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Welcome

Hello and welcome to the second edition of our flagship journal, *European Medical Journal*, for 2017. As always, this unique, mixed therapeutic area publication displays research and studies from across a plethora of fascinating medical topics. In this edition, there is a particular emphasis on medical innovation and technology to improve patient care, as well as peer-reviewed articles from several fields, including cardiology, neurology, and haematology.

Antoniou and Yassin present the featured article for this journal. This looks at 'vaginal rejuvenation' and the safety of current methods and devices for such a procedure, as well as an analysis of regulations in this area.

Due to the large increase of migrants and refugees in the modern era, there is an additional challenge for healthcare systems, especially with the resulting increased risk of chronic illnesses developing. With this in mind, Osae-Larbi discusses ways in which mobile digital health technologies can help tackle this potential burden in the future. In another medical innovations article, Zanini et al. investigate whether it is possible to combine the analgesic effects of looking at one's body with those deriving from the illusion that one's own limb is moving, in order to assist with the goal of developing pain-relieving outcomes. Also from the sphere of innovations, Lauridsen writes about the potential impact the fields of regenerative biology and medicine can have in artificial tissue construction by considering how intrinsic tissue regeneration takes place in nature.

> "As always, this unique, mixed therapeutic area publication displays research and studies from a plethora of fascinating medical topics."

We have a pair of papers from the sphere of allergy and immunology; Brooks and Renaudineau have provided a paper on the nucleolus hypothesis of autoimmune diseases, while Anderson et al. review the incidence, agents, and mechanisms of occupational allergy before going on to detail research needs. From the field of cardiology, Feeman examines the extent to which non-high-density lipoprotein cholesterol is a predictor of the population at risk of atherothrombotic disease. Neurology is also a strong focus: Jeong and Kim provide a thought-provoking piece on intra-arterial thrombectomy and the limitations of extending this therapeutic tool for improved functional outcomes after thrombolysis. Rygiel describes the main mechanisms underlying chronic nociceptive and neuropathic pain, before discussing techniques to help practitioners evaluate and regularly monitor pain, emphasising the importance of collaboration between a team of medical specialists.

We hope that you enjoy this edition of *European Medical Journal* and that the content is both intellectually stimulating and useful to you in your future work. We would like to thank all the authors who have contributed to this edition, as well as our esteemed editorial board for their support and expertise, and we look forward to further editions of this journal in 2017.

Spencer Gore

Director, European Medical Journal

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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). Bosutinib can induce severe skin reactions such as Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis. Bosutinib should be permanently discontinued in patients who experience a severe skin reaction during treatment. Due to the possible occurrence of tumour lysis syndrome (TLS), correction of clinically significant dehydration and treatment of high uric acid levels are recommended prior to initiation of bosutinib (see SmPC section 4.8). Reactivation of hepatitis B (HBV) in patients who are chronic carriers of this virus has occurred after these patients received BCR-ABL tyrosine kinase inhibitors. Some cases resulted in acute hepatic failure or fulminant hepatitis leading to liver transplantation or a fatal outcome. Patients should be tested for HBV infection before initiating treatment with Bosulif. Experts in liver disease and in the treatment of hepatitis B should be consulted before treatment is initiated in patients with positive hepatitis B serology (including those with active disease) and for patients who test positive for HBV infection during treatment. Carriers of HBV who require treatment with Bosulif should be closely monitored for signs and symptoms of active HBV infection throughout therapy and for several months following termination of therapy. 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: 12/2016
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References
1. Pfizer Ltd. Bosulif SPC. Available at: www.medicines.org.uk/emc/

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Pfizer

Foreword

Dr Markus Peck-Radosavljevic

Professor of Medicine and Chairman, Department of Gastroenterology and Hepatology, Endocrinology and Nephrology, Klinikum Klagenfurt am Wörthersee, Klagenfurt, Austria.

Dear Colleagues,

I would like to welcome you to the second edition of the *European Medical Journal* 2017. In this latest edition, we highlight a number of topics related to different areas of medicine and public health.

We begin with an article on the use of digital technology solutions in healthcare to help address chronic illness. Electronic diaries and other forms of communication have become standard practice in clinical trials, but here they are being trialled in the migrant population of Europe to help manage and prevent chronic diseases. Next, we move towards regenerative biology and address artificial tissue construction and three-dimensional (3D) bioprinting. The major issue is that artificial tissues are often not completely reliable or fully functional compared to the original tissues, which is an issue that needs to be resolved before its application can be introduced on a broader scale to the wider public.

> “I am sure that this edition will cover many topics of interest, and I hope that you enjoy reading our latest edition of the *European Medical Journal* 2017.”

Following an article on virtual reality to tackle pain, we present a very important public health topic. In order to compare health outcomes globally, the International Consortium for Health Outcomes Measurement (ICHOM) has engaged in a non-interventional, observational, benchmarking programme to evaluate standard sets of outcomes for important medical conditions. This will enable improved comparisons of the quality of healthcare and health systems globally.

Other relevant topics include a paper on the nucleolus hypothesis for the development of autoimmune diseases. In addition, the extension of the 2016 World Health Organization (WHO) classification for myeloproliferative neoplasms will be discussed, with particular emphasis on the use of new sets of clinical, laboratory, molecular, and pathological criteria. Other topics include epithelial–mesenchymal transition (EMT) in prostate cancer and occupational allergies, amongst others.

I am sure that this edition will cover many topics of interest, and I hope that you enjoy reading our latest edition of the *European Medical Journal* 2017.

Kind regards,

Markus Peck-Radosavljevic

Professor of Medicine and Chairman, Department of Gastroenterology and Hepatology, Endocrinology and Nephrology, Klinikum Klagenfurt am Wörthersee, Klagenfurt, Austria; Fellow, Austrian College of Physicians; Member, 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).

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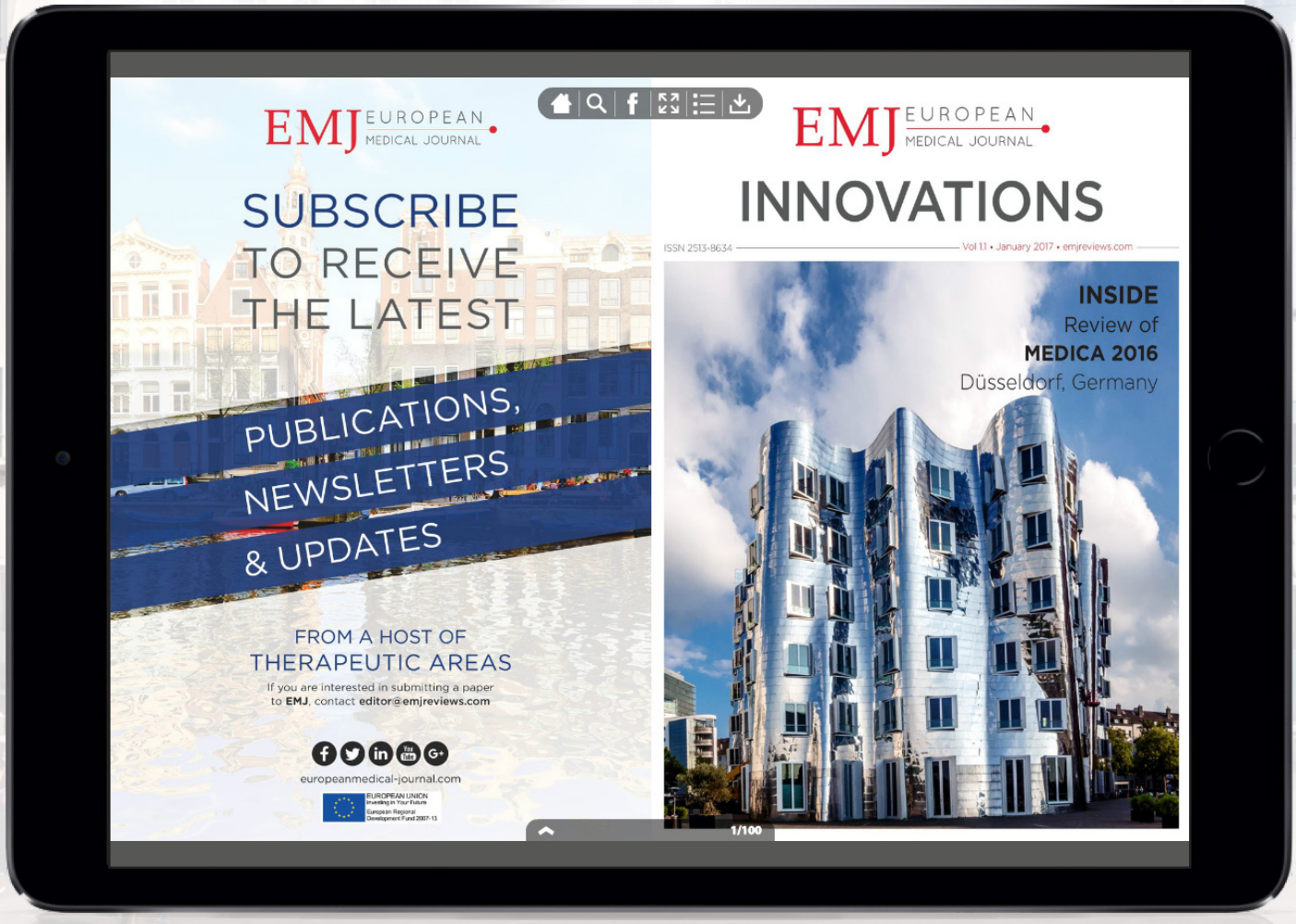

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'VAGINAL REJUVENATION' AND THE REGULATION OF NEW TECHNOLOGIES: CONTROLS ARE STILL LACKING

***Antonios Antoniou, Ahmed Yassin**

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Keywords: Vaginal rejuvenation, new technologies, regulation, registries.

A tour of the stands at the 41st International Urogynaecology Association (IUGA) Annual Meeting in August 2016 revealed a growing number of companies with machines aimed at 'vaginal rejuvenation', an industry-driven misnomer, with promises of tightening of the vagina (for laxity primarily caused by childbirth) and improved sexual satisfaction. A review of the literature regarding the use of energy-based devices, including CO_2-based or erbium:Yttrium-aluminum-garnet (Er:YAG) lasers and radiofrequency-based devices, for the treatment of vaginal rejuvenation, reveal the procedures to be easily performed in the outpatient setting, generally tolerable to the patient, and easy to do. However, most of the studies performed to date are pilot studies with low patient numbers and short follow-up, indicating that both efficacy and safety are yet to be established.[1-4] Moreover, there remain many questions that have yet to be answered, including the possibility of energy transmitted as heat affecting adjacent tissues (such as the cervix, rectum, and bladder) as well as the potential for neoplastic lesions, vulvar dermatoses, and the long-term effects of possible scarring on future obstetric outcome.

It remains unclear as to how online content influences women's consideration and acceptance of female genital cosmetic surgery. Further research is needed into the role the internet plays in its promotion and normalisation, and the consequent effect on patient demand.[5] What is it that drives patients to seek such treatment? Is it the woman herself who seeks better sexual fulfilment or her partner? What is the psychological background of such behaviour? These issues warrant further investigation.

Of greater concern is the fact that there appears to be little worldwide regulation in the sale and use of such devices, with no apparent requirement for the organisation and collection of data in the form of databases or registries. This at least could be used to collect evidence of efficacy and long-term outcomes and to monitor the safety of the devices. These devices are available and have been used by a variety of practitioners including general gynaecologists, urogynaecologists, dermatologists, and plastic surgeons, further complicating matters.

The US Food and Drug Administration (FDA) and the Medicines and Healthcare products Regulatory Agency (MHRA) in the UK regulate the use of medical devices. These regulatory bodies must act in an efficient and timely manner such that patients are not deprived of beneficial innovations, whilst at the same time minimising harm. These bodies have not approved devices for use in this procedure. However, some of the devices used for vaginal rejuvenation have FDA approval for non-genital use. Nonetheless, many sites advertising the procedure misleadingly state that FDA approval has been met. The procedure is also openly advertised without regulation, and is available worldwide.

Although many medical devices for various indications have improved clinical outcomes, not all are beneficial and some have been harmful. Concerns over hip resurfacing techniques and breast implants have raised serious questions about how medical devices are evaluated. Furthermore, it would appear that we have yet to learn the lessons of the vaginal mesh fiasco with industry led introductions of treatments of utero-vaginal prolapse with minimal evidence, and lack of long-term outcomes rather than using a systematic

base of evidence prior to using such products on our unsuspecting and vulnerable patients.[6] The outcome of this has led to a plethora of litigation cases leading the manufacturers to remove their vaginal mesh products from the market.[7] An unfortunate by-product of the vaginal mesh disaster is that evidence-based products such as 'tension-free' vaginal tapes, a treatment for stress urinary incontinence with excellent outcomes and low complication rates, are being unfairly associated with the meshes and mesh kits being used for prolapse treatment, leaving patients with fewer alternative treatments. The Scottish independent review of the use, safety and efficacy of transvaginal mesh implants concluded that robust clinical governance must surround the decision to use the product.[6] It also recommended that the Scottish Government consider alternative methods for the capture of adverse events to further determine the most effective way to ensure complete notification, and that the lack of extended long-term follow-up and related outcome data should be addressed.

The surgical world is a collection of different personalities, some of whom will jump on the latest bandwagon promising innovative new treatments to their patients whilst others at the opposite spectrum wait for an adequate evidence base prior to training and use of new techniques or technologies. What drives this difference in early versus late adopters of new technology? Why are some surgeons much more cautious whilst others will apparently shun the lack of evidence and embrace new treatments? Is it driven by patient demand, financially driven with the promise of increased private income, the desire to use new innovations for the benefit of patients, or inappropriately perceived as being safe? Are patients aware of where their surgeon sits in this debate? More studies are required to investigate the differences between early and late adopters, including surgeon characteristics, their surgical outcomes, complication rates, patient satisfaction surveys, and private practice.

Understanding the considerations that influence physician adoption of new, unproven methodologies is critical to the development of strategies to better align clinical practice with available evidence and to control healthcare costs.[8] Several factors influence this decision, one being physician's peer exposure, since exposure to early or late adopters may influence physician comfort with new innovation.[8]

As a reaction to the analysis of how innovations were taking place in surgery, an expert group was set up within the framework of the Balliol Collaboration to compile recommendations for scientific evaluation of surgical innovations.[9] This group made a series of recommendations including encouragement of the widespread use of prospective databases and registries. Reports of new techniques should be registered as a professional duty, anonymously if necessary, when outcomes are adverse. Protocols for studies should be registered publicly, and randomised trials should be used wherever possible.[9] In the surgical innovation process, registries constitute an important scientific tool that affords insights from the outset and accordingly merits evaluation.[10] This will provide for early identification of any problems or complications on the basis of outcome analysis.

A recent study looking at the regulatory approval of new medical devices suggests that many new devices do receive regulatory approval but often lack clinical trial data supporting their safety and effectiveness.[11] The optimal framework for the regulatory approval of medical innovations remains unclear.[11]

Surgeons should be cautious in the adoption of new technologies. Evidence-based medicine should be used in the decision on when to begin the use of a new methodology and this must only be within the remits of data collection via clinical trials allowing the collection of long-term outcomes of efficacy and safety. Regarding the use of vaginal rejuvenation, the American College of Obstetricians and Gynecologists (ACOG) has stated that these procedures are not medically indicated and the safety and effectiveness has not been demonstrated.[12] This has not precluded its use by gynaecologists or other specialists. Regulation preventing practitioners advertising and offering such interventions to patients outside a trial, for unproven interventions, will help protect patients until safety and efficacy of these devices for use in the vagina is established.

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EDITOR'S PICK

The choice of editor's pick for this edition comes courtesy of Henrik Lauridsen. A stimulating consideration of the nature of regeneration and the impact of humans' low regenerative potential is given throughout this paper. The author provides some excellent examples from the natural world and, in discussing the current technological state of affairs, highlights how far this field has developed. It is certainly thought-provoking to contemplate the potential of artificial tissue regeneration.

A REGENERATIVE BIOLOGY VIEW ON ARTIFICIAL TISSUE CONSTRUCTION AND THREE-DIMENSIONAL BIOPRINTING: WHAT MAY WE LEARN FROM NATURAL REGENERATIVE PHENOMENA?

***Henrik Lauridsen**

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ABSTRACT

The implications of the low tissue regenerative potential in humans are severe and widespread. Several of our major diseases are direct results of this deficiency that leaves us vulnerable to events of tissue damage. This is opposed to some animal groups, such as the urodele amphibians (salamanders), that display distinct tissue regeneration after injury. An important goal of biomedical engineering is the construction of artificial tissue that can ultimately be transplanted into patients, however, such constructs are still in their infancy for more complex structures. Approaches of constructing artificial organ structures by decellularisation/recellularisation procedures and recently with three-dimensional (3D) bioprinting show promising results in obtaining anatomically accurate constructs, however, the function of these artificial tissues is still lacking compared to natural tissues. This review will highlight how the relatively mature fields of regenerative biology and medicine can have potential usage in the younger bioengineering field of artificial tissue construction by drawing on the knowledge of how intrinsic tissue regeneration takes place in nature.

Keywords: Regeneration, three-dimensional (3D) bioprinting, artificial tissue.

THE RECONSTRUCTION OF TISSUE

Tissue regeneration is the process of replacing tissue lost from damage, disease, age, or injury with new tissue. This process is fundamental for life and all animal species to some degree possess mechanisms that can maintain homeostasis and the functional integrity of cells and tissue. Richard J. Goss,[1] one of the pioneers of regenerative research, summed up the relationship between life, death, and regeneration with the words: "If there were no regeneration there could be no life. If everything regenerated there could be no death. All organisms exist between these two extremes. Other things being equal, they tend toward the latter end of the spectrum, never quite achieving immortality because this would be incompatible with reproduction". However true this statement is, regenerative potential varies quite

a bit between different organisms. Humans and mammals, in general, have not been blessed with extensive regenerative potential and tend to resolve tissue damage events by fibrosis and scar formation.[2] This fibrotic situation results from an inflammatory response to injury that generates fibroblastic granulation tissue that is ultimately modelled into an acellular collagenous scar. This maintains the overall integrity of the damaged organ but usually reduces function. On the other hand, 'lower' vertebrate species (e.g. urodele amphibians) do exist with an unsurpassed ability to recapitulate embryonic development and regenerate tissue and even whole organs and appendages to perfection without any scar formation.[3-5] This perplexing conundrum of why mammals have lost this apparently ancestral and seemingly highly beneficial trait has been a driving force in the history of regenerative research. It may be that evolution of warm-blooded mammals susceptible to infections has favoured the individuals with the fastest response to injury, with this being a swift immunological response and subsequent fibrosis to 'seal off' damaged parts rather than regeneration.[6,7] After all, although regeneration of extremities and organs is desirable, it is not essential for reproduction.

The low regenerative potential of humans has far-reaching consequences in medicine. Heart, liver, and renal failure; disorders of the nervous system (e.g. amyotrophic lateral sclerosis, multiple sclerosis, and Parkinson's, Huntington's, and Alzheimer's diseases); and burns and traumatic injuries (to skin, bones muscles joints, ligaments, and tendons) are all examples of diseases and conditions resulting from poor regenerative potential. Other than the personal consequences for patients suffering from these ailments, the cost to society is enormous. In the USA alone the annual cost was estimated to be >$400 billion.[3] Naturally, this has inspired great interest with the field of regenerative medicine seeking to develop and apply future regenerative therapies for human patients. However, it is highly unlikely that regenerative medicine can fulfil this task without a thorough understanding of the basic mechanisms involved in regenerative events. Therefore, advancements in the field of regenerative biology and the understanding of basic molecular and cellular processes during tissue regeneration are a prerequisite for regenerative medicine to develop its full potential.

THE ENGINEERING SOLUTION TO LOW REGENERATIVE POTENTIAL

The lesson from nature is that intrinsic *in situ* regeneration of even highly complex tissues is possible in basic vertebrate models. Most human tissues do in fact replenish themselves to some degree over life; with the strategy to stimulate innate tissue regeneration being one of the most desirable approaches for future regenerative therapies. However, as the key to unlock this intrinsic regenerative potential in humans is yet to be found it is worth considering alternative strategies for rebuilding damaged organs.

A fundamentally different approach of constructing organs or tissue components has arisen in the last decade, namely the *de novo* construction of organs *in vitro* that can later be transplanted into patients. An instructive example is the construction of heart transplants. Since Dr Barnard's successful heart transplantation in the mid-twentieth century the heart transplantation procedure has become a well-established lifesaving treatment to extend and dramatically improve the quality of life for recipients. A patient's heart can either be fully replaced with a donor heart (orthotopic procedure) or supported by an extra heart (heterotopic procedure). Unfortunately, suitable donor hearts, as well as other organs, are a scarce commodity and as the demand is increasing, so is the need for alternative strategies. Two novel engineering approaches to alleviate the problem of scarce donor organs have received much attention in recent years, namely the construction of bioartificial organs either using a decellularisation/repopulation procedure of extracellular matrix scaffolds or bottom-up constructions of artificial organs using three-dimensional (3D) bioprinting.

