[1]:93-101.

ABSTRACT

Wheezing in preschool children is very common, with a wide differential diagnosis. It is essential to be sure of the exact sound that parents are describing; the term 'wheeze' is often applied to non-specific sounds. Structural airway disease such as vascular ring should be considered. Thereafter we propose that umbrella terms for preschool wheeze should be abandoned in favour of 'Hargreave phenotyping', in which the presence and extent of the components of infection, inflammation, variable airflow obstruction, and fixed airflow obstruction are determined as far as is possible, rather than using a general umbrella term such as 'asthma'. The justification for this approach is that it leads to a logical approach to treatment in the disparate airway diseases presenting in the preschool years, and should hopefully prevent over-treatment with inhaled corticosteroids. If, despite this approach, doubt remains as to the nature of the airway disease, then a therapeutic trial of treatment is permissible, but it should be for a short defined period only. In any event, such children should be reviewed regularly to see if treatments need to be changed.

Keywords: Asthma, airway inflammation, inhaled corticosteroid, phenotype, bacterial bronchitis, obliterative bronchiolitis, persistent airflow limitation, airway hyper-responsiveness, bronchomalacia.

BACKGROUND

Approximately one-third of children are diagnosed with wheeze in the first 3 years of life, making wheeze one of the commonest respiratory symptoms.[1] The differential diagnosis of wheeze is wide, and different management strategies are needed depending on the underlying phenotype.[2] Unfortunately, investigative strategies are crude in the extreme, hampering progress both in managing individuals and also in understanding disease groups. The aim of this review is to discuss the reasons as to why preschool children wheeze; to propose a logical, 'Hargreave-driven', clinically relevant approach to phenotyping;[3,4] and to discuss the consequences for management of these wheeze phenotypes (Table 1). There is no point at all in phenotyping or carrying out any other splitting exercise unless there are useful consequences, such as a better understanding of disease or a change in treatment approach.

We will demonstrate that this Hargreave-driven approach to airway disease does have important consequences, and that the use of umbrella terms such as 'asthma' is about as useful as the old, long superseded 'diagnostic' labels of anaemia and arthritis. This approach means that airway disease is described in precise terms, and over-treatment of children with corticosteroids with no evidence of eosinophilic inflammation is avoided. Ideally, treatment in preschool children would be based on objective measurements of lung function and airway inflammation and infection, and this should be our aspiration; however, these tests are rarely available in current clinical practice.

IS IT WHEEZE AT ALL?

The word 'wheeze' is used to describe many different sounds. In one study, there was <50% agreement between parents and clinicians on whether the child wheezed, and only 11% of parents mentioned 'whistling' as part of their description of wheeze.[5] Another study used objective transthoracic recordings of added sounds as the 'gold standard', and showed that there was only 32% agreement between parents and physicians; the objective recording correlated with the physician report. Nurses and parents were equally unreliable.[6] Another study, this time using a video questionnaire as the gold standard, reported that 30% of parents used words other than wheeze to describe wheeze, or wheeze to describe non-wheeze sounds.[7] This same video questionnaire was shown to help to identify upper airway abnormalities such as stridor-causing laryngomalacia, which had been misdiagnosed as wheeze.[8] Clearly if the noise reported is non-specific from the upper airway, management is completely different; and indeed, with the exception of snoring, which should prompt consideration of performing a sleep study, reassurance is all that is required. These findings call into question studies based on tick-box questionnaires, which may make no attempt to determine the sound that is actually heard.

Even if true wheeze is heard, this should not be automatically assumed to be due to bronchospasm. Airway narrowing by mucus will produce true wheeze but does not respond to bronchodilators. Similarly, airway malacia, either related to intrinsic airway wall defects or loss of alveolar tethering points, are also causes of bronchodilator-unresponsive wheeze; indeed, bronchodilators, by reducing airway smooth muscle tone, may actually worsen airway narrowing.[9]

IS WHEEZE DUE TO STRUCTURAL AIRWAY DISEASE?

The differential diagnosis is extensive. Causes of fixed obstruction are summarised in Table 2; a detailed discussion of these possibilities is beyond the scope of this article.