ARTIFICIAL ORGANS BY DECELLULARISATION/ RECELLULARISATION

Bioengineering laboratories around the world have long sought to construct artificial biocompatible scaffolds for cell seeding and transplantable tissue generation. This has led to several successful procedures and especially in orthopaedics multiple useful constructs have been designed.[8] Similar approaches of scaffold construction have been applied for soft tissue construction; however, it is hard to replicate the complex cellular environment found in most organs in simple scaffolds moulded

in the lab. One way to circumvent this limitation is to generate extracellular scaffolds made by decellularisation of true organs that may be too old or do not match the receiver type and hence cannot be used directly for transplantation. This procedure can be a complicated washing process in which all living cells are removed from the organ leaving behind only the supporting extracellular matrix as intact and unmodified as possible.[9] The resulting tissue scaffold is then reseeded with living cells either by immersion in a cell containing medium or more efficiently by perfusion via the skeletonised vasculature of the decellularised organ. As a cell source, either differentiated cells that have previously been dissociated from their host tissue into a single cell state or patient-specific stem cells that have been cultured prior to perfusion, can be applied. The underlying assumption of the decellularisation/recellularisation procedure is that perfused cells migrate through the extracellular matrix scaffold. Differentiated cells should ultimately settle when they reach a suitable environment, whereas stem cells may in fact start to differentiate in a manner defined by cues in the surrounding matrix components.

Following a maturation period of cell proliferation, the final result is a 3D multi-cell type culture that can be thought of as a 'breath of life' into a once dead and skeletonised organ. However, the decellularisation/recellularisation technique does not come without important limitations. In 2008 Ott et al.[10] applied the procedure to recellularise rat hearts using neonatal cardiomyocytes, fibrocytes, endothelial cells, and smooth muscle cells and subsequently transplanted the constructed hearts into host rats. Remarkably, this study demonstrated how contractile and drug responsive hearts could be constructed, however the function of the generated constructs were only ~2% of that of an adult rat heart. In a similar fashion, Lu et al.[11] in 2013 were successful in repopulating decellularised mouse hearts with induced pluripotent stem cell-derived cardiovascular progenitor cells from humans. This attempt also yielded heart constructs that exhibited spontaneous contractions, generated mechanical force, and were responsive to drugs. However, in similar fashion to the attempt by Ott et al.,[10] these constructs only showed minute pump function and were overall unable to propel blood.

The attempts described above to rebuild the heart, as well as other organs,[12] by decellularisation/recellularisation of already existing organs, are interesting because they suggest that this type of organ construction that can be viewed as a very engineering-based mindset (breakdown then build up) and not a biologically inspired way of regenerating organs. However, for now the procedure is insufficient in producing fully functional organs. To overcome this obstacle, repopulation technologies need to consider how organs are constructed during development and in natural regeneration in competent species.

THREE-DIMENSIONAL BIOPRINTING: A POTENTIAL LOOPHOLE FOR TISSUE RECONSTRUCTION

A fundamentally different approach of producing complex organs to that of cell repopulation of scaffolds is 3D bioprinting. The prospects of this technology have been glorified in recent years in TED talks and other quasi-scientific fora; however, the method has in fact shown promising results in terms of producing *de novo* tissue constructs.

As the name suggests, 3D bioprinting originates from 3D printing (also known as rapid prototyping or more precisely as additive manufacturing) of prototypes and models in a large variety of materials such as plastic, wood, ceramics, glass, and metals in industry. 3D printing is additive in nature, i.e. it starts from nothing and ends with a structure after adding layer upon layer. This is opposed to computer numerical control drilling, which performs what can be described as negative manufacturing by carving out a structure from a solid object. From an historical perspective, it is interesting that 3D printing has existed for several decades,[13] but primarily due to proprietary issues gridlocking technological development and competition the technology has only flourished within the last decade with the expiration of early patents. This has resulted in modern day 3D printers becoming better and more affordable at an astonishing pace. 3D printing has been applied in medical and life sciences to create organ models from computed tomography and magnetic resonance imaging (MRI) information and implantable constructs.[14-18] Some major 3D printer manufactures now offer dedicated software and hardware for this field of modelling i.e. complex medical disorders in physical models that surgeons can handle and study even before the first cut is made in surgery.

The 3D printing field is notorious for the lack of consistent nomenclature to describe similar printing technologies; however, the American Society for Testing and Materials (ASTM) currently categorises

3D printing technologies into seven categories: binder jetting, directed energy deposition, material extrusion, material jetting, powder bed fusion, sheet lamination, and vat photopolymerisation.[19] These technologies have all been developed for non-living materials, but in addition, 3D printing has recently evolved into 3D bioprinting in which cells and extracellular matrix can also be used as raw materials for printing.[20] 3D bioprinting relies on two of the seven categories of non-living 3D printing technologies listed above and one additional technology; thus 3D bioprinting can currently be divided into three technologies. The first and simplest is the inkjet method, which is inspired by the material jetting method of 3D printers and is in principle the same inkjet technology applied in desktop paper printers. In this technique, rapid electrical heating or piezoelectric/ultrasound generated pressure pulses are applied to propel cell containing droplets from a nozzle to the build surface. The second 3D bioprinting technique, microextrusion printing, is widely applied and fundamentally applies the same method as material extrusion 3D printers to pneumatically or mechanically dispense a continuous thread of cell containing material. The final and most complex technology is laser-assisted 3D bioprinting in which a laser is briefly and repeatedly focussed on an absorbing substrate coated in cells thereby generating a pressure that propels cell-containing materials onto a collector substrate that becomes coated by cells in a pattern defined by the laser path. Following the initial deposition of cells and substrates, the layered construct generated in all three bioprinting methods is cured and hardened to support its own structure either by designed polymers in the cell medium that respond to cooling, heating, chemical treatment, or more commonly by light-activated polymerisation in a fashion that is comparable to vat photopolymerisation in non-living material 3D printers.

The three 3D bioprinting technologies currently available have specific advantages and limitations. The simplest and most affordable technology is inkjet bioprinting, which also operates at a high printing speed and with a high cell viability because of a relatively gentle printing process. The spatial resolution of the technique is, however, relatively low and cell density in the construct is also low, which is important for the subsequent maturation of the construct. On the other hand, microextrusion based bioprinters provide high-cell densities but at the cost of viability (often <40%), primarily due to sheer stress during deposition, and printing speed is lower than for inkjet printing. Laser-assisted bioprinters are fast and cell viability is very high (>95%) and so is the printing resolution; however, these systems are highly expensive and challenging to maintain.

Early experiments using 3D bioprinting have been reported for a number of tissues and organ structures ranging from skin, blood vessels, trachea, cartilage, kidney, and various cardiac tissue (e.g. myocardium and valves).[20] A central goal of this technology has however been in the construction of cardiac tissue which represents a relatively simple organ (compared to secretory organs often with manifold cell types) and a model system where the success of the construction can easily be tested in terms of function compared to baseline values. Thus, the attempts to construct cardiac tissue serves as a good model for the current status of 3D bioprinting technology. Several impressive attempts of printing different cardiac tissues have been made in recent years.[21] From a regenerative biology viewpoint the attempt by Gaetani et al.[22] in 2012 to apply microextrusion to 3D bioprint small alginate scaffolds with fetal human cardiomyocyte progenitor cells is interesting. These myocardial-like scaffolds both showed high-cell viability and importantly imbedded cardiomyocytes retained their commitment for the cardiac lineage. The implication of this is that the deposited cells in fact remain in their desired lineage and behave in a natural fashion. Another study by Gaebel et al.[23] in 2011 used the laser assisted bioprinting technique on human umbilical vein endothelial cells and human mesenchymal stem cells to construct various capillary like patterns of cells on a polyester urethane urea cardiac patch. Several of these vascular patterns were successful and resulted in the two cell types arranged into a capillary like network. These patches with patterned cells were thereafter cultured and matured and subsequently transplanted *in vivo* into infarcted rat hearts. Intriguingly, the study reported increased vessel formation and additionally a significant functional improvement of the infarcted hearts. The results of this study underline the importance of considering the construction of an appropriate vascular supply in regenerative therapies.

To date, no successful attempts to 3D bioprint complete organ constructs similar to the ones generated by decellularisation/repopulation procedures have been reported, but efforts in using extracellular matrix for building material have been made with success.[24] Assuming that the

3D bioprinting technology eventually matures to the state where the option of full organ printing becomes possible, it is not unlikely that constructs may suffer from the same deficiencies in terms of function and force production as described above for the repopulated heart constructs. To overcome these obstacles, it may be fruitful to consider some aspects of naturally occurring regenerative phenomena and implement these in artificial organ construction.

LEARNING FROM NATURAL REGENERATIVE MECHANISMS

Obviously, not all details of naturally occurring regenerative phenomena have been revealed. In that case artificial tissue construction would be redundant; however, several key aspects of some of these phenomena have been described and potentially hold some information on how to yield functional artificial tissue. Regeneration of extremities, such as the limb in the salamander, is an example of complex tissue regeneration that has been studied in great detail and serves well as an instance for some of these considerations.[3-5,25-28]

The regenerative process of the salamander limb falls into three non-discrete steps: wound healing, blastema formation, and regrowth.[3-5] Within the first couple of hours following amputation of a limb, the wound is sealed with a wound epidermis by migrating cells from the adjacent epidermis. The wound epidermis thickens and becomes several cell layers thick and then forms an apical epidermal cap with a protective outer surface and inner layers that anatomically and functionally resemble the apical ectodermal ridge formed during vertebrate limb development.[29] Signalling from the wound epidermis as well as neurotrophic signalling from severed nerves induces dedifferentiation of differentiated cells adjacent to the amputation site, leading to the formation of a structure termed a blastema within 1–2 weeks containing dedifferentiated cells with varying origin (e.g. connective tissue, muscular tissue, bone, and nerves). Finally, dedifferentiated blastema cells proliferate, redifferentiate, and regrow just part of the missing limb. The conclusion of this mechanism is that a process very similar to embryonic development can be initiated if the right factors are present. In the case of the limb, these are signalling from the wound epidermis, neurotrophic signalling from severed nerves, and the existence of dermal fibroblasts with a different positional identity. If any of these factors are removed, the regenerative process comes to a standstill. On the other hand, if these three factors are expressed artificially it is possible to induce limb regeneration at uncommon places and produce ectopic limbs.[25] The implications of this in an artificial tissue context is that it may not be crucial to construct an anatomical replica of the final organ of interest but rather focus on building a meshwork of cells that can be stimulated to undergo differentiation and growth in a process that recapitulates embryonic development of the organ. This approach has already been implemented in 4D bioprinting, in which printed objects can change functionality and shape after printing by the application of an external stimulus.[30]

Another important aspect of intrinsic limb regeneration is the origin of cells taking part in regeneration. It has long been speculated that blastema cells represent true pluripotent stem cells with the potential to differentiate into any cell type in the regenerating limb. However, in 2009 Kragl et al.[26] demonstrated, using GFP+ transgenic axolotls, that most cell types involved in limb regeneration are lineage restricted. The lesson from this is that artificial tissue endeavours are likely to have a higher chance of being successful if relying on multiple cell types that can differentiate to the exact type of cells needed rather than a single homogenous population of pluripotent stem cells.

CONCLUSION

In terms of tissue regeneration, it is stimulating to think that we have both examples of intrinsic tissue repair found in nature as well as a multitude of toolsets to construct artificial tissues possible with 3D bioprinting being the most sophisticated method to date. Combining the knowledge from natural phenomena with the ingenuity of biomedical engineers it is not unlikely that anatomically correct tissue constructs can be generated within a reasonable timeframe but also constructs that function just as well as their endogenous counterparts.

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EFFICACY OF THE ARTIFICIAL URINARY SPHINCTER ZSI 375 FOR TREATMENT OF POST-RADICAL PROSTATECTOMY INCONTINENCE IN PATIENTS WITH INTRINSIC SPHINCTER DEFICIENCY: A PRELIMINARY STUDY

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ABSTRACT

Objectives: To evaluate the efficacy of the ZSI 375 artificial urinary sphincter (AUS) (Zephyr Artificial Sphincter, Geneva, Switzerland) for the treatment of patients with post-radical prostatectomy urinary incontinence.

Methods: This was a retrospective, non-randomised, multicentre study open to patients with moderate-to-severe urinary incontinence due to intrinsic sphincter deficiency after radical prostatectomy. Efficacy and safety was evaluated on continence status, complications, and surgical revision.

Results: Twenty-seven patients were recruited between September 2013 and April 2016 and followed up to April 2017. Mean age was 67.70 years old (range: 55–78). Twenty-six patients (96.30%) presented with incontinence post-radical prostatectomy and one patient (3.70%) after post-radical prostatectomy plus adjuvant radiotherapy. Follow-up ranged from 12 months–42 months (mean: 27.11). The success rate was 88.90% after 12 months follow-up, 94.12% after 24 months follow-up and 83.33% after 36 months follow-up. Three patients (11.11%) presented with a scrotal infection, two patients (7.41%) suffered a urethral erosion, and two patients (7.41%) had a mechanical failure. The revision rate was 22.22% (six patients).

Conclusion: The follow-up time of this preliminary clinical study was long enough to demonstrate that the ZSI 375 AUS offers a good rate of continence to patients with urinary stress incontinence.

INTRODUCTION

The artificial urinary sphincter (AUS) AMS 800™ (American Medical Systems, Minnetonka, Minnesota, USA), first introduced in 1973, is regarded as the gold standard for the treatment of post-prostatectomy urinary incontinence (UI).[1,2] American Medical Systems proposed five different versions of the device.[3] The last version of the AUS, the AMS 800, was introduced in 1983 and it is still in use today. However, despite good long-term outcomes,[4,5] the preparation and the procedure remain complex with a risk of complications such as erosion, infection, and mechanical failure ranging from 8–45%.[6,7]

Introduction of the antibiotic coating InhibiZone® had no significant impact on AUS infection or explantation rates.[8] To ease AUS preparation and the procedure, the ZSI 375 AUS (Zephyr Artificial Sphincter, Geneva, Switzerland) has been developed (Figures 1 and 2). Manufactured mainly from medical grade silicone rubber, it is a one-piece device made up of two parts pre-connected by kink-resistant tubing. The adjustable cuff is a moulded curve and can be adjusted around the urethra. The pump and the pressure-regulating tank are grouped into the pump-unit and placed in the scrotum. It has no abdominal reservoir to reduce operating time and to avoid abdominal incision.[9] This paper describes the results of our first experience with ZSI 375 device implanted in 27 male patients with stress UI because of an intrinsic sphincter deficiency (ISD) after radical prostatectomy.

PATIENTS AND METHODS

This was a retrospective, non-randomised, multicentre study open to patients with moderate-to-severe UI due to ISD after radical prostatectomy. Moderate incontinence was defined as the use of three pads per day and severe incontinence as the use of four pads and more per day. The patient evaluation comprised a medical history, analysis of voiding (UI episodes, number of pads used per day), clinical examination, cystoscopy, and urodynamic exam to confirm ISD and to exclude bladder overactivity. The device implanted was the ZSI 375 AUS. It was performed through a perineal and inguinal incision as described by Staerman et al.[9] Patients were discharged as soon as they could void spontaneously. The device was activated 8 weeks after the surgical procedure. Follow-up visits were planned 3, 6, and 12 months and then annually after the procedure. Patients had a clinical examination, urine analysis, bladder ultrasonography, and flow rate measurement.

Continence status was based on number of pads used per day with total continence (0 pads per day), social continence (0–1 pad per day) and improvement (2 pads per day and 50% fewer pads than at baseline). Success was defined as social continence plus improvement. All other continence statuses were considered as incontinence. Revision was defined as the device or one of its components needing replacement or temporary removal. During the follow-up period, the issued pressure could be increased by trans-scrotal injection of saline solution into the compensation pouch to improve continence status. The option to increase the issued pressure *in situ* was not considered as a revision.

RESULTS

Twenty-seven male patients with moderate-to-severe UI were treated by implantation of the ZSI 375 AUS between September 2013 and April 2016 and then followed up to April 2017. Patient age at the surgery ranged from 55–78 with a mean age of 67.70 years old. At presentation, 8 patients (29.63%) had moderate incontinence and 19 patients (70.37%) had severe incontinence: mean 5.15 pads per day (range: 3–10 pads per day).

Figure 1: Image of the ZSI 375 artificial sphincter.

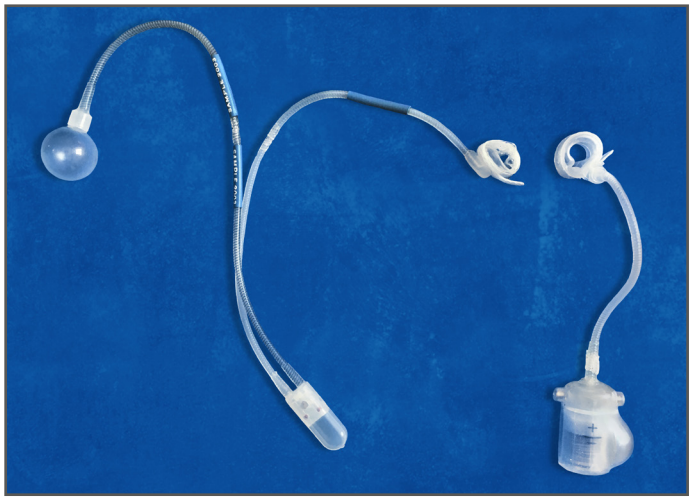

Figure 2: Image of the AMS 800 and ZSI 375 artificial sphincters.

The aetiologies of incontinence were radical prostatectomy in 26 patients (96.30%) and radical prostatectomy with adjuvant radiotherapy in one patient (3.70%). Two AMS 800 devices and one ZSI 375 device had been attempted previously in a total of three patients (11.11%). Three patients (11.11%) were also previously treated for urethral stricture. The ZSI 375 AUS was placed according to Staerman et al.'s[9] surgical technique. The device was activated 8 weeks post-implantation. Twelve months after surgical procedure, 22 out of 27 patients (81.49%) presented a social continence and 2 out of 27 patients (7.41%) were improved. The success rate was 88.90%. Three out of twenty-seven patients (11.11%) were incontinent. Twenty-four months after surgical procedure, 14 out of 17 patients (82.35%) presented with social continence and 2 out of 17 patients (11.77%) were improved. The success rate was 94.12% and one patient (5.88%) was incontinent. Following 36-month follow-up five out of six patients (83.33%) presented social continence, no patient was improved so the success rate was 83.33%. One out of six patients presented incontinence (16.67%) (Table 1). Complications leading to permanent device removal arose in one patient (3.70%) with scrotal infection but without urethral erosion. This occurred within 1 month of AUS insertion and led to immediate removal.

Revision surgery was performed in a total of six patients (revision rate: 22.22%) and the patients enjoyed a social continence after revision. From these six patients, two (7.41%) presented with scrotal infection only, without any urethral erosion associated. The scrotal infections occurred more than 12 months after device insertion and led to an immediate removal of the device. One patient has undergone a new sphincter implantation 3 months after the removal and the second patient waited for 4 months after the removal of his first sphincter. Two patients (7.41%) presented with a urethral erosion. The last two patients (7.41%) from the 'revision group' presented with mechanical failure (Table 2). The patient who underwent pelvic irradiation and the patients with previous AUS did not present any adverse events.

DISCUSSION

Many studies have assessed the outcomes of AMS 800 AUS safety and efficacy but direct comparison is not easy regarding the variability of definitions, perineal, and penoscrotal surgical techniques and selection of patients. The ZSI 375 was designed to simplify AUS preparation and surgical procedure because of the long learning curve needed to achieve mastery.

The capacity to increase or decrease the pressure was an advantage to reach a good rate of continence after device activation. After 12, 24, and 36-month follow-up, our social continence and success rates are in accordance with previous studies about ZSI 375 short-term results, which ranged from 87–94.2%[9-11] and a continence rate for AMS 800 in a 24-month follow-up period of ≤90%.[12,13]

Table 1: Continence rates before and after device implantation.

	Before implantation	≥12 months after implantation	≥24 months after implantation	≥36 months after implantation
Patients, n	27	27	17	6
Pads used/day, n (%)				
None	-	7 (25.93)	5 (29.41)	1 (16.67)
1	-	15 (55.56)	9 (52.94)	4 (66.66)
2	-	2 (7.41)	2 (11.77)	0
3	8 (29.63)	0	0	0
≥4	19 (70.37)	3 (11.11)	1 (5.88)	1 (16.67)
Social continence (0, 1 pad), n (%)	-	22 (81.49)	14 (82.35)	5 (83.33)
Improvement, n (%)		2 (7.41)	2 (11.77)	0
Success, n (%)		25 (88.90)	16 (94.12)	5 (83.33)
Failure, n (%)		3 (11.11)	1 (5.88)	1 (16.67)

n: number of patients.

Table 2: ZSI 375 complications.

Complications	Patients, n (%)
Infections	3 (11.11)
Erosion	2 (7.41)
Mechanical failure	2 (7.41)
Total	7 (25.93)

n: number of patients.

We are in line with the overall continence rate with AMS 800 AUS of 73% and the improved continence rate of 88%.[14,15]

In our study, the short-term complication rate was comparable to that achieved with AMS 800.[12,14,16] ZSI 375 AUS infection occurred in three patients (11.11%). This high rate of infection is not in accordance with AMS 800 device series that range from 0.46–8.5% of cases.[6,12,16,17] Although the implantation technic of the ZSI375 AUS is safe and simple, our infection rate, impacting the revision rate, should be improved with more experience. Urethral erosion was not the most frequently reported complication in the present study. Two patients (7.41%) presented with a cuff erosion, which was in accordance with the AMS cuff erosion rate of 4–12%.[12,14,16,18] Drogo K. Montague[19] proposed that urethral dissection and mobilisation during procedure could be responsible for early cuff erosion and urethral catheter insertion with poor deactivation of the AUS lead to late erosion. Our two erosions occurred 18 months after implantation of the AUS without any catheter insertion. Other factors such as blood perfusion in the urethra could explain our erosions.[20-22] Mechanical failure in our series occurred in two patients (7.41%) 12 and 16 months after the surgical procedure. This was equivalent to the 6% of AMS 800 device mechanical failure with a mean follow-up of 36 months.[6] However, in a report of 530 patients implanted with AMS 800 the 5 years Kaplan–Meier freedom from reoperation was 79.4% for primary cases and 88% for revision surgeries.[7] The follow-up of our series is too short to be compared with the AUS of reference. It should be noted that the dissociation of total failure rate (Table 1) and the failure rate at 12, 24, and 36 months (Table 2) is due to revision. Clemens et al.[17] reported a revision rate after AUS AMS 800 implantation during 5-year follow-up of 50%. Regarding this result, the revision rate of the ZSI 375 must be re-evaluated in the long-term with larger series. Our retrospective study has further limitations; it would have benefited from a prospective approach with patient satisfaction questionnaires and incontinence evaluation using pad weight as recommended by The International Continence Society (ICS) and not pad number.

CONCLUSION

We have described a preliminary clinical result of the AUS ZSI 375. The follow-up time was long enough to demonstrate that ZSI 375 offered acceptable and satisfactory urinary control with the usual AUS adverse events to patients with urinary stress incontinence. The option to adjust pressure *in situ* should reduce the need for surgical revision, but longer-term studies of safety and efficacy, as well as a comparison with other similar devices should be carried out to confirm these good preliminary results.

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DIGITAL TECHNOLOGY HEALTHCARE SOLUTIONS IN AN ERA OF MOVING POPULATIONS AND CHRONIC ILLNESSES: ARE WE BEING REALISTICALLY SMART?

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ABSTRACT

In 2015, the world recorded its highest numbers of international migrants and forced displacement since World War II. With this historic rise in migrants, refugees, and displaced persons around the world, there is a huge risk of chronic illness burden on healthcare systems. Thus, healthcare systems may need to incorporate innovative digital healthcare solutions into their processes and procedures. The purpose of this article is to present the argument that for effective prevention and management of chronic illnesses in ever-increasing migrant societies to be achieved, mobile digital healthcare technologies must be realistically smart and strategically adopted. Beginning with an overview of the current global migration trend, this article considers the implications of this trend for chronic illnesses and the potential for mobile health technologies to support achievement of healthcare outcomes. It highlights three core reasons why digital innovations may be limited as tools for helping to address the global chronic illness challenge and identifies important directions for mobile health technology developers, healthcare professionals, researchers, government and funding agencies, and public health ministries, with a focus on the strategic development and adoption of 'realistically smart' phones. The article concludes with recommendations for research and public health education.

Keywords: Migration, chronic illness, non-communicable, digital health, mobile, smartphone.

INTRODUCTION

All around the world, the high prevalence of chronic illnesses is rapidly shifting the healthcare landscape towards preventive lifestyle behaviours and population health management. Thus, now more than ever, health systems are incorporating into their processes and procedures digital technology healthcare solutions. These are innovative information and computer technologies aimed at achieving one or more health-related goals.[1,2] Common among these goals are: improving access to healthcare information, enhancing the patient experience of healthcare services, improving clinical outcomes, promoting health behaviours and population health, reducing inefficiencies while increasing care quality, and reducing the cost of healthcare.[3] In line with this broad spectrum of goals, digital health technologies involve a wide range of solutions. These include telehealth and telemedicine, mobile health (mHealth), and health information technologies.[3] Some of these solutions (e.g. mobile medical apps) may target specific consumers such as patients and caregivers. Others (e.g. telehealth) may aim at enhancing remote communication, monitoring, and feedback between patients and healthcare professionals (HCPs). Yet still others (e.g. electronic health records) may target information gathering for health administrators, payers, digital health designers, and other stakeholders in the health industry.

Indeed, over the years many healthcare providers and organisations have given increasing consideration to digital healthcare technologies as

tools that hold promise for increasing healthcare outcomes while reducing costs.[2,4-6] Particularly in the area of chronic illnesses, mobile digital devices and software applications (apps) are being explored as ways of achieving both behavioural and clinical outcomes.[7,8] Nonetheless, healthcare systems continue to battle important issues (e.g. inequitable access to healthcare) fundamental to the majority of chronic illnesses that most burden these systems.[9] With the current historic rise in migrants, refugees, and displaced persons around the world, there is an even greater risk of chronic illness burden on healthcare systems.[9]

The purpose of this article is to present an argument to the effect that for effective prevention and management of chronic illnesses in the ever-increasing migrant societies to be achieved, mobile digital healthcare technologies must be realistically smart and strategically adopted. It begins with an overview of the current migration trend around the world. How this trend may contribute to the global burden of chronic non-communicable illnesses, such as diabetes, hypertension, cardiovascular disease, cancers, and mental disorders is then outlined. Evidence of risk factors and prevalence of chronic illnesses in migrants is also highlighted. Next, the paper reviews the role and effectiveness of mHealth technologies in promoting outcomes in chronic illness. This is followed by an outline of three core reasons for which existing mHealth technologies may be limited in effectively tackling any probable chronic illness epidemic[9] as a result of the increasing migration. Finally, important directions for mHealth technology developers, HCPs, researchers, government and funding agencies, and public health organisations are identified, with a focus on the strategic development and adoption of 'realistically smart' phones (RSPs). The paper ends with recommendations for research and public health education efforts.

GLOBAL MIGRATION TRENDS

In 2015, the world recorded its highest ever number of international migrants; 244 million people worldwide moved from their countries of origin to other countries.[10,11] Compared with the 232 million migrants in 2013, 12 million more people migrated in 2015.[10] By the end of the same year, >1.2 million people, largely from Syria, Afghanistan, and Iraq, had sought asylum within the European Union (EU), reflecting >50% the number recorded in 2014 (563,000). Indeed, this is the highest level of forced displacement recorded worldwide since World War II.[10] Unfortunately, while these figures are appalling, they are predicted to further rise in the coming years, as the United Nations (UN) and other world organisations struggle to achieve lasting solutions to the conflicts and persecutions in the Middle East, South Asia, and also parts of Africa such as Nigeria.[12] Within countries, internal migration is also reported to be on the rise.[10] This historic migration trend suggests that many societies are getting increasingly pluralistic and complex. First, there is the presence of various ethnocultural groups in receiving countries and internal regions. Secondly, there is increasing diversity in language, health, educational, religious, and socioeconomic needs of the various ethnocultural groups. Thirdly, and as a matter of urgency, host countries may need to develop and/or amend policies to cater for the health and socioeconomic needs of migrants and for the diversity in needs.

MIGRATION AND CHRONIC ILLNESSES

Whether internal or external, migration generally brings with it an increased potential for infectious disease spread.[9] Yet, with the massive influx of migrants in developed and developing countries, healthcare systems may record higher incidence and prevalence of long-term non-communicable illnesses.[13] Firstly, the steep rise in populations may greatly reduce the capacity of available healthcare resources and services[9] to identify at risk persons, provide timely preventive interventions, and efficiently manage known chronic illness cases for both migrants and residents. Secondly, the surge in language barriers may limit access to healthcare and health-related socioeconomic resources (e.g. housing, employment, and education), particularly for migrants.[14-18] These two fundamental issues facing contemporary healthcare systems may lead to the onset and progression of chronic illnesses in diverse ways. These include increasing waiting times for accessing health screening services, increasing stress from limited access to pertinent socioeconomic resources, and promoting health risk behaviours including smoking, unhealthy eating habits, sedentary behaviours, and alcohol and substance abuse. In addition, they may lead to suboptimum adherence through poor patient–HCP communication and poor understanding of treatment prescriptions. In chronic illnesses, non-adherence to medications and lifestyle recommendations is a major determinant of illness progression.[19,20] Furthermore, language barriers may

reduce access to food labels, which are important for making informed decisions about foods. Inability to do so could have a negative impact on diet quality and hence health. Figure 1 illustrates a theorised model of the relationships between the influx of migrants and prevalence of chronic illnesses.

RISK FACTORS AND PREVALENCE OF CHRONIC ILLNESSES AMONG MIGRANTS

Compared with non-migrants, a number of studies have found a high prevalence of chronic non-communicable illnesses and related risk factors among migrants.[13] These factors include communication difficulties (due to language differences), poor employment, and poor social status.[21-23] Post-migration stress has also been identified as one of the most consistent factors associated with depression, post-traumatic stress disorder, and generalised anxiety disorder.[22] Among resettling migrant women, studies suggest an increased risk of experiencing somatisation and low birth-weight infants.[24] Immigrant women have also been found to be at increased risk of social vulnerability[15] and at more than twice the risk of Canadian-born women for postpartum depression.[21] Often, women migrate during their childbearing years and may lack knowledge about the rights and services available to them.[15,21,25] Among migrant men, a study of Gulf migrants (including emigrants from Kerala, India, to the Gulf countries) and non-migrants in India found that a majority of health risk factors for non-communicable illnesses were significantly higher in migrants.[26,27] With the exception of alcohol use, the prevalence of tobacco use, physical inactivity, poor diet habits, chronic illness history, less sleep, and longer work hours were significantly higher in migrants than non-migrants.[26] Accordingly, hypertension and abdominal obesity were significantly more prevalent among migrants than non-migrants.[26,27] A similar study found that participants with a history of migration (largely from Kerala to the Gulf countries) had a higher prevalence of diabetes, hypertension, and cardiac problems.[28]

Chronic illnesses currently constitute the most prevalent and costly of all health problems in both developed and developing countries,[4,13,29] with morbidity and mortality from chronic illnesses exceeding that from infectious diseases.[29] Coupled with the generally strained healthcare facilities and resources worldwide, it is of utmost importance that smart yet realistic mHealth innovations are adopted to help address the core health needs of contemporary pluralistic societies. Specifically, the need for: i) innovative language-bridging solutions; ii) increased access to preventive healthcare information and services irrespective of socioeconomic status; iii) wider promotion of healthy lifestyles and behaviour change techniques; iv) enhanced self-monitoring and self-management behaviours; and v) increased access to essential health-related socioeconomic resources (housing, education, employment, support networks). This task may seem enormous and costly, yet countries that have embraced pluralism and fostered integration of migrants via effective policies and programmes, referred to as multicultural countries, tend to report higher health as well as economic status.[30]

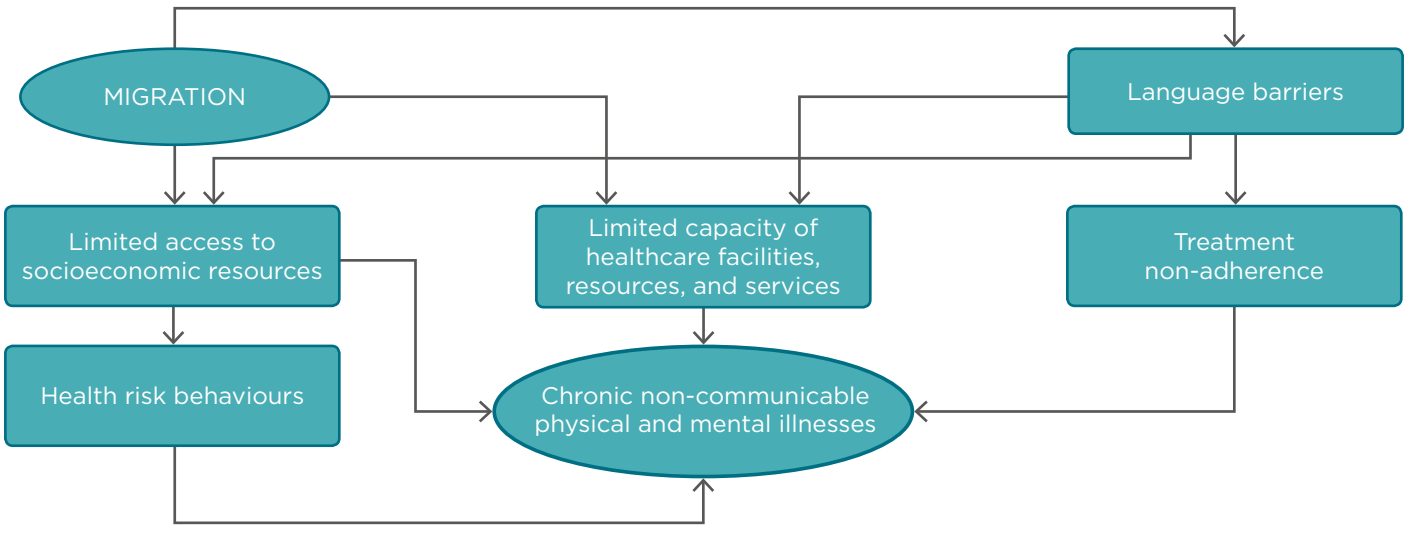

Figure 1: Hypothesised relationships between migration influx and chronic illnesses.

THE ROLE AND SUCCESS OF MOBILE DIGITAL INTERVENTIONS IN HEALTHCARE

Over the last two decades, well over 100,000 health and medical apps have been designed for use on mobile smartphones, tablet computers, laptops, and wearable devices.[31] Presently, consumers can use mHealth technologies to access a wide range of clinical and public health services.[32] For instance, smartphones together with wearable wireless sensors can be used to measure, monitor, and manage vital body signs (e.g. blood glucose levels), which are critical in chronic illness management.[8,32-34] With the increasing movement of populations to developed and developing countries, where resources are typically fewer and concentrated in urban areas, mHealth devices and apps may offer opportune avenues for meeting the critical health needs of modern societies.[7,34,35] Their capacity for continuous interactive wireless communication means that health interventions can be accessed independent of geographic location.[34,35] Intervention content can also be delivered and updated consistently across users.[35] These devices allow users to privately and anonymously access personalised interventions at their own convenience and pace.[34,35] As interventions are accessed independent of expensive therapy consultations, mHealth innovations are potentially cost effective.[34] Furthermore, they have the potential to revolutionise healthcare research, data analysis, and service delivery via real-time data collection.[35]

Empirically, a recent systematic review of 30 randomised controlled trials (RCTs) found that telemonitoring interventions significantly reduced the odds of mortality and hospitalisation among people with heart failure, compared to standard post-discharge care.[36] Telemonitoring interventions may include HCPs monitoring symptoms through live telephone calls with patients. Patients may also enter data on signs and symptoms into an electronic communication device (e.g. smartphone) to be downloaded and viewed later by HCPs.[37] Among a cohort of 17,025 veteran patients, a home telehealth programme was found to reduce the number of bed days of care by 25% and the number of hospital days by 19%. The average cost per patient ($1,600.00 per annum) was reported to be substantially lower than nursing home care.[38] A recent comparison of patients on the programme to a matched control group indicated further reductions in hospitalisations, healthcare costs, and mortality rates for programme participants.[39] In men who have sex with men, a tailored and interactive text-messaging intervention (a set of messages addressing nine topic areas) significantly improved medication adherence, human immunodeficiency virus (HIV) knowledge, and social support post-intervention, whereas there were significant reductions in viral load and the number of sex partners.[40] Findings of a systematic review of the effectiveness of communication technologies among adolescents with diabetes showed that 10 of 18 studies found positive improvements in blood glucose levels, although four studies reported detrimental increases in blood glucose levels.[41] Increased frequency of patient–HCP contact was found in 15 studies.[41] In a recent systematic review of 42 controlled trials of mobile technology-based health interventions, modest benefits were found for SMS reminders on appointment attendance.[1] In developing countries, a review of mHealth intervention studies (largely SMS messages) reported enhanced mass delivery of health information, remote patient monitoring, self-management, and data monitoring systems for diagnosis and treatment.[42] A similar review including nine RCTs found that mHealth interventions (e.g. mobile phone-based interactive software plus management feedback) positively improved chronic illness outcomes. These included clinic attendance, medication adherence, pulmonary function (in asthma patients), emergency visits, hospitalisations, and cost-effectiveness.[43]

Many of these reviews indicate high heterogeneity in study design (e.g. from RCTs to pilot studies[42]) and quality. Yet, generally, the extant literature over the years seems to assert that mHealth innovations are potentially effective strategies for promoting healthcare.[1,7,32-43] In certain countries, some mHealth technologies have received approval from government agencies such as the US Food and Drug Administration (FDA).[32]

MOBILE DIGITAL TECHNOLOGIES AND CONTEMPORARY HEALTH NEEDS OF PLURALISTIC SOCIETIES

Despite their potential healthcare and public health benefits, the question remains of whether innovations are being realistically leveraged to the probable epidemic of chronic illnesses[9,13] facing contemporary societies. It is argued that unless mHealth is fully accessible to migrants, as well as average residents, ethnocultural minorities, and the

most vulnerable across societies, this could breed a digital divide that may severely intensify the global health challenge of socioeconomic inequalities.[35,44] As it stands, it is worth asking how many of the world's population can afford a smartphone. On one hand, most smartphones are overly sophisticated, replete with superfluous apps, many of which consumers may hardly make use of, and are unreasonably expensive. On the other hand, low-end mobile phones tend to lack the basic features and capacity for internet-based healthcare interventions. There is also the question of what percentage of the world's population has access to a wireless internet connection? Even in the UK, reports indicate that about a fifth of the adult population has absolutely no home internet access.[35] This suggests that in low-income countries, more people may have no access to internet-based health interventions. Furthermore, although mHealth innovations are penetrating most modern societies, few public health education efforts are aimed at supporting consumers to effectively use these technologies. Among socioeconomically disadvantaged groups, who generally record lower educational levels, this may also contribute to a digital divide and wider gaps in health inequalities.[35,44] Lack of mHealth education may further heighten migrants' fears that data could lead to deportation, xenophobic, or discriminatory attitudes.[45,46] So what is the way forward in effectively harnessing mHealth technologies? Rather than simply increasing access, a strategic and multidisciplinary effort is required.

DIRECTIONS FOR DEVELOPMENT AND ADOPTION OF 'REALISTICALLY SMART' MOBILE PHONES

To equitably promote clinical outcomes and public health across the globe via digital health, three things need to be critically considered. First, mobile phones, the most widely available technology, should be designed to realistically address the critical health needs of contemporary pluralistic societies. More practically, RSPs, in addition to the basic features of a mobile phone should have: i) capacity for wireless internet connection; ii) a built-in/affixed sensor for measuring multiple vital health information (e.g. temperature, blood pressure, blood oxygen, glucose levels, and heart rhythm); and iii) a core set of medical and health apps preinstalled. The core set of apps must be approved by appropriate institutions (e.g. food and drug agencies) and help to bridge language barriers, provide clinical diagnosis and treatment support for common health problems, encourage healthy lifestyles and provide evidence-based techniques for risk behaviour change, support self-monitoring and self-management behaviours, and alert users of vital health-related opportunities for socioeconomic resources. For example, RSPs could include an app that easily translates words, sentences, and inscriptions on snapped images from dominant languages into numerous local dialects and vice-versa. This could particularly support migrants and linguistic minority groups to better engage with health information and communicate with HCPs. The RSP could also include an app that picks up all job openings and socioeconomic opportunities within the residing country of the user daily, and display this information in a selected language option.