WHEEZE IN THE FIRST YEAR OF LIFE

Wheeze in the first year of life is common throughout the world, at least as determined by questionnaires, with all the caveats set out above. In one international study of >30,000 infants, 45.2% had at least one episode of 'wheeze', and 20.3% had recurrent 'wheeze'.[9,10] The nature of the noise and the pathophysiology was unclear. However, a study of 53 infants aged 3–26 months, who were investigated with infant pulmonary function tests and rigid bronchoscopy for severe respiratory symptoms including wheeze, had no evidence of eosinophilic airway inflammation or remodelling.[11] This was true even in the subgroup that was atopic and had airflow obstruction acutely reversible with short-acting β2 agonist. Given that this group of infants must have been the most severe tip of the iceberg, and yet had no airway eosinophilia, prescribing inhaled corticosteroids (ICS) to wheezy children in this age group does not seem logical. Despite this, 46.1% of the 30,000 infants reported above were prescribed ICS. The Hargreave phenotype is non-inflammatory, likely variable due to bronchoconstriction and possibly fixed airflow obstruction, triggered by viruses; the role of bacteria is unclear, but the finding in the COPSAC data that early bacterial colonisation of the nasopharynx is associated with later wheeze suggests that there may be a role for bacteria.[12] Indeed, in older children bacterial infection may be at least as common as viruses in triggering acute asthma attacks.[13] However, as yet there is no evidence to deploy antibiotics in preschool children, except in the presence of clear-cut evidence of a bacterial infection.

MULTIPLE TRIGGER WHEEZE AND EPISODIC VIRAL WHEEZE

Approximately 30% of all children have at least one episode of wheeze in the first 3 years of life. The paper from Tucson delineating the patterns of transient, persistent, and late-onset wheeze[1] has to some extent been superceded by more detailed data from the ALSPAC group,[14] confirmed in the PIAMA[15] and Southampton birth cohorts[16] in which more temporal phenotypes have been described. This combination of birth cohorts has been a powerful tool in genetic and other studies, and will undoubtedly lead to more discoveries, but as yet it has not told us much about how to treat these infants. What we do know is that no treatment prevents progression from early wheeze to school-age atopic asthma; three well-conducted randomised controlled trials have shown that early use of ICS does not modify disease progression.[17-19]

Table 1: Components of the proposed Hargreave phenotypes.
Note that inflammation may be a beneficial response to infection, or may be exaggerated and detrimental to the host.

Component	Extramural	Intramural	Intraluminal
Fixed airflow obstruction	Loss of alveolar tethering	Reduced airway calibre (developmental or environmental)	-
Variable airflow obstruction	Loss of alveolar tethering	Airway smooth muscle (bronchospasm) Airway oedema	Mucus and other airway secretions
Inflammation	Role poorly explored	Cellular pattern: eosinophilic, neutrophilic, both, neither: likely many pathways Neurogenic Other	Cellular pattern: eosinophilic, neutrophilic, both, neither: likely many pathways Neurogenic Other
Infection	-	Latent viral in particular	Any combination of bacterial, viral, fungal

Table 2: Differential diagnosis of wheeze and other sounds that may be confused with wheeze.

Upper airway disease - adenotonsillar hypertrophy, rhinosinusitis, postnasal drip, subglottic stenosis, laryngomalacia, vocal cord paresis.

Congenital structural bronchial disease - complete cartilage rings, cysts, webs.

Bronchial/tracheal compression - vascular rings and sling, enlarged cardiac chamber, lymph nodes enlarged by tuberculosis or lymphoma.

Endobronchial disease - foreign body, tumour.

Oesophageal/swallowing problems - reflux, incoordinate swallow, laryngeal cleft, or tracheoesophageal fistula.

Causes of pulmonary suppuration - cystic fibrosis, primary ciliary dyskinesia, persistent bacterial bronchitis, any systemic immunodeficiency.

Miscellaneous - bronchopulmonary dysplasia, congenital or acquired tracheomalacia, pulmonary oedema.