Secondly, just as going to school is a human right and as such governments strive to bring education to all, so is access to health a human right. Therefore, in this era of digitisation, governments must equally strive to partner with phone developers, app designers, HCPs, and healthcare researchers with a goal to invest in affordable RSPs and gather evidence of their influence on clinical outcomes and public health. Furthermore, the onus lies on governments to make wireless internet widely and freely accessible so that all persons, irrespective of socioeconomic status or geographic location, can access essential internet-based health interventions.

Finally, to prevent creating a new cause of health inequalities and rather promote digital health for all persons, there must be a revolution in public health education efforts across the globe. Public health ministries need to focus on actively training the public to use information and communication technologies. Such education efforts should be drastic enough to ensure that all persons, irrespective of adult age or socioeconomic background, have the knowledge and skills required to connect to the internet, access digital health interventions, take the necessary health actions at any stage of the care continuum, and seek support with security and privacy concerns. It is only when this revolution in public health education efforts parallels the revolution in digital technology healthcare solutions can maximum uptake of these innovations be realised.

LIMITATIONS

Few studies have considered the prevalence of chronic physical illnesses compared with mental illnesses in migrant populations and subpopulations (e.g. reproductive health problems in women migrants). Despite the numerous evaluation studies in mHealth, evidence of long-term, large-scale effectiveness and cost-effectiveness, as well as continued engagement with mHealth programmes, is still not uniformly conclusive across disease areas, innovation types, subpopulations, and countries. While it is largely asserted that developing countries may benefit greatly from mHealth, efficacy, cost-efficacy, and practicality (e.g. concerns about data security) are extremely lacking in these regions.

CONCLUSION

As the world grows progressively diverse due to the continuous rise in migration, there is an increased potential for development of chronic illnesses through reduced access to healthcare information and services and socioeconomic resources. Mobile digital healthcare technologies, owing to their availability and functionality, have the capacity to support achievement of clinical and public health outcomes. Nonetheless, it is important that they are realistically designed and strategically adopted to avoid a digital divide, whereby those most in need of healthcare education and services are the least likely to access digital health interventions. It is recommended that government agencies support intercultural research that provides rigorous evidence for mHealth innovations across regions and sub-regions. Community platforms for updating especially low socioeconomic-status groups on mHealth research (including phenomenological reports from mHealth users) and for soliciting input for further research may help alleviate security concerns and boost adoption of these potentially beneficial technologies.

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COMPREHENSIVE ASSESSMENT AND PHARMACOTHERAPY OF PATIENTS WITH CHRONIC PAIN SYNDROME DUE TO DIABETIC NEUROPATHY AND POSTHERPETIC NEURALGIA

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ABSTRACT

Management of chronic pain, usually associated with comorbid conditions, remains challenging. General practitioners, together with multidisciplinary teams of specialists, play an important role in diagnosing and treating patients with chronic pain of different origin. This article outlines the main mechanisms underlying chronic nociceptive and neuropathic pain and describes some helpful techniques to initially evaluate and regularly monitor pain. Pharmacologic treatment options, including their benefits and adverse effects, with particular emphasis on the management of diabetic neuropathy and postherpetic neuralgia, commonly seen in the primary care practice setting, are presented.

Chronic pain, regardless of its cause, is a disease syndrome and, as such, requires a comprehensive, individualised approach to each patient reaching beyond symptom control. Collaboration of physicians (general practitioners, diabetologists, neurologists, and pain specialists), pharmacists, and nurses in the management of patients with diabetic neuropathy and postherpetic neuralgia improves patient safety and contributes to better adherence to therapeutic regimens, which leads to more favourable outcomes and improved quality of life.

Keywords: Chronic pain, diabetic neuropathy, postherpetic neuralgia (PHN), pharmacotherapy, patient management.

INTRODUCTION

Pain is a physiological part of the protective mechanism that allows individuals to survive. In fact, pain evaluation is so important that it has been called the fifth vital sign that needs to be monitored on a regular basis, alongside pulse rate, arterial blood pressure, respiratory rate, and body temperature.[1,2] Since the perception of pain is subjective, there is no particular objective test that can accurately measure the type and intensity of pain experienced by a patient. Therefore, patient self-reporting is usually considered to be a key component of pain evaluation.[1,2] However, these reports must be linked with a detailed medical interview, physical examination, laboratory and imaging test results, as well as with patient scores on standard pain scales.[1] Also, chronic pain needs to be evaluated at regular intervals of time and should be precisely documented in the patient's records to ensure that every member of the treatment team, including general practitioners (GPs), consulting specialists (diabetologists, neurologists, or pain specialists), pharmacists, nurses, rehabilitation therapists, or other providers, has access to current information, which will allow precise monitoring of the patient's response to treatment.[1,3]

In contrast to acute pain evaluation, which is often brief and direct, assessment of chronic pain (nociceptive or neuropathic), particularly if it is combined with different comorbid conditions or remains refractory to standard initial treatment, is very complex. The International Association for the Study of Pain (IASP) defines neuropathic pain as "a pain arising as a direct consequence of

a lesion or disease affecting the somatosensory system".[2] Assessment of neuropathic pain requires multidisciplinary knowledge and experience, co-operation within a treatment team, and a professional relationship with each patient based on mutual trust and respect. Many GPs are in a unique position to establish long-term relationships with their patients that facilitate good communication.[1,3]

INITIAL ASSESSMENT OF PATIENT SUFFERING FROM CHRONIC PAIN

Initial assessment of every patient suffering from chronic pain is focussed on obtaining a detailed history covering basic characteristics of the patient's pain, such as its type (nociceptive versus neuropathic), localisation, frequency, duration, and aggravating and alleviating factors.[1,3] In addition, it is crucial to briefly analyse some other related domains of the patient's life, including his/her physical and psychosocial wellbeing (including family and occupational situation), functional status, comorbidities, mental and emotional status, present or past psychiatric disorders, and current or past substance abuse problems.[1,3]

The physical exam should be focussed on musculoskeletal and neurological systems. Diagnostic studies need to be analysed in comparison to past medical records, if available. A goal of such a comprehensive assessment is a correct diagnosis and a reasonable management plan. It should be emphasised that simple steps, including obtaining a patient's detailed history and performing a thorough physical exam, are often instrumental for detecting potential causes of a patient's pain.[1-3] At this point, knowing the right questions to ask can lead to finding the root cause(s) of the patient's pain, indicating further appropriate directions for diagnosis and treatment. There are several questions that are particularly helpful at the beginning of diagnostic work-up:

- Using a body diagram (front and back), can you point to the exact site of your pain?
- Does your pain spread or radiate? Where?
- What things relieve or worsen it?
- When did the pain begin?
- What was done about it?
- Have you been using any medications or procedures? What kind?
- Did any of those interventions work?
- What medications (over-the-counter or prescription), vitamins, or dietary supplements, are you currently taking?
- Do you drink alcohol?
- Do you smoke?
- Do you use any drugs?

These direct questions, in addition to obtaining precise characteristics of the pain, can also determine whether there are any psychosocial issues that might interfere with a future therapy plan.[1,3] As mentioned before, the physical exam is an integral component of the comprehensive pain assessment. In addition to taking vital signs, it should include evaluation of mental status and mood, posture (e.g. guarding or splinting), neurological status (e.g. sensory or motor dysfunctions), and signs of sympathetic dysfunction or musculoskeletal abnormalities.[1,3]

In general, laboratory and radiographic evaluations are performed in cases in which previous evaluations were inadequate or the patient's quantity or quality of pain has changed.[1,3] Although the experience of pain is subjective, several validated instruments exist, including both one-dimensional and multi-dimensional questionnaires that are designed to help measure the intensity and type of the patient's pain. Since chronic pain requires regular monitoring, any chosen evaluation method, depending on the patient's age and communication/language skills, needs to be used consistently during each follow-up visit.[1,4]

Typical one-dimensional pain scales that are most useful in a daily practice include:

- The Numeric Rating Scale (NRS): the patient rates his/her pain intensity on a scale of 0 (no pain) to 10 (worst imaginable pain). Patients may also use the scale at home to record pain intensity at different times in relation to their activity levels and applied treatments, which should be documented in a pain journal. This kind of documentation is very helpful in determining pain patterns.[1,4]
- The Visual Analogue Scale (VAS): allows a more descriptive rating of pain, since the patient marks the line at the point between 0 and 10 that best describes his/her pain intensity.[1,4]
- The Wong–Baker FACES® pain rating scale: uses facial expressions to depict different degrees of pain intensity. It is particularly useful for assessing children and cognitively impaired or elderly patients (who often have some language barriers or difficulty communicating verbally).[1,4]
- The Verbal Rating Scale (VRS).[1,4]

Some other validated, multi-dimensional pain assessment tools include:

- The McGill Pain Questionnaire (MPQ): covers three dimensions of pain: sensory, affective, and evaluative. The patient selects words that best describe the quality of their pain from a pre-defined list, such as burning, shooting, throbbing, aching, and pins and needles.[5]
- The Brief Pain Inventory-Short Form (BPI-SF), a nine-item self-report questionnaire (scored on a 0–10 scale), examines pain severity and its interference with emotional and physical functioning. For the BPI-SF, the arithmetic mean of four pain severity items yields a general pain severity score; the mean of seven interference values yields a general interference score and other items inform about the pain location and the use of pain relief strategies.[6]
- The Douleur Neuropathique 4 (DN4) questionnaire: a tool aimed at screening neuropathic pain that is used to help distinguish between neuropathic and nociceptive pain. It is made up of seven items related to pain symptoms and three items related to physical evaluation. DN4 is correlated with the medical diagnosis.[7]

These tools can provide a comprehensive profile of the patient's pain, whilst also taking into consideration emotional distress, and the degree to which the individual can function in various domains of life. In addition, several multi-dimensional tools exist, for example the Neuropathic Pain Scale (NPS), Treatment Outcomes of Pain Survey (TOPS), and the Oswestry Disability Index (ODI). These can be usedto assess specific types of pain or to assess pain in terms of functional status among large groups ofpatients. However, these tools are usually reserved for the research setting.[1,4]

NOCICEPTIVE PAIN PATHWAY

In order to better understand the development of two main types of pain, nociceptive and neuropathic, with their respective specific symptoms commonly experienced by patients, it is important to briefly review the normal anatomy and physiology of pain pathways. Nociceptive pain pathways allow individuals to perceive pain and avoid further injury after a noxious stimulus, such as heat, cold, or mechanical trauma activates receptors in tissues (e.g. skin). This noxious stimulus is then transduced into incoming signals that travel toward the central nervous system along small, unmyelinated C fibres. The C fibres make synaptic connections in the dorsal horn of the spinal cord. The second-order neurons cross the midline and travel up the contralateral spinal tract to the thalamus and other parts of the brain so that the pain sensation can be perceived.[1,4] It should be emphasised that the intensity of the incoming noxious signals can be modulated by a descending inhibitory tract. For instance, opioids bind to specific receptors in the brain and cause an increase in descending inhibition, to reduce the incoming pain signals. This, in turn, allows patients to decrease the degree of pain that they feel.[1,4]

ALLODYNIA AND HYPERALGESIA

The normal anatomy and physiology of a pain pathway can be depicted as the stimulus intensity increasing from a light touch to a pinch causing a person to start perceiving pain, which can be rated in intensity on a numeric scale from 0–10. At this point, injured tissues become sensitised, and the pain perception is often greater.[1,4] For example, upon a non-painful stimulus, such as a light touch, the injured area can cause a mild-to-moderate pain sensation called allodynia. Similarly, a mildly painful stimulus, such as a light pinch, may cause in these circumstances severe pain, called hyperalgesia. This response is normal to ensure protection of the injured area until it heals. However, persistence of the allodynia and hyperalgesia after the tissue injury has healed is characteristic of neuropathic pain.[2,4]

NEUROPATHIC PAIN PATHWAY

The management of chronic neuropathic pain, whether peripheral or central, is clinically challenging and often frustrating because of the complex pathophysiology of neuropathic pain transmission.[2,4] Neuropathic pain is a common form of chronic pain, which results from damage to the peripheral nervous system, often due to diabetic neuropathy, postherpetic neuralgia (PHN), or chronic lumbar radiculopathy.[1,4] In addition, neuropathic pain can also be caused by injury to the central nervous system, which can occur in: stroke, spinal cord injury, and multiple sclerosis (MS).[1,4]

In general, symptoms of neuropathy can be categorised as:[2,8]

- Negative, resulting in partial or total loss of sensation
- Positive, including various types of abnormal or unpleasant sensations (due to normal stimuli), such as:

- Dysaesthesia: burning/stabbing
- Paraesthesia: tingling or prickling (pins and needles)
- Allodynia: painful sensation due to a mild stimulus; not normally associated with pain
- Hyperalgesia: exaggerated response to painful stimulus

CAUSES, MECHANISMS, AND SYMPTOMS OF NEUROPATHIC PAIN

Neuropathic pain represents a chronic condition that is often difficult to diagnose and to treat due to complex interrelations between the causes, mechanisms, and symptoms of various diseases underlying this type of pain.[2,8] In neuropathic pain, neurons in the spinal cord often become hyper-responsive, leading to excessive pain sensation (hypersensitivity) and extension of pain beyond the region of original damage due to injury or disease. Moreover, changes in sodium and potassium channels after a nerve injury increase cell membrane excitability and cause paraesthesias.[8] The sensitisation of both central and peripheral neurons alters perception of pain and increases sensitivity to temperature and to touch. Peripheral nerve injury may also reduce inhibitory cerebral influence on dorsal horn neurons.[1,8]

DISTAL SYMMETRIC POLYNEUROPATHY

Distal symmetric polyneuropathy (DSPN) is the presence of symptoms and signs of peripheral nerve dysfunction in patients with diabetes (after the exclusion of other causes). DSPN has a multifactorial pathogenesis (e.g. oxidative and inflammatory stress associated with metabolic disorders) and is the most common diabetic neuropathy. DSPN that involves small-diameter Type A delta and Type C sensory fibres usually results in symptoms such as painful paraesthesia, which is perceived as burning, stabbing, crushing, cramping, or aching sensations (often aggravated at night).[9] These symptoms typically develop in the hands in a glove-like distribution. In addition, paraesthesias and the loss of sensation in the feet, in a sock-like distribution, creates a risk of developing foot ulcers that can be complicated by gangrene.[9] Moreover, frequently coinciding decreased ankle and knee reflexes, weakness of foot muscles, and impaired proprioception can affect the gait, and cause sensory ataxia. Furthermore, many patients experience symptoms related to the autonomic nervous system, such as resting tachycardia, orthostatic hypotension, bladder or bowel dysfunction, anhidrosis, and sluggish reaction of pupils to light.[9]

A neurologic evaluation is necessary to determine severity and to rule out other causes of neuropathy. Management of each diabetic patient with DSPN, in addition to his/her appropriate blood glucose levels, lipid levels, and blood pressure control, needs to take into consideration any medical or mental comorbidities, possible treatment side effects, and some other individual factors (e.g. socio-economic).

PHARMACOTHERAPY FOR DIABETIC NEUROPATHY

Pharmacotherapy for diabetic neuropathy includes various medications from the following groups:[9,10]

- Antidepressants
  - Tricyclic (e.g. amitriptyline, imipramine, desipramine)[11]
  - Selective serotonin/norepinephrine reuptake inhibitors (SNRIs) (e.g. duloxetine, venlafaxine)
  - Selective serotonin reuptake inhibitors (e.g. paroxetine, citalopram)
- Anticonvulsants (e.g. gabapentin, pregabalin, sodium valproate, phenytoin, carbamazepine)
- Non-steroidal anti-inflammatory drugs (e.g. ibuprofen, naproxen)
- Analgesics
  - Topical (e.g. lidocaine gel [5%], capsaicin)
  - Opioids (e.g. tramadol, morphine sulfate, or oxycodone). It should be emphasised that add-on therapy with opioids may be necessary in some patients, who failed to respond to other treatments; however, in such cases, a referral to specialised pain clinics and close medical supervision are recommended).

A choice of medication from any of these groups (or combination therapy) for the management of diabetic neuropathy should be made after considering the individual patient's clinical status, goals, and needs.[9,10] Unfortunately, adverse effects of these medications (see next section) are common, and thus the GP's familiarity with the most typical side effects is important in order to achieve realistic treatment goals whilst maintaining the patient's comfort. In addition, educating the patients about some of the possible adverse effects of the otherwise beneficial therapy may help

them to be more motivated and adherent to the treatment regimen.[10,12]

POSSIBLE ADVERSE EFFECTS OF MEDICATIONS USED IN TREATMENT OF DIABETIC NEUROPATHY

In general, the anticonvulsants and antidepressants commonly used for the treatment of DSPN decrease neuronal excitability, leading to a reduction of pain. However, they are not free of adverse effects.[11] Second-generation anticonvulsants (e.g. gabapentin, pregabalin) can cause somnolence, dizziness, fatigue, headache, confusion, diarrhoea, nausea, weight gain, peripheral oedema, and thrombocytopenia/neutropenia.[12] First-generation anticonvulsants (e.g. sodium valproate) can cause tremors, hepatotoxicity, peripheral oedema, weight gain, hair loss, pancreatitis, and interactions with tricyclic antidepressants.[12] SNRIs (e.g. venlafaxine, duloxetine) can cause poor appetite, weight loss, insomnia, drowsiness, dizziness, fatigue, headache, mydriasis, nausea/vomiting, and urinary retention.[12] Tricyclic antidepressants (e.g. nortriptyline, amitriptyline) can cause dry mouth, constipation, blurred vision, dizziness, drowsiness, and increased heart rate.[12]

ADDITIONAL MEDICATIONS BEING CONSIDERED FOR NEUROPATHIC PAIN

Current recommendations of the American Diabetes Association (ADA) are designed to help primary care physicians focus on effective management of neuropathies in patients with Type 2 diabetes mellitus (T2DM).[13] In addition to recently completed or ongoing clinical trials in the area of diabetic neuropathy, investigating SNRIs (e.g. duloxetine) or anticonvulsants (e.g. pregabalin) in different diabetic populations, there is still a growing need to find more effective therapeutic approaches to DSPN that are less toxic. Some of them are briefly described below.

Alpha-Lipoic Acid

Alpha-lipoic acid (ALA) is an antioxidant that, according to a multicentre placebo-controlled trial, was found to cause short-term symptomatic relief of neuropathy symptoms in patients with T2DM (its recommended dose is 600 mg/day for 3 weeks).[14]

Actovegin

Actovegin is a deproteinised derivative of calf blood, containing inorganic substances (electrolytes and trace elements), organic components (amino acids, oligopeptides, nucleosides, and glycosphingolipids), and inositol phospho-oligosaccharides that may enhance insulin actions (central or peripheral). Based on the randomised clinical trial by Ziegler et al.,[15] it was found that upon therapy with actovegin (e.g. intravenous [2,000 mg/day], and then oral [1,800 mg/day] administration over 5 months) both sensory functions and quality of life of patients with T2DM and polyneuropathy were improved.

Aldose Reductase Inhibitors

Aldose reductase inhibitors (e.g. alrestatin, sorbinil, tolrestat, and epalrestat) block the rate-limiting enzyme in the polyol pathway, which is activated by hyperglycaemia. However, these medications are not currently US Food and Drug Administration (FDA) approved. Epalrestat, which reduces intracellular sorbitol accumulation, has been shown to improve motor and sensory nerve conduction as well as diabetic neuropathy symptoms (e.g. pain, hyperaesthesia, numbness, coldness of extremities, dizziness, and orthostatic fainting) compared to baseline or placebo. At present, epalrestat is marketed only in Japan and its recommended dose is 150 mg/day for 12 weeks.[16]

POSTHERPETIC NEURALGIA

Brief Aetiology and Clinical Manifestations of Herpes Zoster

Herpes zoster (or shingles) reflects the reactivation of the varicella-zoster virus. After the primary varicella infection (chickenpox), the virus remains quiescent in the dorsal root ganglia, often for many decades, kept in check by the normal immune system.[17,18] However, with ageing, or due to immunosuppression associated with illness, the virus can multiply.

The clinical manifestations of herpes zoster can be divided into three phases:

- Pre-eruptive or preherpetic neuralgia
- Acute eruptive phase: unilateral patchy erythema, herpetiform vesicles that rupture, crust, and involute, and severe pain (acute herpetic neuralgia)
- Chronic or post-herpetic neuralgia[17,18]

When the pain associated with an acute herpes zoster outbreak persists for more than 2 months, it is called PHN, which is caused by viral-mediated damage to peripheral afferent neurons. Immediate

treatment with antiviral medications can reduce the severity of zoster and the risk of future PHN. However, this neuralgia may persist for a few years.[17,18]

The pain of PHN is usually severe and is often characterised by constant aching or burning, electric shock-like sensation, allodynia, and hyperalgesia.[17,18]

Treatment of Postherpetic Neuralgia

The recommended medications, used to treat PHN include:[17,18]

- Antidepressants (tricyclic): nortriptyline (which has fewer cardiac side effects), desipramine, amitriptyline
- Anticonvulsants: gabapentin, pregabalin
- Opioids (only in selected, severe cases; during acute phase of herpes zoster)
- Lidocaine topical preparations
- Capsaicin topical preparations

The adverse effects of medications used in the therapy of PHN and diabetic neuropathy are identical. Similarly, the side effects of lidocaine and capsaicin topical preparations, used in the management of both of these conditions, may include abnormal skin sensations (e.g. burning or change in hot or cold sensation), redness, or swelling at the application site.[17,18] Since there is only limited evidence to guide the selection of different pharmaceutical agents, the adverse effects of these medications should help with choosing the most appropriate and available agent by considering the benefits and risks for each individual patient. Furthermore, the FDA has approved a live attenuated varicella-zoster virus vaccine, Zostavax®, that has been used since 2006, and has demonstrated a decrease in the incidence rate of herpes zoster. Zostavax reduces the risk of developing shingles by 51%, and PHN by 67%. It is administered in one dose injection. Currently, Zostavax is approved for use in patients >50 years old, and is considered to be cost-effective.[19]

SUMMARY

Pain assessment is one of the competencies that GPs need to master in order to correctly diagnose and effectively treat patients with chronic pain of different origin. In many cases, chronic pain can be alleviated, at least to some degree, with appropriately targeted therapeutic options, even if the patient may have to try several different modalities before finding one that works best. In clinical practice, two main categories of chronic pain, neuropathic and nociceptive, often overlap. Moreover, many medical comorbidities, mental disorders, stress, or environmental factors (at work and at home) can complicate the management of chronic pain. For these reasons, chronic pain as a disease syndrome, should be addressed within a biopsychosocial, interdisciplinary model of care, which considers each patient's psychosomatic, occupational, environmental, family, and social conditions. Finally, it should be emphasised that a team collaboration between physicians (e.g. GPs, diabetologists, neurologists, psychiatrists, rehabilitation, and pain specialists), pharmacists, and nurses who manage patients with diabetic neuropathy and PHN improves patient safety and contributes to better adherence to medical regimens. This, in turn, leads to more favourable outcomes and better quality of life.

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BENCHMARKING OUTCOMES THAT MATTER MOST TO PATIENTS: THE GLOBE PROGRAMME

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ABSTRACT

Significant variation in health outcomes exists around the world. The International Consortium for Health Outcomes Measurement (ICHOM) has developed Standard Sets of outcomes for important medical conditions and populations to enable outcome measurement and comparision in order to understand variation and stimulate improvement. ICHOM has recently launched a prospective, non-interventional, observational pilot benchmarking programme. This article reviews the pilot methods, timelines, expected outputs, lessons learnt to date, and the next steps. We believe this programme is truly innovative as it will be the first global initiative in Standard Sets benchmarking, provider engagement, and risk-adjustment on the outcomes of care of most importance to patients. It has the potential to bring significant changes to compare the quality of healthcare and health systems around the world and ultimately to improve patient care.

Keywords: Outcomes, benchmarking, standardisation, patient reported outcome measure (PROM), patient-centred, value-based healthcare.

INTRODUCTION TO VALUE-BASED HEALTHCARE

In 2012 the International Consortium for Health Outcomes Measurement (ICHOM) was founded as a non-profit organisation to develop Standard Sets of outcome measures for different medical conditions and populations, as well as drive their adoption by healthcare institutions. It is our belief that the systematic measurement of Standard Sets of outcomes by institutions around the world will enable, for the first time, global outcome comparisons. We think this will catalyse a new wave of innovative learning for healthcare professionals, as institutions will be able to see where the greatest outcomes are being achieved and then learn from the processes that they have resulted from, as well as really inform patient choice. ICHOM has now created Standard Sets of outcomes that matter most to patients for 21 medical conditions, including prostate cancer, cataracts (CAT), hip and knee osteoarthritis (HKO), and stroke amongst many others.

The concept of value-based healthcare[1,2] has been defined by Prof Michael E. Porter, Bishop William Lawrence University Professor, Harvard Business School, Boston, USA, and Prof Elizabeth Teisberg, Full Professor, Dell Medical School, University of Texas, Austin, USA, as the patient outcomes

achieved per dollar expended.[3,4] Outcomes are the results people care about most when seeking treatment, including functional improvement and the ability to live 'normal', productive lives. Current metrics for care across many conditions tend to capture processes and costs and do not measure whether they achieve the outcomes which matter most to patients. The process of developing a Standard Set has been previously described.[5-7] In developing globally-agreed-upon Standard Sets of outcomes for different medical conditions and patient populations, we aim to enable providers to measure the most important outcomes for their patients and then compare themselves, in a consistent manner, with other countries around the world.[8]

The application of performance benchmarking, transparency in reporting, improved performance, and collaborative learning, gained from the processes that the best performers share, ultimately informs patient choice towards value. The ICHOM Global Outcomes Benchmarking (GLOBE) programme was launched in May 2016 and aims to globally compare standardised outcomes between international partners to enable the identification of treatment paradigms that are more effective, leading to improvements in healthcare outcomes. The launch of two prospective, non-interventional, observational pilot studies using the ICHOM HKO,[9,10] and CAT[11,12] Standard Sets which include patient-reported outcome measurement (PROM) instruments, assess the feasibility of collecting outcome data from multiple international institutions, and provide risk-adjusted benchmarks.

Table 1: Five notable initiatives to systematically collect standardised datasets.

Initiative (alphabetically)	Scope	Mechanism of change	Summary of results	Further reading
Cystic Fibrosis Foundation (CFF) Patient Registry	Sub-national, USA (120 CFF-accredited care centres)	PB; PR; BP/G	Since 1995, the annual incidence of *Pseudomonas*, a serious and difficult-to-treat hospital-acquired infection has declined by >14%.	Cystic Fibrosis Foundation[13]
Dutch Surgical Colorectal Audit (DSCA)	National, Netherlands (all Dutch hospitals)	PB	From 2009–2011, the rate of complications and re-interventions after colon and rectal resections improved significantly. Postoperative mortality rates (in-hospital and 30-day) also improved significantly for both procedures.	Van Leersum et al. 2013[14]
European Registry of Quality Outcomes in Cataract and Refractive Surgery (EUREQUO)	Regional, Europe (Surgeons from 15 countries)	BP/G	Between 2009 and 2016, surgeons from 15 countries contributed 2.24 million surgeries to the EUREQUO database resulting in the use of 523,921 cataract extractions in 2012 as a basis for the development of evidence-based guidelines around the cataract surgery process.	Lundström et al. 2012[15]
The Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART)	National, Sweden (all hospitals caring for patients with acute coronary artery disease)	PR; BP/G	Between 1998 and 2009, the average 30-day mortality rate for patients who had an acute heart attack decreased by 65% and the 1-year mortality rate decreased by 49%.	Jernberg et al. 2011[16]
Swedish Hip Arthroplasty Register (SHAR)	National, Sweden (all public and private orthopedic units)	BP/G	Thirty years of data on total hip arthroplasty have helped identify best clinical practices and high-performing implants, resulting in a revision rate of just 10%.	Kärrholm et al. 2007[17]

PB: performance benchmarking; PR: public reporting; BP/G: identification and dissemination of Best Practices or Clinical Guidelines.
Adapted from Larsson et al.[18]

The GLOBE pilot programme will bring together highly motivated participants including institutions, hospitals, and registries that are keen to compare outcomes and learn from each other.

THE GLOBAL OUTCOMES BENCHMARKING PROGRAMME

The idea of performance benchmarking as a mechanism to drive improvement is by no means a new one. A myriad of examples in the literature indicate that, when thoughtfully organised and well managed, the systematic collection of standardised data sets accelerates improvement upon measurement of process, structure, and outcome indicators which inherently reduces total cost (see Table 1).

At its most basic level, benchmarking sets the bar with regards to what is achievable, providing motivation for clinicians, care teams, and institutions to adopt best practices and/or evidence-based guidelines, or to innovate on their own. When combined with efforts to identify the underlying factors that lead to high performance, and co-ordinated quality improvement activities focussed on disseminating these lessons, the benefits of benchmarking can be amplified.

ICHOM's GLOBE programme builds on available examples in two important ways. Firstly, it will be the primary truly global initiative, unlike numerous existing national or regional benchmarking programmes. By including institutions from around the world, we expect to see wider variation with regard to both processes and outcomes, thereby expanding the possible research questions that can be investigated and increasing the likelihood of discovering new best practices in pockets of innovation and excellence. Secondly, this will be the first initiative to emphasise PROM such as mobility, pain, and quality of life. This is critically important if we are to guide improvement efforts in a way that is compatible with patients' preferences and values.

Global Outcomes Benchmarking Pilot Programme Objectives

The GLOBE pilot programme is a proof-of-concept initiative that aims to test the feasibility of setting up an international benchmarking programme based on the ICHOM Standard Sets. The pilot has five primary objectives:

a. Identify and overcome legal and technical hurdles to aggregating data from an international set of providers
b. Assess the appropriateness of the ICHOM CAT, and HKO sets for benchmarking and define the required changes
c. Design risk adjustment methodologies to adequately adjust participant outcomes between sites
d. Deliver risk-adjusted outcomes, reporting to participating institutions/hospitals so that they can understand their relative performance
e. Determine how pilot sites can leverage outcomes data to learn from one another

Pilot Overview

International Consortium for Health Outcomes Measurement community

ICHOM supports institutions, hospitals, and organisations in implementing Standard Sets of outcomes and develops case studies of examples of outcome measurement around the world. These have been described in further detail in case studies,[19-21] and an example of implementation has been described in Box 1. The importance of supporting implementation of the Standard Sets is two-fold: scaling the effective use of standard PROM instruments for outcomes; and for decision-making in performance benchmarking, transparency, and narrowing variation toward improved outcomes. The programme design and success of the GLOBE pilot programme is largely based on the ICHOM community of implementers of ICHOM Standard Sets.

Conditions

The two ICHOM Standard sets, HKO[9] and CAT,[10] were chosen for initial examination in the GLOBE pilot. According to a 2010 assessment, 51% of world blindness is caused by CAT,[22] and HKO has been ranked as the 11th highest contributor to global disability in the 2010 Global Burden of Disease.[23] It is therefore globally appropriate to start with these two conditions. Using the lessons derived from this pilot, ICHOM intends to launch benchmarking programmes across additional Standard Sets going forward.

Participants

The GLOBE pilot participants were recruited from ICHOM's international group of strategic partners, members of Standard Set implemention communities, the working groups of the ICHOM

HKO, and CAT Standard Sets, and from the ICHOM global community. Participants determined which hospitals within their institutions would be participating. All self-identified as being interested in the ICHOM GLOBE pilot given their engagement with the ICHOM community. Participating institutions were required to sign a memorandum of understanding (MOU) governing data privacy and security, namely requiring the de-identification of data gathered by participating institutions, prior to transmission of data. As the need and requirements for ethics boards and patient consent varies by country, the responsibility of acquiring regulatory approvals and/or ethics committee review for participation rested with the institution. Each institution sought approval for i) the use of data for benchmarking analysis and ii) the transfer of de-identified patient data outside the operating jurisdiction. The project is best classified as a service evalution or clinical audit that does not require informed consent as no patient care is impacted. The GLOBE programme provides a resource guide, study plan, data dictionary, and MOU to aid approval processes.

As of December 2016, the institutions identified in Figure 1 had agreed to contribute data to the pilot. Due to the popularity of the programme, recruitment is ongoing for both pilots at the time of this publication.

Outcomes collected

The endpoints collected are based on the ICHOM HKO and CAT Standard Sets that have been previously detailed and published.[10,12] Standard Sets include baseline conditions and risk factors to enable meaningful case-mix adjustment globally, ensuring that comparisons of outcomes account for differences in patient populations across not just providers but also countries and regions. High-level treatment variables are included to allow stratification of outcomes by major treatment types. Additional measurement time points have been added, where appropriate, to decrease the timeline needed to observe outcome differences between sites. Additionally, a pilot objective is to test the Standard Sets for the purposes of detailed benchmarking and identify required modifications to ensure the ICHOM HKO, and CAT sets can be used for benchmarking going forward.

ABUHB is an integrated payer-provider in rural Wales, UK. In 2014, ABUHB's senior management decided to use VBHC as the vehicle for achieving better outcomes at lower cost under the Welsh government's new policy for healthcare services, Prudent Healthcare. ABHUB undertook the following steps to achieve implementation success:

1. **Securing support from the workforce:** Senior management spent a significant amount of time engaging their staff in the VBHC approach. This included meetings and organisation-wide events with staff from all disciplines, from clinical teams to IT teams.
2. **Picking a pilot site:** ABUHB decided to pilot VBHC by measuring the ICHOM Parkinson's disease Standard Set. This was one of the options that included fewer measurement tools and time points than other Standard Sets and which would be implemented in one of the most enthusiastic clinical departments.
3. **Forming the VBHC team:** To ensure dedicated support and resourcing, two multidisciplinary VBHC teams were formed: a steering committee and a project team. The steering committee provided management oversight, whereas the project team drove the project on a day-to-day basis. Both teams included doctors, nurses, IT experts, and administration/project managers.
4. **Process mapping:** The team started by deconstructing clinic flow from patient, clinician, and informatics perspectives. This provided an opportunity to identify suitable time points for data collection that would cause minimal disruption to normal clinic operations. As part of this, the team carried out a gap analysis to determine what, where, and how each metric was measured or would be measured.
5. **Building an IT platform:** ABUHB's clinicians and IT team worked closely to develop eforms for data capture, which would be completed by patients in the clinic waiting room on iPads prior to their consultations.
6. **Collecting data and refining the model:** On deployment of the eforms, the Parkinson's disease clinic started collecting data on a small cohort of patients. The process was monitored closely, discussed at weekly VBHC project team meetings, and the data collection model continuously improved until outcomes were being measured seamlessly.
7. **Scale:** Following success of the pilot, the data collection infrastructure was scaled to more patients in the Parkinson's disease clinic. ABUHB are also now independently replicating their Parkinson's disease Standard Set measurement methodology for further Standard Sets (e.g. Heart Failure) in other departments.

Box 1: Implementation Example: ABUHB and the implementation of ICHOM Standard Sets.
ABUHB: Aneurin Bevan University Health Board; VBHC: value-based healthcare; ICHOM: International Consortium for Health Outcomes Measurement.

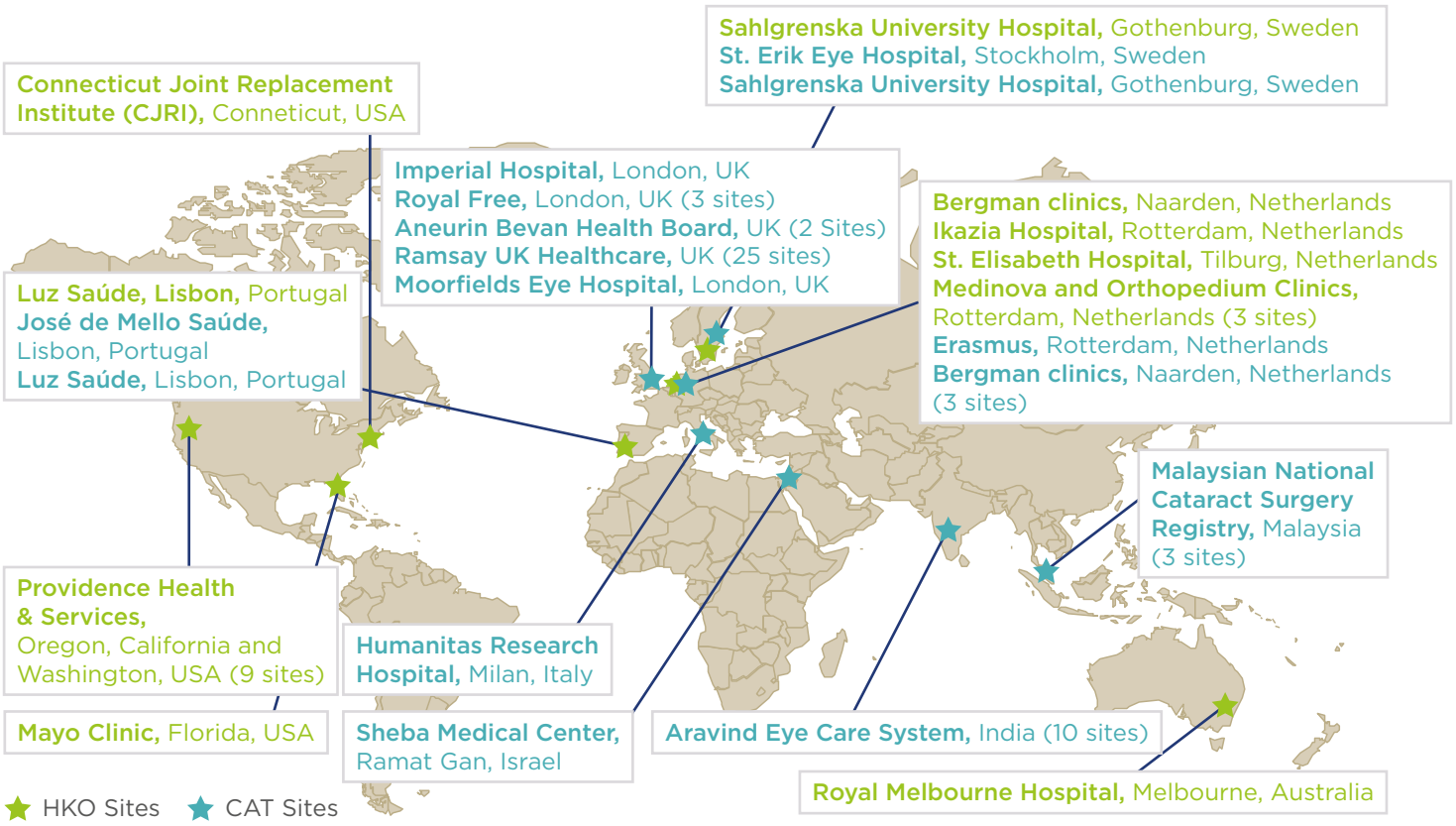

Figure 1: Hip and knee osteoartritis and cataract GLOBE pilot participants as of December 2016.
GLOBE: The ICHOM Global Outcomes Benchmarking (GLOBE) programme; ICHOM: International Consortium for Health Outcomes Measurement.

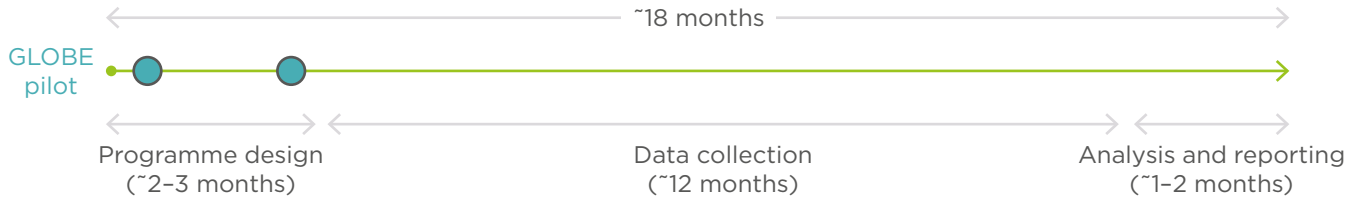

Figure 2: Overview of pilot phases for the HKO programme.
HKO: hip and knee osteoarthritis.

Methodology

Both the CAT and HKO programmes are prospective, non-interventional observational studies that are expected to run for 15 and 18 months respectively. Each pilot is divided into three main phases: Programme Design, Data Collection, and Analysis and Reporting, each with a specific goal (Figure 2).

Programme design

The aim of the Programme Design phase was to recruit potential pilot participants and provide a framework to work together to prepare sites to sign up for the pilot. There are four major engagement steps that were explained to onboard pilot participants:

a. Onboarding session: For each interested institution, a call was set up that included representation from the hospital leadership, clinical and programme management teams to discuss the pilot methodology and objectives. The aim of this session was to assess alignment with pilot objectives and ensure adequate resources were available at the hospital site to execute the pilot objectives.
b. Gap analysis assessment: All interested sites completed a gap analysis which assessed their sites' overall alignment with the ICHOM

Standard Sets. Sites were vetted to ensure that they collected the correct outcomes, which were based on the ICHOM defined outcome definitions, at acceptable time points. Sites that diverged from the ICHOM Standard Set recommendations or collected an incomplete set were coached on what gaps needed addressing to participate in the pilot.

c. Legal session: An exploratory discussion was held with interested sites to understand the legal and regulatory requirements for their participation in the pilot programme. These discussions were used to inform the legal contract ICHOM developed for this pilot discussed in Box 2.

d. Technical: A technical discussion was held with a member of the Technical/Informatics department to provide an overview of the data transfer process and assess if sites were technically able to extract and transfer the complete ICHOM Standard Set data.