Hence it is logical to consider only symptoms when planning treatment, and to this end the ERS Task Force[2] has proposed the use of two categories:

- Episodic viral wheeze (EVW) – the child wheezes only at the time of a usually clinically diagnosed viral upper respiratory tract infection and is symptom-free between viral colds
- Multiple trigger wheeze (MTW) – the child wheezes at the time of viral colds, but also between colds, for example with excited behaviour, aeroallergen exposure, and cold, smoky atmospheres

Of note, the atopic status of the preschool child does not help predict response to ICS. These phenotypes have limitations, but these have been exaggerated. There are objective differences between them: MTW has worse airflow obstruction and gas mixing, and evidence of eosinophilic airway inflammation as judged by exhaled nitric oxide (eNO), compared with EVW.[20] There is evidence of fixed airflow obstruction, as well as bronchial hyper-responsiveness, in preschool wheeze phenotypes, although often studies do not discriminate between EVW and MTW.[1,21-24] It is true that they are not fixed, and may change over time,[25] but in this case the treatment approach changes, and this is standard in paediatric conditions as the child develops. It is also true that an infant may have interval symptoms that the family do not appreciate and are only recognised when they are treated. Despite this, they are useful to guide treatment,

accepting that regular review and possible change of treatment in the future may be necessary.

EPISODIC VIRAL WHEEZE

Invasive studies including bronchoalveolar lavage (BAL) and endobronchial biopsy have shown no evidence of eosinophilic airway inflammation.[11] Indeed, BAL studies have demonstrated that non-atopic, EVW infants have predominantly neutrophilic inflammation.[26,27] There is likely to be neutrophilic inflammation at the time of acute viral infection, but this is likely to be a beneficial response. Non-invasive studies have shown that there is less airway obstruction, less impairment of gas mixing, and lower fractional eNO than in MTW.[28] No study has shown that prophylactic ICS has prevented episodic symptoms, and treatment should therefore be intermittent; trials of inhaled β2 agonists and anticholinergics are the first line if therapy is needed at all.[29] The data on the intermittent use of leukotriene antagonists are controversial, and although two early trials showed benefits from this approach,[30,31] two significantly larger studies failed to confirm these findings.[32,33] Nonetheless, anecdotally some children respond; however, parents must be warned about the behavioural side effects of montelukast. Intermittent ICS just at the time of viral colds may be indicated; there are proof-of-concept studies supporting this approach, but the dose and duration of therapy are unclear. The frequency of viral colds needs to be considered; if very high doses of ICS are given, then the cumulative dose over the winter may be considerable. A trial of continuous ICS should only be given if the paediatrician suspects that interval symptoms are being underplayed by the family. The Hargreave phenotype of EVW is non-inflammatory (or at least non-eosinophilic), possible fixed airflow obstruction, with variable airflow obstruction that may be due to bronchospasm, but could be due to airway oedema or mucus. The acute triggers are viral and likely bacterial infection.

MULTIPLE TRIGGER WHEEZE

The presumption from invasive studies is that these children have classical eosinophilic inflammation and should be treated along the same lines as school-age children.[11] However, other than in a research context, objective measurement of response is difficult to measure, and a three-stage therapeutic trial is recommended (below). The Hargreave phenotype of MTW is eosinophilic inflammation, likely some fixed but also variable airflow obstruction, the latter due to broncho-constriction but likely also a component of airway oedema and mucus, with acute viral and likely bacterial triggers.

POST-BRONCHIOLITIS WHEEZE

Infants who are admitted to hospital with respiratory syncytial virus (RSV) bronchiolitis are especially likely to have prolonged cough and wheeze afterwards. There is controversy over whether in fact RSV causes asthma, or if severe RSV bronchiolitis is a sign that the infant had preceding risk factors that are a marker for risk of asthma onset, or in fact that the symptoms regress.[34] Prospective data from Perth, Australia, showed impaired lung function in babies who went on to develop bronchiolitis, and this tracked into mid-childhood.[35] We also know that none of the myriad studies showed any evidence of benefit from ICS either in the acute phase of the illness or after discharge from follow-up.[36,37] The best evidence is that, for most infants, symptoms gradually regress over time, but those who are at high risk of asthma due to preceding factors such as a strong atopic history and maternal smoking may obviously develop asthma.[38] Hence, the Hargreaves phenotype is fixed airflow obstruction, with no evidence of chronic inflammation. There may be episodes of variable airflow obstruction due to any or all of bronchospasm, airway oedema, and mucus triggered by viral and possibly bacterial infection. There is no reason to treat with ICS; if it is thought that the child is developing true eosinophilic asthma, then a therapeutic trial of ICS should be at least considered, but only using the three-step protocol discussed below.

PERSISTENT BACTERIAL BRONCHITIS

This condition is poorly understood but was the commonest cause of cough in one large series investigated in a tertiary hospital; nearly 40% of >100 children were given this diagnosis after investigation. Of note, half had received an initial diagnosis of asthma, which was the final diagnosis in only about 5%.[29] Persistent bacterial bronchitis (PBB) is largely a disease of preschool children, and is characterised by neutrophilic airway inflammation and chronic infection with organisms such as *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae*.[39,40]

It is a diagnosis of exclusion; more serious conditions such as bronchiectasis and aspiration syndromes must be considered. The pathophysiological basis is not known but it presumably reflects a local defect in mucosal defence because there is no systemic infection.[41] It is interesting to speculate how much may be iatrogenic; ICS are widely used in children with respiratory symptoms, and are known to increase the risk of pneumonia,[42] tuberculosis,[43] and atypical mycobacterial infection in adults.[44] Could it be that the treatment of non-specific respiratory symptoms with ICS promotes mucosal immunosuppression and low-grade infection?

Whatever the pathophysiology, the Hargreave phenotype of PBB is neutrophilic inflammation and airflow obstruction due to mucus. If PBB is suspected clinically, it is reasonable to give a 2-week trial of co-amoxiclav.[45] If there is no response or a rapid relapse after treatment, then it is wise to consider further investigations to exclude bronchiectasis or another underlying cause.[46] The presumption that PBB is a precursor of idiopathic bronchiectasis has not been tested, but aggressive treatment with antibiotics (courses may need to be prolonged and repeated) and airway clearance should be instituted until the problem resolves.

CYSTIC FIBROSIS, PRIMARY CILIARY DYSKINESIA, BRONCHIECTASIS

These conditions are included because the same phenotypic considerations apply to their management, and also because, in the case of cystic fibrosis (CF) and primary ciliary dyskinesia (PCD), they would likely still be categorised under the same non-specific umbrella as PBB and probably other causes of preschool respiratory symptoms because the diagnosis is made from specific tests.[47,48] The detailed management is beyond the scope of this article, but the Hargreave phenotype is neutrophilic inflammation, chronic bacterial infection, and fixed and variable airflow obstruction, and antibiotics and airway clearance are the mainstays of treatment.

CF and PCD point the way towards that which we should aspire to for other infant and preschool respiratory diseases. Even Hargreave phenotyping is at best crude, albeit a lot better than categorising conditions together under the same umbrella. CF and PCD are diagnosed using very specific tests, and this is needed for other conditions. The use of the terms EVW and MTW may represent an advance, but they can hardly be considered 21st-century diagnostic terms.

OBLITERATIVE BRONCHIOLITIS

In preschool children, this is usually the result of a preceding severe infection, usually adenovirus or *Mycoplasma pneumoniae*.[49] The Hargreave phenotype is fixed airway obstruction with no reversibility or inflammation unless coincidentally the child has a second airway disease such as coincident atopic asthma.

SICKLE CELL ANAEMIA

Sickle cell anaemia (SCD) is another condition in which asthma is said to be common, but without much evidence of clarity of thought about what the nature of the airway disease is. The nature of SCD airway disease is controversial. One recent study in SCD children with only very mild pulmonary vascular disease demonstrated that they had mild airway obstruction, but no evidence of variable airflow obstruction or eosinophilic inflammation (at least as shown by eNO) when compared with ethnically matched controls.[50] Other studies have shown increased airway responsiveness in SCD, including acute bronchodilator responsiveness, but no convincing evidence of eosinophilic airway inflammatiion.[51,52] As with obliterative bronchiolitis, these children may have another coincident airway disease such as atopic asthma that should be treated on merit, but pure SCD airway disease appears not to have the Hargreave phenotype of eosinophilic airway inflammation. There is certainly fixed obstruction; the data on variable airflow obstruction are controversial.

POST PREMATURE BIRTH

A description of the early pathophysiology of neonatal lung disease of prematurity is beyond the scope of this review. There is ample evidence that infant survivors of premature birth have evidence of fixed airflow obstruction, even if they do not require ventilation.[53,54] The decrements with prematurity are improving as intensive care becomes more sophisticated. These babies at follow-up into childhood have increased respiratory symptoms, acute bronchodilator responsiveness, and may have airway reactivity.[55] However, they have no evidence of airway inflammation; exhaled breath temperature and eNO are both normal.

Table 3: Phenotyping preschool paediatric wheezing disorders.
There are multiple different paediatric pathways to wheeze, and the approaches should be pathway-specific.