Data collection

During the data collection phase participants submit data through a secure ftp protocol to ICHOM's data partner's (ICON) server on a monthly basis and receive validation or data quality reports post-data submission. This process is followed so that institutions and their hospital sites can receive timely feedback on their collection and make any required adjustments as early as possible in the pilot. An example of the data collection process for the CAT pilot is shown in Figure 3.

Analysis and reporting

The final phase of the pilot will focus on i) testing and implementing risk adjustment techniques to allow for adjusted comparisons between institutions and their hospital sites, ii) assessing potential visualisation techniques to allow for comparisons between providers, and iii) developing reporting options to showcase relative performance and facilitate collaborative sharing of best practices.

To encourage participation in the CAT and HKO pilots from a range of international institutions from different legal and regulatory environments, a legal agreement was developed that a) satisfied the requirements of data transfer across participating jurisdictions and b) satisfied regulatory/ethical requirements across participating jurisdictions. The agreement has now been successfully adopted in >10 countries. To achieve this there were five key considerations that had to be accounted for:

1. **Data Ownership**
   A key component of the agreement with participating institutions is that ICHOM does not take ownership of any data transferred during the course of the pilot. The participating institutions maintain the role of data owner or controller depending on the jurisdiction in which it operates.
2. **Data Type**
   Only de-identified patient data is utilised in the pilot and transferred from participating institutions to ICHOM. Provider sites are responsible for providing a unique ID that allows for the longitudinal tracking of patients' data over time that blinds ICHOM from any identifiable patient information.
3. **ICHOM's Role**
   ICHOM and its affiliates function as a data processor and will only access, use, manage, disclose to Third Parties, transfer internationally, or otherwise process participating institutions' (participant) data in accordance with participant instructions. The participant authorises ICHOM to utilise the data to complete the required analyses to meet the jointly agreed upon pilot objectives.
4. **Reporting Requirements**
   ICHOM agreed to develop a benchmarking report(s) to provide comparisons of outcomes at the hospital level in which participants will be compared to others in the pilot. Reports created for external disclosure or publication will disclose the names of participating institutions but will not attribute results directly to participants unless the participant approves such disclosure in writing prior to report disclosure or submission for publication.
5. **Regulatory Approval**
   ICHOM required each participant to seek the appropriate regulatory approval to take part in the pilot that was relevant in their operating jurisdiction. The level of required regulatory review and submission requirements were assessed by the participant to allow sites in different regulatory environments to follow an approval process best suited to their region.

Box 2: Developing a legal framework for data transfer.
CAT: cataract; HKO: hip and knee osteoarthritis; ICHOM: The International Consortium for Health Outcomes Measurement.

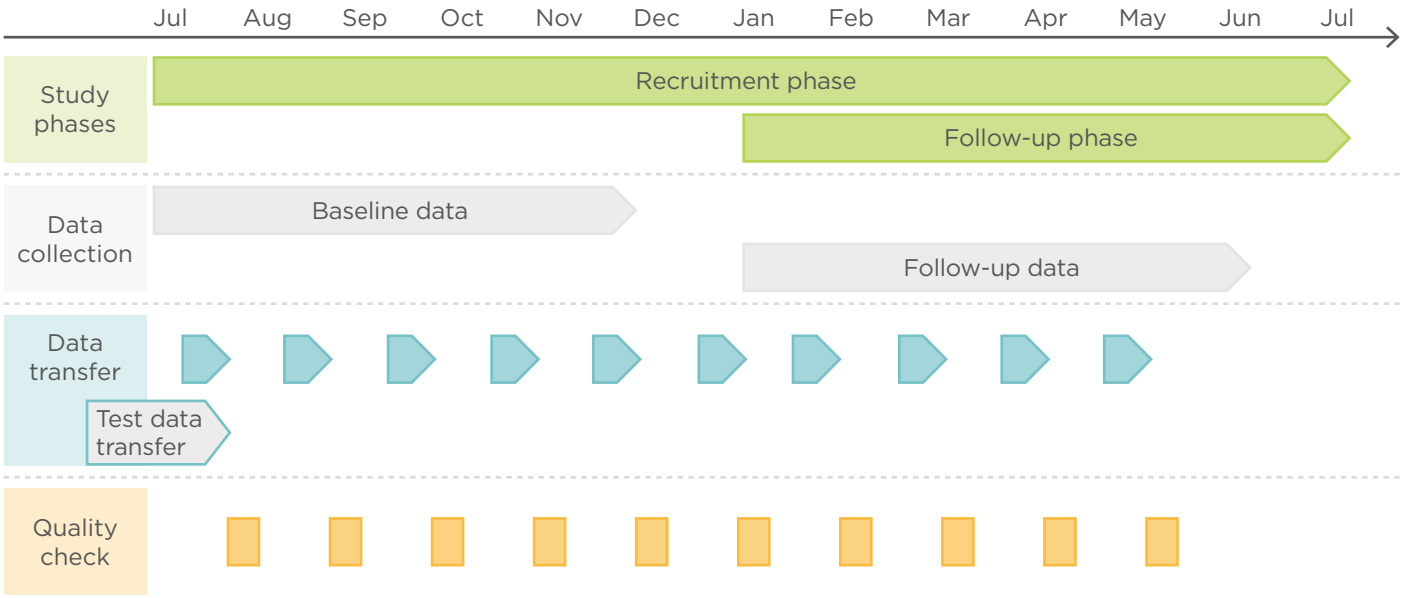

Figure 3: Overview of the CAT pilot data collection phase.
CAT: cataract.

CURRENT STATUS AND INITIAL LESSONS

Currently the HKO and CAT pilots are in the data collection phase with both expected to deliver results by the end of 2017, after which each pilot is expected to transition into a fully defined benchmarking programme. To date, the pilots have overcome a number of potential hurdles including, but not limited to, defining a legal framework (Box 2) to allow for data sharing, developing a data transfer process that accommodates institutions with different collection systems and technical capabilities, and identifying new variable definitions/requirements to help capture process differences between sites.

EXPECTED OUTPUTS AND NEXT STEPS

The HKO and CAT pilots will provide ICHOM with important lessons to allow it to develop a benchmarking programme that draws outcomes data from an international set of contributors and both analyses and reports back to providers on their relative performance. Lessons learned from evaluating risk adjustment within these two pilot conditions will also inform future Standard Sets and improve upon an ongoing benchmarking programme.

The risk-adjusted benchmarking performance data will provide an immense opportunity to improve patient care by identifying differences in hospital performance that can be used to facilitate learning programmes between institutions. Over time, the programme will likely incorporate additional data sources and expand its scope of analyses. Potential options include but are not limited to the identification of determinants of hospital performance, an understanding of individualised treatment response at the patient level, or an assessment of the most efficacious set of medical interventions for a given patient subpopulation. Outcomes data sharing programmes like this will provide the largest repository of structured data that has been collected to date, linking both underlying patient characteristics and treatment options to patient outcomes. In this exciting future, we expect the ICHOM GLOBE programme to serve a pivotal role in facilitating, sharing, and utilisation of data at an international level.

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EPITHELIAL-MESENCHYMAL TRANSITION IN DOCETAXEL-RESISTANT PROSTATE CANCER

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ABSTRACT

Castration-resistant prostate cancer (CRPCa) is an advanced stage of prostate cancer in which a tumour progresses even under androgen deprivation. Treatment alternatives for CRPCa remain very limited and mostly rely on docetaxel-based chemotherapy. Despite being shown to increase patients' overall survival, docetaxel's clinical efficacy is impaired by development of chemoresistance. Most patients do not respond to docetaxel treatment and even those initially responsive ultimately develop resistance. Recently, chemoresistance was found to be closely related to epithelial-mesenchymal transition (EMT), a process in which epithelial cells transition into a mesenchymal phenotype. In fact, EMT markers are overexpressed in prostate cancer and are correlated to a higher Gleason score. For this reason, new therapeutic strategies are being studied to inhibit this process in several cancers. However, the clinical usefulness of targeting EMT as a way to overcome docetaxel resistance in CRPCa is still questionable and suffers from some significant limitations. This review briefly summarises the most common mechanisms of EMT-induced chemoresistance and evaluates its use as a new approach to overcome docetaxel resistance in CRPCa.

Keywords: Castration-resistant prostate cancer (CRPCa), epithelial-mesenchymal transition (EMT), docetaxel, chemoresistance.

INTRODUCTION

Prostate cancer is the most common non-cutaneous cancer affecting men in Europe.[1] Its incidence rates vary by >7-fold throughout the continent, with the highest rates found in northern and western countries, such as Norway and France,[1,2] and have been continuously increasing over the past few decades, owing to the widespread use of prostate-specific antigen testing in prostate cancer screening.[3] The well-established risk factors for prostate cancer include age, race/ethnicity (e.g. African ancestry), and family history of the disease.[4]

Despite having an impressive 5-year survival rate of almost 100% when diagnosed in its early stages,[5] prostate cancer remains the third leading cause of death from cancer in European men, preceded only by lung and colorectal cancer.[2] The main reason for its high lethality is the development of castration-resistant prostate cancer (CRPCa), an advanced stage of prostate cancer in which a tumour progresses even under androgen deprivation.[6] About 80–90% of the patients who receive hormonal therapy will eventually progress to CRPCa and the overall survival after tumour recurrence drastically decreases to 1–2 years.[7] Treatment alternatives for CRPCa remain very limited and mostly rely on docetaxel-based chemotherapy.[8] However, some patients do not respond to docetaxel treatment and even those initially responsive ultimately develop docetaxel resistance, which is considered the main reason for CRPCa high mortality.[9]

Recently, epithelial-mesenchymal transition (EMT) emerged as a key mediator of drug resistance in several cancers, including CRPCa.[10] Due to the current urgency in developing new therapeutic options for CRPCa, this review briefly summarises the most common mechanisms of EMT-induced chemoresistance and evaluates its use as a new approach to overcome docetaxel resistance in CRPCa.

EPITHELIAL-MESENCHYMAL TRANSITION

EMT is a process in which epithelial cells transition into a mesenchymal phenotype.[11] While epithelial cells are known to show apical–basal polarity and adherence to a basement membrane, mesenchymal cells are multipolar, have a spindle shaped morphology, and present motility. Thus, during this process, epithelial cells have to undergo significant transcriptional and non-transcriptional changes that reorganise their cytoskeletal architecture, resulting in altered cell shape, increased motility, and in some cases the ability to degrade extracellular matrix proteins.[11] This switch is co-ordinated by a crosstalk between several intracellular signalling pathways, including Wnt, Notch, phosphatidylinositol-3-kinase (PI3K/Akt), Hedgehog, and transforming growth factor beta (TGF-β), that activate a number of transcription factors involved in the acquisition of the mesenchymal phenotype, such as SNAIL, ZEB, and TWIST.[11] The most remarkable hallmark of EMT is the loss of E-cadherin adhesion molecules, concomitant with the overexpression of mesenchymal markers such of N-cadherin and vimentin.[11-13]

Based on its biological functions, EMT is often classified into three different subtypes.[14] The first concerns its role during embryonic development. EMT is involved in several steps of embryogenesis, including embryo implantation, placenta formation, and generation of the mesoderm, which eventually gives rise to primary mesenchyme and migratory neural crest cells. These are able to dissociate from the neural fold and disperse to other parts of the embryo.[14]

The second EMT subtype is involved with wounding, tissue regeneration, and fibrosis. Under the influence of growth factors produced by macrophages during inflammation and tissue injury, epithelial cells can undergo EMT to originate new fibroblasts and contribute to organ fibrosis.[14] Fibroblasts originating from EMT express several mesenchymal markers, such as alpha-smooth muscle actin (α-SMA), fibroblast-specific protein 1 (FSP1), and collagen Type 1.[15] In fact, Iwano et al.[16] demonstrated that a substantial amount of interstitial kidney fibroblasts were actually originated from tubular epithelia at the site of injury.

Finally, the third subtype is related to EMT's role in cancer metastasis and progression. Metastasis is a multi-step process that ultimately leads to the dissemination of primary tumours and is a major cause of cancer mortality. In order to successfully colonise further tissues and form metastasis, cancer cells firstly have to acquire the capability to detach from basal membrane, migrate through adjacent tissues, and enter into the vasculature to finally reach distal sites via haematogenous or lymphatic spread.[17,18] EMT is required to enable these changes in cell adhesion and motility during tumour invasion, and therefore represents a critical regulator of the initiating events of the metastatic cascade.[19] For this reason, EMT involvement in metastasis and cancer progression has been extensively investigated.[15]

The Emerging Role of Epithelial-Mesenchymal Transition in Chemoresistance

The link between EMT and chemoresistance has been suggested for decades, since Sommers et al.[20] reported in the early 1990s that metastatic breast cancer cell lines resistant to the chemotherapy drugs doxorubicin and vinblastine presented loss of E-cadherin and the expression of the mesenchymal marker vimentin. This function of EMT was, however, somewhat expected, since most signalling pathways that regulate the EMT process have long been associated with chemoresistance in a variety of human cancers.[21-23]

Despite the accumulating evidence of EMT importance to chemoresistance, it was not until the recent publication of two studies that the interest in this particular role of EMT emerged. In the first one, Fischer et al.[24] developed a transgenic mouse model that was able to track EMT process during breast-to-lung metastasis. Using a FSP1 promoter-driven Cre recombinase, they created a mesenchymal-specific fluorescent marker that labelled breast cancer cells with a red fluorescent marker, which turns green when cells undergo EMT. Surprisingly, they found that the majority of cells within the primary tumour and the lung metastasis had not undergone EMT. However, after treatment with cyclophosphamide, it was observed that most cells that formed metastasis after tumour relapse had undergone EMT. Gene expression profile analysis revealed that these cells presented elevated expression of chemoresistance-related genes.

In the second study, Zheng et al.[25] created a genetically engineered mouse model of pancreatic ductal adenocarcinoma that harboured a tissue-specific deletion of either SNAIL or TWIST, two EMT-inducing transcription factors. Despite having no effect on metastasis formation, EMT inhibition

enhanced mice sensitivity to the chemotherapy drug gemcitabine, resulting in increased overall survival. Tumour cells derived from EMT-deficient mice presented elevated expression of equilibrative nucleotide transporter 1 (ENT1) and concentrative nucleoside transporter 3 (CNT3), which are known to promote gemcitabine sensitivity in human pancreatic cancer.

Mechanisms of Epithelial-Mesenchymal Transition-Induced Chemoresistance

The mechanisms that drive EMT-induced chemoresistance are yet to be fully elucidated. However, compelling evidence shows that EMT regulates at least two major mechanisms of drug resistance in human cancers. The first involves the activity of ABC transporters, a large family of transmembrane proteins responsible for ATP-dependent efflux of many cytotoxic compounds, including drugs. The best characterised ABC transporters involved in chemoresistance are ATP-binding cassette sub-family B member 1 (ABCB1), also known as P-glycoprotein, ATP-binding cassette subfamily C member 1 (ABCC1), and ATP-binding cassette subfamily G member 2 (ABCG2). These transporters are overexpressed in several human cancers and are responsible for the efflux of a wide range of chemotherapy drugs, resulting in decreased drug intracellular concentrations and ultimately lead to drug insensitivity.[26] Overexpression of ABC transporters is the predominant mechanism of multidrug resistance (MDR), the major cause of chemotherapy failure,[27] and EMT plays an important role in the regulation of their expression. Breast cancer cells overexpressing EMT transcription factors TWIST, SNAIL, and FOXC2 displayed increased levels of several ABC transporters and tolerated a 10-fold higher concentration of chemotherapy. Conversely, knockdown of EMT transcription factors reduced the expression of ABC transporters in these cells and restored their sensitivity to chemotherapeutic treatment. Saxena et al.[28] also demonstrated that the promoter sequence of ABC transporters has binding sites for the EMT transcription factors SNAIL, TWIST, and FOXC2, enabling EMT to directly regulate their expression.[28] Therefore, the overexpression of these transcription factors during EMT leads to enhanced promoter activity of ABC transporters, followed by the acquisition of MDR.[28]

EMT also promotes chemoresistance by inhibiting TGF-β-induced apoptosis. Controversially, TGF-β is involved in the control of both apoptosis and EMT and the choice of which way to follow seems to be closely related to cell cycle stage.[29] TGF-β-induced EMT mostly occurs during G1/S phase and results in decreased TGF-β-induced caspase activity and apoptosis.[30] Cells undergoing EMT also show overexpression of anti-apoptotic B cell lymphoma-extra large (Bcl-xL) and downregulation of pro-apoptotic proteins.[31] On the other hand, cells at G2/M phase mostly undergo TGF-β-induced apoptosis and display high caspase activity.[30] In order to evade apoptosis, SNAIL transcription factor promotes G1/S cell cycle arrest by repressing cyclin D2 transcription and increasing the expression of p21Cip1, a cyclin-dependent kinase that impairs cell cycle progression.[32] Of note, SNAIL-expressing cells have a 3-fold reduction in caspase-3 activity and are resistant to TNF-α-induced apoptosis.[32]

EPITHELIAL-MESENCHYMAL TRANSITION IN DOCETAXEL-RESISTANT CASTRATION-RESISTANT PROSTATE CANCER

Androgen deprivation therapy (ADT) is the standard treatment option for advanced prostate cancer.[8] However, tumour regression after castration is only temporary and in 80–90% of patients the tumour relapses and progresses to castration resistance.[7] One of the most remarkable features of prostate cancer transition to a castration-resistant status is the aberrant signalling of the androgen receptor (AR). CRPCa cells not only continue to express AR but are characterised by the amplification and hyperactivation of the receptor, which makes them hypersensitive to low androgen levels.[33] AR signalling is activated in these cells partly by intratumoural androgen synthesis, enabling tumour growth even with very low serum testosterone levels.[33]

Using LuCaP35 xenografts, Sun et al.[34] observed that after surgical castration tumours presented decreased levels of E-cadherin and increased expression of N-cadherin, vimentin and EMT-inducing transcription factors, including ZEB and TWIST.[34] It is noteworthy that castrated tumours also had increased expression of the stem cell markers WNT5a and WNT5b. Gene expression profiles of patients who had undergone ADT also revealed an increased expression of mesenchymal markers when compared to untreated patients.[34] Searching for markers of castration resistance in prostate cancer, Tanaka et al.[35] compared gene expression between hormone-sensitive and

castration-resistant LAPC9 xenografts and also observed higher expression of N-cadherin in the resistant mice. N-cadherin expression was also progressively raised after mice castration. Likewise, while androgen-sensitive LNCaP cell line does not express this mesenchymal marker, its androgen-insensitive clone LNCaP-CL1 does, reinforcing the theory that androgen deprivation might be related to EMT.[35] However, the precise molecular mechanisms that link CRPCa to EMT are still unclear.

Furthermore, when cultured in low hormone medium, androgen-sensitive LNCaP cells underwent EMT and acquired chemoresistance, while treatment with testosterone fully restored their sensitivity to docetaxel.[34] Conversely, EMT was also induced by ADT conditions in androgen-independent PC-3 cells and was reversed when cells were exposed to testosterone.[36] PC-3 cells that underwent castration-induced EMT also showed increased migration and invasion capabilities.[36]

Interestingly, ZEB1 was overexpressed in the LuCaP35 xenografts, in human prostates, and in LNCaP cells following castration.[34] ZEB1 represses E-cadherin and is a key transcriptional regulator of EMT in prostate cancer. Its expression correlates directly with Gleason score and with invasiveness, and migratory abilities of prostate cancer cells.[37] Taken together, these findings indicate that EMT not only increases prostate cancer progression but can actually be induced by castration, possibly by the upregulation of ZEB1 expression. Additionally, these results raise the question as to whether patients can actually benefit from being treated with docetaxel-based chemotherapy prior to ADT.

Castration-induced EMT is particularly concerning as it enables cancer cells to acquire stem cell-like features that determine chemotherapy failure.[10] According to the cancer stem cell (CSC) theory of cancer, only a few niche cells, CSCs, within the heterogeneous tumour mass are capable of unlimited self-renewal, which makes them most likely to acquire mutations and to be responsible for initiating tumour development.[38] Collins et al.[39] discovered that a few epithelial basal cells on adult prostate tissue highly express the integrin α2β1, a stem cell marker in prostate cancer. These cells presented high clonogenic capability and were able to generate a prostate-specific differentiated epithelium *in vivo* when injected into nude mice. However, it is still a matter of ongoing discussion whether prostate CSCs derive from basal or luminal epithelium.[40] Although the link between EMT and CSCs is still uncertain, it is known that cancer cells with EMT phenotype often display stem cell–progenitor cell-like properties. It has been reported that PC-3 cells that have undergone EMT acquired self-renewal ability by the upregulation of the transcription factors SOX2, NANOG, and OCT4, that are known to induce the reprogramming of differentiated cells to display a stem-like phenotype.[41] Also, Puhr et al.[42] and Marín-Aguilera et al.[43] induced docetaxel resistance in castration-resistant DU145 and PC3 cell lines and observed that resistant cells had increased expression of both mesenchymal and stem-cell like markers.