Disease	Inflammation? (Eosinophilic, neutrophilic, both)	Variable airflow obstruction? (Bronchospasm, oedema, mucus, loss of tone, loss of alveolar tethering)	Fixed airflow obstruction	Infection? (Bacterial, viral, both)	Treatment
Wheeze in the first year of life	No	+/-	+/-	Likely viral and bacterial triggers	Trial bronchodilators, discontinue if ineffective
Multiple trigger wheeze	Eosinophilic	Yes, bronchoconstriction	+/-	Likely viral and bacterial triggers	Inhaled steroids, bronchodilators
Episodic viral wheeze	None chronic	Yes, bronchoconstriction, airway oedema, mucus	+/-	Likely viral and bacterial triggers	Bronchodilators. Inhaled steroids ineffective
Post-bronchiolitis wheeze	None chronic	Probably, nature unclear	+/-	Likely viral and bacterial triggers	Bronchodilators. Inhaled steroids ineffective
Persistent bacterial bronchitis	Neutrophilic, bacteria	Mucus	No	Bacterial, viral	Antibiotics, Airway clearance
Cystic fibrosis, primary ciliary dyskinesia, bronchiectasis	Neutrophilic	Yes, mucus obstruction	Usually	Bacterial predominant	Antibiotics, mucolytics, airway clearance
Obliterative bronchiolitis	No	Mucus	Yes	No	Airway clearance
Obliterative bronchiolitis	None	No	Yes	No	Supportive
Sickle cell anaemia	None	Yes, bronchoconstriction	Yes	Not known	Bronchodilators
Post-premature birth	None	Yes, bronchoconstriction	Yes	Not known	Bronchodilators
Primary airway malacia	None	Yes, loss of airway wall tone	+/-	Secondary	Airway clearance, CPAP, antibiotics, mucolytics, tracheostomy
Post-NEHI wheeze	None	Yes, bronchoconstriction	Yes	Not known	Bronchodilators

NEHI: neuro-endocrine cell hyperplasia; CPAP: continuous positive airway pressure.

There may be evidence of oxidative stress.[56] There is no evidence that these babies respond to ICS, and the Hargreave phenotype is fixed and variable airflow obstruction, with no inflammation.

These children may, of course, have more than one cause of airway obstruction, and iatrogenic large airway disease must not be disregarded. Repeated or prolonged intubation may lead to subglottic stenosis, and damage to the left recurrent laryngeal nerve during surgery to ligate a patent ductus arteriosus may leave the child with a vocal cord palsy.

The importance of phenotyping is illustrated by the problems of the so-called late-preterm delivery. There is evidence of persistent and fixed airflow obstruction in babies born as late as 32 weeks

gestation and an increased burden of asthma even in babies delivered at 37-38 weeks gestation.[57] This begs the question as to what sort of asthma is being diagnosed; the evidence that these babies have eosinophilic inflammation as a result of late prematurity is missing, but there is a real risk that ICS will be prescribed indiscriminately, especially to these late-premature babies whose Hargreave phenotype is the same as for the more preterm infants.

AIRWAY MALACIA

Airway malacia may be primary, related either to loss of airway wall tone or reduction in the number of alveolar attachments holding open the airways; it may be part of a syndrome, such as Ehlers–Danlos; or it may be secondary to airway compression by blood vessels or a mass, and persist even after relief of the external pressure. In any event, the Hargreave phenotype is just variable airflow obstruction with no inflammation. There may be a secondary infection and inflammation as a result of poor distal airway drainage that may need treatment, but asthma medications are ineffective.

WHEEZE AND CHILDHOOD INTERSTITIAL LUNG DISEASE

The presentation of childhood interstitial lung disease is non-specific, but interestingly, wheeze was reported at presentation in >20% of nearly 200 children.[58] Neuroendocrine cell hyperplasia of infancy typically presents with respiratory distress and oxygen dependency in the early weeks of life. High-resolution computed tomography shows typical appearances of ground-glass opacification in the lingula and right middle lobes, and also in a perihilar distribution.[59] Lung biopsy appearances are usually normal, except for increased numbers of cells positive for the neuropeptide bombesin in the distal airways.[60] Infant lung function shows hyperinflation. The prognosis is good, although some may have prolonged oxygen dependency. However, a small follow-up series showed that six of nine children had non-atopic asthma and those tested had some evidence of variable airflow obstruction.[61] Despite an absence of evidence of inflammation, prescriptions of ICS/long acting β2 agonists were given to 50%. Not enough is known to Hargreaves phenotype these children, but given that they present with airflow obstruction early on, at a time when there is little if any evidence of airway inflammation, the presumption must be that this is non-inflammatory, fixed, and possibly variable airflow obstruction.

DOES IT MATTER?

Table 3 summarises the various wheezing syndromes, and, more importantly, shows how lines of treatment can and should be determined by the Hargreave phenotype.[62] Of course, there may be some overlap, and it may also be difficult to differentiate between, for example, post-bronchiolitis, episodic viral, and multiple trigger wheeze. The cardinal principle when starting a treatment for any child with a wheezing disorder is to constantly re-evaluate whether there is any response to treatment, whatever it is, and discontinue it if there is any doubt about benefit.

It is wise to use a three-stage protocol for any trial of medium-term (i.e. non-acute) treatment since the natural history of many airway diseases is spontaneous remission. So, for example, if a trial of ICS is being given to a child with a presumptive diagnosis of MTW,[63] the steps would be:

1. Commence treatment at a relatively high dose (e.g. fluticasone 150 µg b.i.d. via an appropriate spacer); if the child does not respond to this dose, then the airway disease is unlikely to be steroid-responsive. If a low dose is used, then time may be wasted by going on to a higher dose before steroid insensitivity is diagnosed
2. After a fixed (arbitrary) period, around 4-8 weeks, stop treatment. If the child has not improved, do not escalate ICS therapy but reconsider the diagnosis. If the child appears to have improved, then it is unclear at this stage if this is spontaneous or treatment-related
3. Only restart ICS treatment if symptoms return, and then keep titrating the dose to the minimum needed to control symptoms; regular review is mandatory

Clearly in older children, who can perform lung function testing, objective documentation of response is mandatory. However, in preschoolers this protocol, which is of course not evidence-based, will avoid the error of labelling children as having a steroid-sensitive airway disease and continuing treatment, whereas in fact they had merely spontaneously improved.

SUMMARY AND CONCLUSION

Here we have proposed a framework for the assessment, and more importantly treatment, of preschool airway disease. We propose that the components of airway diseases are considered separately and that each are treated on merit, rather than applying umbrella terms such as 'asthma'. We accept that studies are needed to confirm that this is a useful approach in routine clinical practice. In particular, the phrase 'Dr-diagnosed asthma' should vanish from the literature, particularly for preschool children. It is clear that our means of assessing airway disease in the clinic are very crude; in particular, airway inflammation and airway obstruction are usually not measured at all, and upper airway surrogates are used for lower airway infection. Clearly an infant who has a clear clinical picture and responds to appropriate therapy does not need elaborate testing, but for more problematic cases we should deploy existing tests, such as induced sputum for lower airway inflammation and infection, eNO, and peripheral blood eosinophil count. There is also an urgent need to put our assessments of these infants on a more scientific basis by developing new, clinically applicable tests, to bring the ideas of Freddie Hargreave[64] into the preschool years. Clearly his ideas are the start of a process, and in the future, refinements of his thinking will be introduced, but these ideas are far better than the current lumping of wheezing syndromes. The ultimate aim of all of this is, of course, improved outcomes for these infants, to which we all aspire.

REFERENCES

1. Martinez FD et al. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med. 1995;332(3):133-8.

2. Brand PL et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J. 2008;32(4): 1096-110.

3. Hargreave FE, Nair P. The definition and diagnosis of asthma. Clin Exp Allergy. 2009;39(11):1652-8.

4. Bush A et al. The asthmas in 2015 and beyond: a Lancet Commission. Lancet. 2015;385(9975):1273-5.

5. Cane RS et al. What do parents of wheezy children understand by "wheeze"? Arch Dis Child. 2000;82(4):327-32.

6. Levy ML et al. Wheeze detection: recordings vs. assessment of physician and parent. J Asthma. 2004;41(8):845-53.

7. Cane RS, McKenzie SA. Parents' interpretations of children's respiratory symptoms on video. Arch Dis Child. 2001;84(1):31-4.

8. Saglani S et al. A video questionnaire identifies upper airway abnormalities in preschool children with reported wheeze. Arch Dis Child. 2005;90(9):961-4.

9. Panitch HB et al. Effect of altering smooth muscle tone on maximal expiratory flows in patients with tracheomalacia. Pediatr Pulmonol. 1990;9(3):170-6.