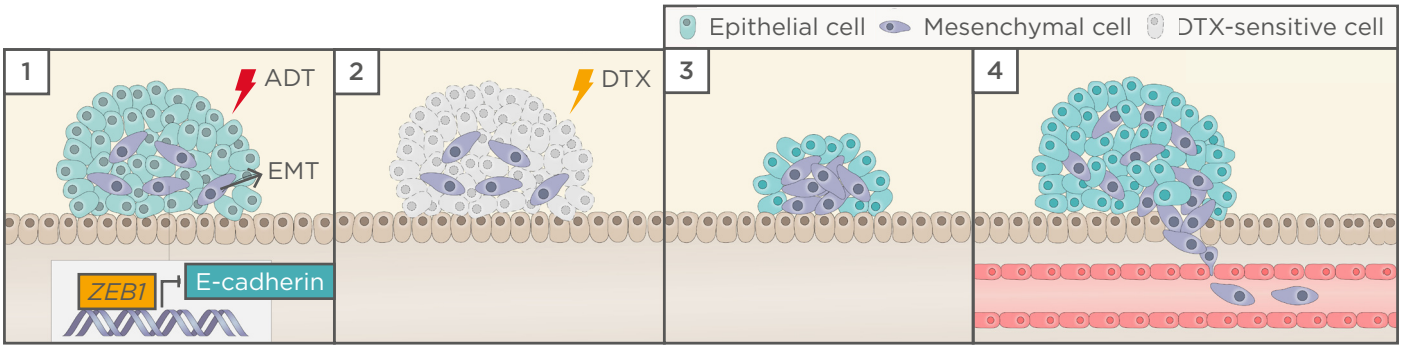

Figure 1: Hypothetical model of castration-induced epithelial-mesenchymal transition (EMT).
1) ADT triggers EMT in prostate cancer cells, possibly by the upregulation of *ZEB1* expression; 2) When the tumour relapses, cells that underwent EMT after castration acquired stem cell-like features and become resistant to docetaxel-based chemotherapy; 3) EMT remanescent cells proliferate, causing tumour relapse; and 4) EMT enable cells to leave the primary tumour, migrate through adjacent tissues, enter into the vasculature, and form metastasis.
ADT: androgen deprivation therapy; DTX: docetaxel.

The expression of EMT genes also correlated with worse clinical outcome and shorter time until tumour recurrence in patients that received docetaxel treatment,[43] supporting the idea that targeting EMT might be a potential strategy for overcoming docetaxel resistance in CRPCa to increase patients' survival. The proposed model of castration-induced EMT is summarised in Figure 1.

Targeting Epithelial-Mesenchymal Transition in Docetaxel-Resistant Castration-Resistant Prostate Cancer

So far, EMT-targeting strategies have mostly relied on the inhibition of its related transcription factors and signalling pathways. For instance, Hedgehog signalling blockade by GDC-0449 antagonist induced apoptosis and reversed docetaxel resistance of PC3 cells.[44] Combined treatment with GDC-0449 and docetaxel also showed greater anti-tumoural growth inhibitory effect on PC3 cell xenografts.[44] Likewise, inhibition of PI3K/Akt, Notch, and Hedgehog signallings increased docetaxel sensitivity and promoted cell apoptosis in prostate cancer resistant cells.[45,46] Another target of particular interest is human macrophage inhibitory cytokine-1 (MIC-1), a member of the TGF-β protein family. MIC-1 expression progressively raises as prostate cancer progresses to metastatic state and correlates to the acquisition of EMT features and docetaxel resistance in PC3 cells.[47-50] MIC-1 knockdown in these cells increased docetaxel cytotoxic effects by promoting both mitochondrial and caspase dependent apoptosis.[50] Recently, Kawamura et al.[51] demonstrated that CRISPR/Cas9-mediated gene knockout of *NANOG* genes in DU145 cells significantly increased docetaxel sensivity. *NANOG* genes are major regulators of stem cells pluripotency and positively regulate the EMT process via STAT3-induced SNAIL activation.[51,52] *NANOG* is overexpressed in prostate cancer and is associated with acquisition of stem cell characteristics, increased Gleason score, and poor prognosis.[51,53] Of note, inhibition of ZEB1 in docetaxel-resistant lung cancer cells also significantly enhanced chemosensitivity.[54]

Another approach is to reverse the mechanisms of EMT-induced chemoresistance. *ABCB1* is frequently involved in acquired docetaxel resistance in CRPCa and is upregulated in docetaxel-resistant cell lines.[55] *ABCB1* shRNA-mediated knockdown was able to sensitise castration-resistant C4-2B prostate cancer cells to docetaxel treatment.[55] The flavone apigenin, a natural inhibitor of ABCB1 expression, also efficiently restored docetaxel sensitivity in these cells and might be considered as a natural source to modulate MDR in CRPCa. Recently, the anti-androgens abiraterone acetate and enzalutamide were shown to inhibit ABCB1 activity and therefore reduce its efflux activity.[56] Combined treatment with both drugs sensitised DU145 cells to docetaxel and might be considered as a promising strategy to overcome chemoresistance in CRPCa.[56] However, it is noteworthy that polymorphisms in *ABCB1* largely affect the efficacy and toxicity of ABCB1 inhibitors and currently represent a significant barrier towards their clinical use.[57]

Finally, it is noteworthy that microRNA (miRNA)-based cancer therapy has become a research field of growing interest. miRNAs are small non-coding RNAs that control post-transcriptional regulation of gene expression. Some miRNAs are known to be involved in EMT regulation in several human cancers through the regulation of the expression of epithelial and mesenchymal markers, transcription factors, and signalling pathways that promote EMT.[58] While some miRNAs exert oncogenic activity by promoting EMT, others act as tumour suppressors by inhibiting it.[58] Several miRNAs related to EMT that are downregulated in CRPCa are being investigated as novel therapeutic targets, most notably miR-205 and miR-200 families. Transfection with miR-200c and miR-205 in PC-3 and DU-145 cells, which inherently express low levels of both miRNAs, resulted in restored E-cadherin expression and reduced migration and invasive capabilities.[42,59] Furthermore, miR-205 induced the actual reversal of EMT, a process known as mesenchymal–epithelial transition (MET), in DU-145 cells, through regulation of genes involved in epithelial organisation and cell-cell adhesion, such as *ZEB*.[59] Moreover, transfection of miR-205 in the docetaxel-resistant WPE1-NB26 prostate cancer cell line was able to reduce chemoresistance and enhance docetaxel-induced apoptosis,[60] providing encouraging evidence on the use of nanotechnology-based strategies for CRPCa treatment.

CONCLUSIONS AND FUTURE DIRECTIONS

EMT has recently emerged as a promising topic on chemoresistance research and several strategies to impede this process are being studied as potential pharmacological approaches against chemoresistance in a broad range of human

cancers, including CRPCa. However, the clinical efficacy of targeting EMT as a way to overcome docetaxel resistance in CRPCa is still questionable and presents with some significant limitations. Firstly, it is highly debatable that the simple inhibition of a specific target would successfully suppress a dynamic process such as EMT, which is regulated by multiple genes and intracellular signalling pathways. Instead, it is more likely that the inhibition of one target would be easily compensated by the activity of another EMT regulator, that exerts similar or redundant functions. Moreover, it should be considered that EMT inhibitors might exert limited benefits in advanced patients that have already undergone this transition. Patients with CRPCa would perhaps benefit more from therapies focussed on reversing EMT, therefore inducing MET, rather than inhibiting it. Instead, targeting EMT might perhaps be a more suitable approach for patients that have recently undergone ADT, as a way to prevent the progression to CRPCa and chemoresistance development. Finally, another concern relates to thesafety and clinical use of EMT targets, which is yet to be investigated. Despite these limitations, EMT remains a promising target among the current lack of therapeutic strategies for CRPCa treatment and may become clinically suitable upon further research.

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CONCERNS ABOUT THE USE OF NON-HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AS A LIPID PREDICTOR

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ABSTRACT

Introduction: Non-high-density lipoprotein (non-HDL) cholesterol is the sum of low-density lipoprotein (LDL) cholesterol and very-low-density lipoprotein (VLDL) cholesterol, and is usually approximated by the total cholesterol minus HDL-cholesterol. The National Lipid Association (NLA) has advocated the use of non-HDL cholesterol as its favoured lipid predictor. Cut-off points are based on LDL cholesterol values, with a lower end at 100 mg/dL (2.50 mmol/L) and a higher end at 190 mg/dL (4.75 mmol/L), adding 30 mg/dL (0.75 mmol/L) to keep triglyceride (TG) levels <150 mg/dL (1.70 mmol/L).

Objectives: The author will demonstrate that the use of non-HDL cholesterol has not been fully considered.

Methods: The author will examine a general population lipid database to demonstrate the frequency of distribution of non-HDL cholesterol in the part of the population that was known to have developed a form of atherothrombotic disease (ATD) and in the part that was not known to have done so. The effect of stratifying each non-HDL cholesterol quintile in terms of another lipid predictor that does not involve VLDL-cholesterol or TG will be demonstrated. The other risk predictor is the cholesterol retention fraction (CRF) defined as (LDL-HDL)/LDL.

Findings: All non-HDL cholesterol quintiles above the lowest quintile had higher frequencies in the ATD population than in the non-ATD population. The highest two quintiles had frequencies in the ATD population that are 2.5-times as high as those in the non-ATD population, whereas in the middle two quintiles, the frequency in the ATD population was minimally higher than in the non-ATD population. In the lowest quintile, the frequency is much higher in the non-ATD population than in the ATD population. At any non-HDL cholesterol quintile, the average age of ATD onset depends on cigarette smoking (not discussed here) and the CRF. Higher CRF levels equate to an earlier average age of ATD onset and lower levels of CRF equate to a later onset. A 75-year-old male who was a hypertensive diabetic and a former smoker was not on statins because of low lipid levels, had clean arteries on angiography, whereas a 45-year-old normotensive, non-smoking patient with severe dyslipidaemia (obtained at first encounter) had a massive stroke due to carotid stenosis. Both had non-HDL cholesterol levels in the intermediate ATD risk quintiles.

Conclusions: Non-HDL cholesterol is not the optimal predictor of the population at risk of atherothrombotic disease and its use should be reconsidered.

Keywords: Risk predictors, non-high-density lipoprotein (non-HDL) cholesterol, atherothrombotic disease (ATD), cholesterol retention fraction (CRF).

INTRODUCTION

The National Lipid Association (NLA) has recently advocated non-high-density lipoprotein (non-HDL) cholesterol as a prime lipid predictor for the prediction of the population at risk of atherothrombotic disease (ATD).[1] Non-HDL cholesterol is the sum of the low-density lipoprotein (LDL) cholesterol and the very-low-density lipoprotein (VLDL) cholesterol. Non-HDL cholesterol can also be approximated by total cholesterol (TC) minus HDL cholesterol.

While the concept of non-HDL cholesterol is not new, there are no randomised controlled clinical trials to validate non-HDL cholesterol as either a lipid predictor or a guide to the treatment of dyslipidaemia to stabilise/regress plaque or decrease ATD events. Non-HDL cholesterol was mentioned in the write-up of the Helsinki Heart Study but, as the authors stated, the aim of the study was to prove the lipid regulatory hypothesis of Dr Esko Nikkila, which stated that maximal regression of atherosclerosis occurs when LDL cholesterol is lowered simultaneously with HDL cholesterol being raised.[2]

The purpose of this paper is to discuss the limitations of non-HDL cholesterol in terms of the Bowling Green Study (BGS) database. The author will show that non-HDL cholesterol is a suboptimal predictor compared with the cholesterol retention fraction (CRF) (defined as [LDL-HDL]/LDL) and that the use of non-HDL cholesterol as a lipid predictor should be reconsidered.

MATERIAL AND METHODS

The author established the BGS of the primary and secondary prevention of ATD initially as a means of predicting the population at risk of ATD, and subsequently as a guide to the maximal stabilisation/regression of plaque and maximal reduction of ATD events.[3-5] When establishing the BGS, the author created an age-sex database of all patients in his practice who had had a lipid profile during the BGS study timeframe of 4th November 1974–4th November 2003. During the years prior to 1st January 1978, only TC and triglyceride (TG) levels were available, but in the years following 1st January 1978, HDL cholesterol and LDL cholesterol were also available. HDL cholesterol was measured by the precipitation method and LDL cholesterol was calculated by the Friedewald formula.[6] Since LDL cholesterol calculations are relatively accurate above 400 mg/dL (4.5 mmol/L), the author accepted LDL cholesterol calculations up to TG levels of 500 mg/dL (5.6 mmol/L).[7] This point may be controversial, and indeed the author spoke to Dr Friedewald a number of years ago on this matter. Dr Friedewald insisted that his formula should not be used when TG levels exceeded 400 mg/dL (4.5 mmol/L). However, Wilson et al.[7] have shown that LDL cholesterol calculations are relatively accurate in the 400–500 mg/dL (4.5–5.6 mmol/L) region and the author has elected to follow WIlson et al. in this matter so that more CRF determinations are available. Two-hour postprandial blood sugar levels were also obtained in most cases of lipid screening. Cigarette smoking status was not routinely ascertained until 1984, when it became clear that cigarette smoking played a fundamental role in the pathogenesis of ATD events. Blood pressure determination, height, and weight were measured at every office visit, and BMI was calculated on the initial visit and periodically thereafter.

The author's practice of family medicine in northwest Ohio is located in Bowling Green, the county seat of Wood County. Wood County is largely a rural county, with many professional occupations in agriculture and small businesses. The largest employer is Bowling Green State University and the population of Wood County was about 128,000 at the time of the last census, of which about 30,000 live in Bowling Green. The population is mainly Anglo-Europeans in ancestry and the chief minority group is Latin American. African-Americans and Asian-Americans are not present in large numbers.

Risk factors for ATD were not well understood in the 1970s and 1980s and because many dyslipidaemic patients refused therapy or had an inadequate response to therapy, a number of the author's patients developed some form of ATD. The BGS was not a randomised controlled clinical trial. All dyslipidaemic, hypertensive, and diabetic patients were offered therapy; some accepted, but many declined. All cigarette smokers were advised to quit smoking.

All known ATD risk factors were examined, including dyslipidaemia, cigarette smoking, hypertension, diabetes mellitus, obesity, age, sex, and occasionally a hypercoagulable state. In searching for the best lipid predictor, the author noted the promotion of the Framingham Fraction (TC:HDL) but declined its use since it was based on non-fasting blood. However, in 1981, an article was published asking if the LDL:HDL ratio was the best lipid predictor. From this suggestion was born the concept of the CRF (defined as [LDL-HDL]/LDL), which sought to determine what percentage of cholesterol entering the artery stays within the artery wall. After evaluation, the BGS determined the CRF to be its best lipid predictor.[8]

The determination of the population developing some form of clinical ATD was based on clinical findings. For example, acute myocardial infarction (MI) was based on the acute onset of typical

mid-sternal chest pain (described as a pressure, heaviness, or an ache, with or without radiation) and was virtually always accompanied by electrocardiographic and/or cardiac enzyme changes. Sudden unexpected death was included within the MI category. On the other hand, angina pectoris displayed a similar pain without electrocardiographic/enzyme changes, and was of a chronic, recurrent nature. A stroke was diagnosed in patients with acute neurologic changes, either sensory or motor, lasting ≥1 hour. A transient ischaemic attack had similar findings, but those findings had to be resolved within 1 hour. Aneurysms were generally found on radiographic testing. About 40% of patients presented with a history of an ATD event, and in such cases the author attempted to authenticate the diagnoses, though often this was not possible.

RESULTS

In the BGS population database, the frequency distribution of non-HDL cholesterol is determined in terms of biological sex and in terms of the frequency of each quintile in the ATD and non-ATD populations, using the quintiles suggested by the NLA (Table 1). It will be noted that the quintiles given in this paper are different than those recommended by the NLA. The reason for this is that the manufacturers of the auto-analysers changed the methodology by which they measured HDL cholesterol. The older precipitation method of HDL cholesterol measurement was the gold standard utilised by laboratories worldwide and was the method used to measure HDL cholesterol in all of the atherosclerosis regression studies conducted prior to the year 1999. The newer enzymatic method has been used in most of the atherosclerosis regression studies undertaken since the year 1999. The two methods give differing values for HDL cholesterol: the value obtained using the enzymatic method is an order of 10 mg/dL (0.26 mmol/L) higher than that obtained using the precipitation method. Consequently, the calculated LDL cholesterol using the enzymatic method will be in the order of 10 mg/dL (0.26 mmol/L) lower than that obtained using the precipitation methodology.[9] This makes the comparison of databases obtained before and after the methodology conversion difficult unless a conversion factor is utilised. For this study, the conversion factor is: HDL cholesterol (precipitation method)=(HDL cholesterol [enzymatic method] minus 12)/0.93.

The NLA guidelines for non-HDL cholesterol are based on LDL cholesterol goals, with the upper-most quintile being defined by the LDL cholesterol level establishing familial hypercholesterolaemia (i.e. 190 mg/dL [4.9 mmol/L]) and the lowest quintile defining an optimal LDL cholesterol level, at which little LDL cholesterol enters the artery wall (i.e. 99 mg/dL [2.6 mmol/L]). The non-HDL cholesterol values in between these two extremes were divided into three groups to make up the other three quintiles. The NLA then added 30 mg/dL (0.77 mmol/L) to each quintile to correct for the cholesterol carried by VLDL.

The BGS database was compiled mainly before the HDL cholesterol measurement conversion and hence its regional laboratory utilised the precipitation methodology. As a result, a LDL cholesterol value (using the enzymatic methodology) of 190 mg/dL (4.5 mmol/L) is equivalent to a LDL cholesterol of 200 mg/dL (5.2 mmol/L) in the BGS database. Similarly, a LDL cholesterol (using the enzymatic methodology) 100 mg/dL (2.6 mmol/L) is equivalent to a LDL cholesterol of 110 mg/dL (2.8 mmol/L) in the BGS database. Adding the 30 mg/dL (0.77 mmol/L) to each number gives the results depicted in Table 1.

The non-HDL cholesterol quintiles depicted in Table 1 can be graded into ATD risk categories on the basis of frequencies within the ATD and non-ATD populations. The frequency of the two highest quintiles (>200 mg/dL [5.2 mmol/L]) in the total ATD population was 36% (225/626) and in the total non-ATD population was 14% (369/2,660). Since the frequency of the two highest quintiles in the ATD population was about 2.5-times as high as in the non-ATD population it is reasonable to attribute the highest ATD risk to these two quintiles, though in a graded fashion. Similarly, the frequency of the middle two non-HDL cholesterol quintiles in the ATD population was 49% (308/626) and 40% (1,075/2,660) in the non-ATD population. There appears to be a graded intermediate ATD risk for non-HDL cholesterol in the 140–199 mg/dL (3.6–5.2 mmol/L) range. On the other hand, the frequency of the lowest quintile of non-HDL cholesterol in the ATD population was 15% (93/626) and 46% in the non-ATD population.

The range of CRF values in any given non-HDL cholesterol quintile is widely variable. Table 2 shows the average ages of ATD onset for each CRF-non-HDL cholesterol cohort in the BGS ATD population. The data clearly showed that at any quintile of non-HDL cholesterol, the average age

of ATD onset is determined by the associated CRF value, with earlier ages of ATD onset occurring when CRF levels are higher and later ages of ATD onset occurring when CRF values are lower, in a linear fashion. The converse is not true: at any level of CRF, non-HDL cholesterol does not predict the average age of ATD onset. These findings are also true for LDL cholesterol.

Table 1: Non-high-density lipoprotein cholesterol distribution in Bowling Green Study atherothrombotic disease population versus non-atherothrombotic disease population 1974–2003.

Non-HDL cholesterol category	Male		Female		∑	
	ATD	NATD	ATD	NATD	ATD	NATD
≥230 mg/dL (5.9 mmol/L)	48 (14%)	73 (6%)	66 (23%)	57 (4%)	114 (18%)	130 (5%)
200–229 mg/dL (5.2-5.9 mmol/L)	65 (19%)	124 (10%)	46 (16%)	115 (8%)	111 (18%)	239 (9%)
170–199 mg/dL (4.4-5.2 mmol/L)	80 (24%)	236 (19%)	66 (23%)	187 (13%)	146 (23%)	423 (16%)
140–169 mg/dL (3.6-4.4 mmol/L)	90 (27%)	308 (24%)	72 (25%)	344 (25%)	162 (26%)	652 (25%)
≤139 (3.6 mmol/L)	56 (17%)	529 (42%)	37 (13%)	687 (49%)	93 (15%)	1,216 (46%)
∑	**339**	**1,270**	**287**	**1,390**	**626**	**2,660**

The freestanding number in each column represents the actual number of patients. The number in parentheses represents the percentage of the group (column). Non-HDL cholesterol categories based on BGS equivalences. To determine comparative NLA values, subtract 10 mg/dL (0.26 mmol/L).
HDL: high density lipoprotein; BGS: Bowling Green Study; ADT: atherothrombotic disease; NATD: non-atherothrombotic disease.