10. Mallol J et al. International prevalence of recurrent wheezing during the first year of life: variability, treatment patterns and use of health resources. Thorax. 2010;65(11):1004-9.

11. Saglani S et al. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. Am J Respir Crit Care Med. 2005;171(7):722-7.

12. Bisgaard H et al. Childhood asthma after bacterial colonization of the airway in neonates. N Engl J Med. 2007;357(15):1487-95.

13. Bisgaard H et al. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. BMJ. 2010;341:c4978.

14. Sherriff A et al. Risk factor associations with wheezing patterns in children followed longitudinally from birth to 3(1/2) years. Int J Epidemiol. 2001;30(6): 1473-84.

15. Savenije OE et al. Comparison of childhood wheezing phenotypes in 2 birth cohorts: ALSPAC and PIAMA. J Allergy Clin Immunol. 2011;127(6):1505-12.e14.

16. Collins SA et al. Validation of novel wheeze phenotypes using longitudinal airway function and atopic sensitization data in the first 6 years of life: evidence from the Southampton Women's survey. Pediatr Pulmonol. 2013;48(7):683-92.

17. The Childhood Asthma Management Program Research Group. Long-term effects of budesonide or nedocromil in children with asthma. N Engl J Med. 2000;343(15):1054-63.

18. Bisgaard H et al. Intermittent inhaled corticosteroids in infants with episodic wheezing. N Engl J Med. 2006;354(19):1998-2005.

19. Guilbert TW et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med. 2006;354(19):1985-97.

20. Sonnappa S et al. Symptom-pattern phenotype and pulmonary function in preschool wheezers. J Allergy Clin Immunol. 2010;126(3):519-26 e1-7.

21. Bisgaard H et al. Interaction between asthma and lung function growth in early life. Am J Respir Crit Care Med. 2012;185(11):1183-9.

22. Palmer LJ et al. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. Am J Respir Crit Care Med. 2001;163(1):37-42.

23. Clarke JR et al. Bronchial responsiveness in the neonatal period as a risk factor for wheezing in infancy. Am J Respir Crit Care Med. 1995;151(5):1434-40.

24. Martinez FD et al. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N Engl J Med. 1988;319(17):1112-7.

25. Schultz A et al. The transient value of classifying preschool wheeze into episodic viral wheeze and multiple trigger wheeze. Acta Paediatr. 2010;99(1):56-60.

26. Marguet C et al. Bronchoalveolar cell profiles in children with asthma, infantile wheeze, chronic cough, or cystic fibrosis. Am J Respir Crit Care Med. 1999;159(5 Pt 1):1533-40.

27. Najafi N et al. Differential cytology of bronchoalveolar lavage fluid in asthmatic children. Pediatr Pulmonol. 2003;35(4):302-8.

28. Moeller A et al. Exhaled nitric oxide distinguishes between subgroups of preschool children with respiratory symptoms. J Allergy Clin Immunol. 2008;121(3):705-9.

29. Bush AJ et al. Managing wheeze in preschool children. BMJ. 2014;348:g15.

30. Bacharier LB et al. Episodic use of an inhaled corticosteroid or leukotriene

receptor antagonist in preschool children with moderate-to-severe intermittent wheezing. J Allergy Clin Immunol. 2008;122(6):1127-1135.e8.

31. Robertson CF et al. Short-course montelukast for intermittent asthma in children: a randomized controlled trial. Am J Respir Crit Care Med. 2007;175(4): 323-9.

32. Valovirta E et al. Intermittent or daily montelukast versus placebo for episodic asthma in children. Ann Allergy Asthma Immunol. 2011;106(6):518-26.

33. Nwokoro C et al. Intermittent montelukast in children aged ten months to 5 years with wheeze (WAIT trial): a multicentre, randomised, placebo-controlled trial. Lancet Respir Med. 2014;2(10):796-803.

34. Bont L et al. Long-term consequences of respiratory syncytial virus (RSV) bronchiolitis. Paediatr Respir Rev. 2000;1(3):221-7.

35. Turner SW et al. Reduced lung function both before bronchiolitis and at 11 years. Arch Dis Child. 2002;87(5):417-20.

36. Ermers MJ et al. The effect of high dose inhaled corticosteroids on wheeze in infants after respiratory syncytial virus infection: randomised double blind placebo controlled trial. BMJ. 2009;338:b897.

37. Zomer-Kooijker K et al. Lack of long-term effects of high-dose inhaled beclomethasone for respiratory syncytial virus bronchiolitis: a randomized placebo-controlled trial. Pediatr Infect Dis J. 2014;33(1):19-23.

38. Castro-Rodriguez JA et al. A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Respir Crit Care Med. 2000;162(4 Pt 1):1403-6.

39. Marchant JM et al. Evaluation and outcome of young children with chronic cough. Chest. 2006;129(5):1132-41.

40. Chang AB et al. Bronchoscopic findings in children with non-cystic fibrosis chronic suppurative lung disease. Thorax. 2002;57(11):935-8.

41. Cole P. The damaging role of bacteria in chronic lung infection. J Antimicrob Chemother. 1997;40 Suppl A:5-10.

42. Suissa S et al. Inhaled corticosteroids in COPD and the risk of serious pneumonia. Thorax. 2013;68(11):1029-36.

43. Lee CH et al. Use of inhaled corticosteroids and the risk of tuberculosis. Thorax. 2013;68(12):1105-13.

44. Fritscher LG et al. Nontuberculous mycobacterial infection as a cause of difficult-to-control asthma: a case-control study. Chest. 2011;139(1):23-7.

45. Marchant J et al. Randomised controlled trial of amoxycillin clavulanate in children with chronic wet cough. Thorax. 2012;67(8):689-93.

46. Chang AB et al. Chronic wet cough: Protracted bronchitis, chronic suppurative lung disease and bronchiectasis. Pediatr Pulmonol. 2008;43(6):519-31.

47. Barbato A et al. Primary ciliary dyskinesia: a consensus statement on diagnostic and treatment approaches in children. Eur Respir J. 2009;34(6): 1264-76.

48. Farrell PM et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. J Pediatr. 2008;153(2):S4-S14.

49. Cullinan P, Bush A. Growing old(er) with postinfectious bronchiolitis obliterans. Thorax. 2015;70(2):103-4.

50. Chaudry RA et al. Reduced forced expiratory flow but not increased exhaled nitric oxide or airway responsiveness to methacholine characterises paediatric sickle cell airway disease. Thorax. 2014;69(6):580-5.

51. Leong MA et al. Airway hyperreactivity in children with sickle cell disease. J Pediatr. 1997;131(2):278-83.

52. Knight-Madden JM et al. Asthma in children with sickle cell disease and its association with acute chest syndrome. Thorax. 2005;60(3):206-10.

53. Doyle LW et al. Bronchopulmonary dysplasia in very low birth weight subjects and lung function in late adolescence. Pediatrics. 2006;118(1):108-13.

54. Greenough A. Long-term respiratory consequences of premature birth at less than 32 weeks of gestation. Early Hum Dev. 2013;89 Suppl 2:S25-7.

55. Narang I et al. Airway function measurements and the long-term follow-up of survivors of preterm birth with and without chronic lung disease. Pediatr Pulmonol. 2006;41(6):497-508.

56. Filippone M et al. Evidence of unexpected oxidative stress in airways of adolescents born very pre-term. Eur Respir J. 2012;40(5):1253-9.

57. Kotecha SJ et al. Effect of late preterm birth on longitudinal lung spirometry in school age children and adolescents. Thorax. 2012;67(1):54-61.

58. Bush A. Diagnosis of interstitial lung disease. Pediatr Pulmonol. 1996;22(2): 81-2.

59. Brody AS et al. Neuroendocrine cell hyperplasia of infancy: diagnosis with high-resolution CT. AJR Am J Roentgenol. 2010;194(1):238-44.

60. Deterding RR et al. Persistent tachypnea of infancy is associated with neuroendocrine cell hyperplasia. Pediatr Pulmonol. 2005;40(2):157-65.

61. Popler J et al. Familial neuroendocrine cell hyperplasia of infancy. Pediatr Pulmonol. 2010;45(8):749-55.

62. Hargreave FE et al. Asthma as a disease concept. Lancet. 2006;368(9545):1415-6.

63. Castro-Rodriguez JA, GJ Rodrigo. Efficacy of inhaled corticosteroids in infants and preschoolers with recurrent wheezing and asthma: a systematic review with meta-analysis. Pediatrics. 2009;123(3):e519-25.

64. O'Byrne PM et al. In memoriam: Frederick E Hargreave (1938-2011). Thorax. 2011;66(12):1101-2.