Table 2: Cholesterol retention fraction distribution in non-high density lipoprotein cholesterol atherothrombotic disease Bowling Green Study General Population: 1974–2003.

Average age of atherothrombotic disease onset cholesterol retention fraction							
Non-HDL cholesterol (mg/dL)	≥0.80	0.75–0.79	0.70–0.074	0.65–0.69	0.60–0.64	≤0.59	∑
≥230	54	27	11	8	1	-	101
	3,067	1,737	739	494	58	-	6,095
	57	64	67	62	58	-	60
200–229	23	33	28	17	5	3	109
	1,312	2,166	1,940	1,076	341	243	7,078
	57	66	69	63	68	81	65
170–199	17	33	38	18	12	21	139
	1,014	1,834	2,428	1,288	863	1,510	8,937
	60	56	64	72	72	72	64
140–169	7	14	27	32	30	48	158
	390	852	1,763	2,189	2,052	3,431	10,677
	56	61	65	68	68	71	68
≤139	-	3	5	13	13	60	94
	-	149	274	828	775	4,035	6,061
	-	50	55	64	60	67	64
∑	101	110	109	88	61	132	601
	5,783	6,738	7,144	5,875	4,089	9,219	38,848
	57	61	66	67	67	70	65

Non-HDL: non-high-density lipoprotein.

Table 3: Cholesterol retention fraction versus non-high-density lipoprotein cholesterol the Bowling Green Study general population: 1974–2003.

		Average age of atherothrombotic disease onset cholesterol retention fraction zones			
		Red	Yellow	Green	Σ
Non-HDL cholesterol	Red	176	31	3	210
		10,961	1,969	243	13,173
		62	64	81	63
	Yellow	136	92	69	297
		8,281	6,392	4,941	19,614
		61	69	72	66
	Green	8	26	60	94
		423	1,603	4,035	6,061
		53	62	67	64
	Σ	320	139	132	601
		19,665	9,964	9,219	38,848
		61	67	70	65

Non-HDL: non-high-density lipoprotein.

Using the BGS database, a table can be set up for both CRF and non-HDL cholesterol, placing the highest risk cohorts in the 'red' zone, intermediate risk cohorts in the 'yellow' zone, and the lowest risk cohorts in the 'green' zone. The highest risk zone for CRF is ≥0.70; the intermediate risk zone, 0.60–0.69; and the lowest risk zone, ≤0.59. For non-HDL cholesterol, the highest risk zone is ≤200 mg/dL (5.2 mmol/L); the intermediate risk zone, 140–199 mg/dL (3.6–5.2 mmol/L); the lowest risk zone, ≤139 mg/dL (3.6 mmol/L). The result of this analysis can be seen in Table 3. At any risk zone of non-HDL cholesterol, the risk zone for the CRF determines the average age of ATD onset. (The red zone parameters for the enzymatic method of HDL cholesterol measurement and the NLA quintiles are CRF of 0.60 or higher and non-HDL cholesterol of 190 mg/dl [5.1 mmol/L]; the yellow zone, CRF=0.50–0.59 and non-HDL cholesterol= 130–189 mg/dL [3.4–5.1 mmol/L]; the green zone, CRF of 0.49 and lower and non-HDL cholesterol of 129 mg/dL [3.4 mmol/L] or lower.)

The author would like to make note of a comparative case that occurred in his practice. A 75-year-old male, who was an insulin-dependent diabetic, hypertensive, former smoker with acceptable lipids (TC=189 mg/dL [4.9 mmol/L], HDL cholesterol= 36 mg/dL [0.9 mmol/L], LDL cholesterol=110 mg/dL [2.8 mmol/L], TG=214 mg/dL [2.4 mmol/L], non-HDL cholesterol=153 mg/dL [4.0 mmol/L], and CRF=0.67) at 43 years of age, further LDL cholesterol lowering occurring naturally over the subsequent years. His lipids were followed over time as the author followed the patient's blood sugar level and blood pressure; since his LDL cholesterol fell to very low levels, the patient was not placed on a statin. At 75 years of age the patient needed to have orthopaedic surgery and cardiac clearance was requested. An angiogram was performed and his coronary arteries were found to be devoid of plaque. Another patient who came into the author's practice at the age of 45 years, having suffered a massive stroke due to carotid stenosis, had avoided seeing physicians in the belief that he was doing well. He had never smoked cigarettes and had his blood pressure taken with good results: 120/80 mmHg. He also had a TC with a value of 200 mg/dL (5.2 mmol/L). The author attended the patient in the casualty department of the local hospital and obtained a full lipid profile, which was abnormal: TC=200 mg/dL (5.2 mmol/L), HDL cholesterol=25 mg/dl (0.6 mmol/L), LDL cholesterol=145 mg/dL (3.7 mmol/L), non-HDL cholesterol=175 mg/dL (4.5 mmol/L), and TG=150 mg/dL (1.7 mmol/L). His CRF was 0.83. The non-HDL cholesterol values for both patients were in the intermediate risk range, yet in the former patient, statin therapy was held as the author followed the patient's lipids and when the LDL cholesterol fell of its own accord, never initiated since the CRF fell

below 0.59 (precipitation method but 0.49 using the enzymatic method), whereas the patient who had the stroke had a CRF that demanded immediate treatment long before his acute ATD event.

DISCUSSION

Non-HDL cholesterol represents the sum of all of the apolipoprotein (apoB) carrying particles in the blood, and hence has been referred to as the new atherogenic dyslipidaemia.[1] Non-HDL cholesterol has been advocated as a first-line lipid predictor by the NLA. This position is based on numerous studies, usually comparing non-HDL cholesterol with LDL cholesterol or apoB.[10-19] Non-HDL cholesterol has been advanced as a surrogate for apoB[20] but may in fact be superior to apoB in predicting the population at risk of ATD.[21,22]

The use of lipid ratios was first suggested by Kannel et al.[23] in 1979, describing the findings of the Framingham Heart Study.[23] Initially advocated was the ratio between TC and HDL cholesterol (TC:HDL). Since the bulk of lipid profiles at that time were performed on non-fasting blood, LDL cholesterol calculations were not available to Kannel et al. Some studies, done with fasting blood, have proposed the apoB/apoA-1 ratio instead.[24-26] The apoB/apoA-1 ratio has not been shown to be superior to non-HDL cholesterol as a predictor[27-29] and non-HDL cholesterol has not been shown to be superior to TC: HDL.[30] Other studies have compared the predictive abilities of the LDL:HDL cholesterol ratio to apoB, and the ratio is superior to apoB.[31]

Elshazly et al.[32] have analysed eight intravascular ultrasound studies involving 4,917 patients and found that the TC:HDL cholesterol ratio was a better predictor of plaque stabilisation/regression and major adverse cardiovascular events than LDL cholesterol, non-HDL cholesterol, and apoB. This finding adds to the author's call to use lipid ratios in the evaluation of ATD risk and to guide therapy to maximally stabilise/regress atherosclerotic plaques.[33]

The author has advocated the use of the CRF as a lipid predictor[3-5] and has shown the superiority of the CRF as a lipid predictor.[8] The ATD risk predicted by the CRF is graded with the lowest (minimal) risk afforded by a CRF of ≤0.59 and the highest risk by a CRF of ≥0.80. CRF values in the 0.70–0.79 range represent a high-risk category, though in a graded manner. CRF values in the 0.60–0.69 range represent a lower risk category, though again in a graded manner. (These values are based on the precipitation method of HDL cholesterol measurement. If the enzymatic method is used, then the highest risk category is ≥0.70, the high-risk category is 0.60–0.69, the lower risk category, 0.50–0.59, and the minimal risk category is ≤0.49).

Treatment to stabilise/regress plaque in eight published angiographic regression trials depends on correction of hypertension and the dyslipidaemia risk factor.[33] Using the CRF as the lipid arm and systolic blood pressure (SBP) as the barometric arm, a graph can be generated with the CRF on the ordinate and SBP on the abscissa. Additionally, a threshold line can be generated with CRF-SBP loci of (0.74,100) and (0.49,140) above which lies the CRF-SBP plots of 85% of the ATD patients in the author's ATD database. (These co-ordinates are based on the precipitation method of HDL cholesterol measurement; if the enzymatic method is used, the co-ordinates are [0.62,100] and [0.40,140].) Any therapy that brings the patient's CRF-SBP plot below the threshold line results in plaque stabilisation/regression in a minimum average of 75%.[33] If LDL cholesterol goals are used instead, then any therapy that brings the LDL cholesterol level to <80 mg/dL (precipitation method of HDL cholesterol measurement but 70 mg/dL when the enzymatic method is used) results in stabilisation/regression of plaque in 93% of cases, and this is the case at any LDL cholesterol level below this threshold.[34]

The Program on the Surgical Control of the Hyperlipidemias (POSCH) was not structured to control blood pressure. As a result, significant plaque stabilisation/regression occurred in the face of marked hypertension. Consequently, at least in POSCH, plaque stabilisation/regression is purely a function of lipid changes. When LDL cholesterol levels are brought down to 99 mg/dL (2.5 mmol/L) or lower or when CRF levels are brought down to ≤0.59, plaque stabilisation/regression occurs in 99% of cases.[35] Moreover, in POSCH, changes in the CRF perfectly predicted plaque outcomes. If the CRF rose at 1 year, then invariably angiographic plaque progressed at 3 years. If the CRF fell at 1 year, then invariably angiographic plaque stabilised/regressed at 3 years.[35]

Table 2 and Table 3 reveal that at any level of non-HDL cholesterol, a higher CRF portends an earlier age of ATD onset, while a lower CRF portends a later age of ATD onset in a linear fashion. The converse is not true: at any CRF value, a higher

non-HDL cholesterol does not necessarily portend an earlier age of ATD onset and a lower non-HDL cholesterol does not necessarily portend a later age of ATD onset.

LDL cholesterol has long been the focus of interventional lipidologists for the prevention of primary or secondary ATD.[36] The author has resurrected the Lipid Regulatory Hypothesis of Dr Esko Nikkila, and has shown that, at least in the POSCH[37] and the National Heart Lung and Blood Institute (NHLBI) Type II Coronary Intervention Study,[38] raising HDL cholesterol and lowering LDL cholesterol are both important in the stabilisation/regression of plaque.

One limitation of these findings is that they are based on a real-world observational study. During the study (4th November 1974–4th November 2003), full patient follow-up was not available. This is unfortunate, but unavoidable, in everyday practice of medicine in the USA. However, every case of ATD known to the author or reported to the author is reported here. The strengths of this study are that it represents the family practice of a single physician in a single location in a rural county with a relatively stable population over a long period of time. This study was undertaken at a time when lipid testing was not routinely done and the concept of treating dyslipidaemia was neither widely established nor accepted by either local patients or physicians.

Future directions of research involving non-HDL cholesterol should involve a broader look at all putative lipid predictors. The virtual reluctance of most investigators to use lipid ratios must be abandoned and lipid ratios given their place amongst the array of lipid predictors.

CONCLUSION

The concept of non-HDL cholesterol as a lipid predictor should be reconsidered due to the evidence of this study. While no randomised controlled clinical trial has been performed to validate the CRF, the outcomes of eight angiographic regression trials have been presented by the author. It would seem reasonable to dispense with non-HDL cholesterol and go back to the Framingham Heart Study concept of lipid ratios to determine the population at risk of ATD. The CRF is thus advocated as a lipid predictor worthy of note.

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OCCUPATIONAL ALLERGY

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ABSTRACT

An estimated 11 million workers in the USA are potentially exposed to agents that can become a cause of allergic diseases such as occupational asthma and allergic contact dermatitis, which can adversely affect health and well-being. Hundreds of chemicals (e.g. metals, epoxy and acrylic resins, rubber additives, and chemical intermediates) and proteins (e.g. natural rubber latex, plant proteins, mould, animal dander) present in virtually every industry have been identified as causes of allergic disease. In general, allergens can be classified as low molecular weight (chemical) allergens and high molecular weight (protein) allergens. These agents are capable of inducing immunological responses that are both immunoglobulin E and non-immunoglobulin E-mediated. Interestingly, the same chemical can induce diverse immune responses in different individuals. As new hazards continue to emerge, it is critical to understand the immunological mechanisms of occupational allergic disease. Specific understanding of these mechanisms has direct implications in hazard identification, hazard communication, and risk assessment. Such efforts will ultimately assist in the development of risk management strategies capable of controlling workplace exposures to allergens to prevent the induction of sensitisation in naïve individuals and inhibit elicitation of allergic responses. The purpose of this short review is to give a brief synopsis of the incidence, agents, mechanisms, and research needs related to occupational allergy.

Keywords: Allergic contact dermatitis (ACD), occupational allergy, asthma.

INTRODUCTION: INCIDENCE OF OCCUPATIONAL ALLERGY

Occupational immune diseases are among the most common illnesses that affect workers. An estimated 11 million workers in the USA, across every industrial sector, are potentially exposed to agents that can produce allergic diseases including: asthma, allergic contact dermatitis (ACD), urticaria, allergic rhinitis, eczema, and folliculitis.[1] Significantly, occupational exposures are responsible for approximately 9–25% of all adult onset asthma cases,[2,3] while ACD represents 20% of all work-related cutaneous disorders.[4] These diseases can adversely affect an individual's health and capacity to perform at work, resulting in significant economic losses.[5,6] Similar findings have been reported in Europe and other developed nations where occupational allergens are a recognised health hazard.[7]

Occupational asthma and ACD have been reported to show increased incidence in healthcare workers;[8,9] hairdressers and cosmetologists;[10,11] individuals working in manufacturing and automotive industries;[12,13] cleaning and janitorial staff;[14,15] food processing and packaging workers;[16,17] animal handlers;[18] and individuals working with metals,[19] compared to individuals in other occupational sectors. Over 250 causative agents of occupational asthma have been reported[20] and approximately 400 allergens are available for patch testing in humans,[4] demonstrating the breadth of potential allergens found in the workplace.

Table 1: Common occupational allergens.

Agent	Occupation/industry
High molecular weight allergens	
Flour dust	Food processing, bakers, grain handlers
Enzymes	Detergents, food, bakers
Plant products	Healthcare, food, agriculture
Wood dust	Furniture, sawmill
Animal products and dander	Farmers, food, veterinary, laboratory
Low molecular weight allergens	
Isocyanates	Manufacturing, spray paint, plastics, polyurethane, plastics
Anhydrides	Chemical manufacturing, flame retardants, epoxy adhesives, plastics
Amines	Chemical manufacturing, spray painting, welding, metalworking
Metals	Paints, metal plating, welding
Plastics	Adhesive, textiles, coatings
Dyes	Hairdressing, food, photography, textiles
Antimicrobials and biocides	Healthcare, janitorial, food, disinfectants

TYPES OF DISEASE

For the purpose of this manuscript, only immunological allergic diseases will be reviewed. The severity of allergic disease can be influenced by several factors including the route of exposure, the source of exposure, the environment, and genetics. Allergic diseases are characterised by a latency period between exposures (sensitisation) and symptoms (elicitation) and may involve immunoglobulin E (IgE) and non-IgE-mediated responses. In the context of hypersensitivity or allergic reactions there are four basic hypersensitivity reactions as originally classified by Gell and Coombs in 1963.[21] The distinct responses were characterised based on the primary effector molecules and immune cells involved in each reaction. Type I and Type IV (referred to as IgE and non-IgE-mediated, respectively) are the most common hypersensitivity reactions in the occupational setting. While in recent years these classification schemes have been further subcategorised, the importance of the role of the innate immune system in allergy is increasingly being recognised. These concepts are beyond the scope of this review.[22,23]

Immunoglobulin E-Mediated

An IgE-mediated allergic reaction is mediated by IgE antibody and mast cells and is sometimes called immediate-type hypersensitivity (Type I). It involves the initiation of T helper 2 cytokines, such as interleukin (IL)-4 and IL-13, leading to IgE production by B cells. Once IgE is produced and secreted, it binds to mast cells and basophils. Upon activation, these cells degranulate and release soluble allergic mediators, such as histamine and leukotrienes, which act on smooth muscles, sensory nerves, mucous glands, arteries, and eosinophils.[24] Common clinical outcomes of an IgE-mediated reaction are increased vascular permeability, smooth muscle cell contraction, and vasodilation. IgE-mediated reactions manifest within minutes to hours of exposure. Depending on the site(s) and frequency of allergen exposure, these reactions may occur in one or more organs resulting in diseases such asthma, allergic rhinitis, urticaria, and anaphylaxis.

Non-Immunoglobulin E-Mediated

A non-IgE-mediated or delayed type hypersensitivity response (Type IV) is T cell-mediated and characterised by excessive inflammation. The most distinctive feature of a non-IgE-mediated hypersensitivity response is the delay observed between allergen exposure and immune response. Following sensitisation, subsequent exposures result in elicitation of the non-IgE-mediated response, characterised by the secretion of proinflammatory cytokines (granulocyte-macrophage colony-stimulating factor, interferon-γ IL-3, IL-12, and tumour necrosis factor-β) that activate and recruit macrophages and other immune cells. Due to the time it takes for these

cytokines to attract and activate macrophages at sites of exposure, the effector phase typically occurs 24 hours following exposure and it generally peaks at 48–72 hours after exposure.[24] ACD is an example of a non-IgE-mediated hypersensitivity reaction.

OCCUPATIONAL ALLERGENS

Occupational allergens encompass a wide variety of substances. This includes both proteins and chemicals, high and low molecular weight (HMW/LMW) compounds, and natural and synthetic products (Table 1). Some of the most common allergens are wheat and enzymes (bakeries); latex, antimicrobials, and biocides (healthcare workers); isocyanates and anhydrides (manufacturing); nickel and cobalt (metal workers); and persulphates (hairdressers). Typically, occupational allergens are classified as either HMW >5 kDa, or LMW <5 kDa, and their size is thought to play a significant role in their allergenicity and mechanism of action. Protein allergens are usually HMW, while chemical allergens are LMW. HMW agents act as complete antigens and are innately immunogenic, whereas LMW chemicals must first react with autologous or heterologous proteins to form a hapten-complex before they can act as a functioning allergen. IgE responses are most commonly seen following HMW antigen exposure but can also be seen following LMW exposures. Metal ions such as nickel, cobalt, and chromium are among some of the most common triggers of ACD.[25] However, much less is known about the immunological responses to metals.[26] In addition to frequent exposure, other factors, such as predisposing skin injuries, atopy, and genetics, may influence an individual's susceptibility to developing allergies.

Low Molecular Weight Occupational Allergens

LMW chemical allergens are diverse in structure, reactivity, and application; however, there are several common attributes that are associated with immunogenicity including: haptenation potential (protein reactivity), ability to access the epithelium, and irritancy potential.[27,28] Thousands of chemicals have been identified as causative agents of skin sensitisation resulting in ACD, while substantially fewer chemical allergens (<100) have been identified as causative agents of asthma.[29] For the majority of LMW sensitisers, the immunologic response has no proven mechanisms and often can result in non-IgE and IgE-mediated responses.[27]

One of the most common occupationally-relevant chemical allergens, toluene diisocyanate (TDI), is a highly reactive chemical utilised in the automobile industry and in the manufacture of polyurethane foams, paints, elastomers, and coatings. TDI is a potent allergen and exposure can lead to a variety of diseases, including asthma, rhinitis, and ACD.[20,30] The incidence of asthma related to occupational TDI exposure has been estimated at ≤5.5% for the total workforce.[13] Based on the majority of the available epidemiological data, persulphate salts are reported as another common occupational allergen and may cause ACD, urticaria, rhinitis, and asthma.[10,11] Persulphate salts (ammonium, potassium, and sodium) are inorganic salts used as oxidising agents in hair bleaches and hair-colouring preparations at concentrations of ≤60%.[31] TDI and persulphate salts are generally classified as IgE-mediated sensitisers but may also induce a non-IgE-mediated response.[32,33] However, while animal studies support an IgE-mediated mechanism, TDI asthmatics often have no measurable TDI-specific IgE. Similar findings have been reported for persulphate.[34,35] The complete immunological mechanisms of sensitisation for these chemicals and other LMW sensitisers are not fully understood.

Numerous LMW chemical allergens are used in the healthcare profession. These include biocides (formaldehyde, glutaraldehyde, and orthophthaldehyde) commonly used to sterilise medical devices that are sensitive to normal heat or steam sterilisation processes and as disinfectants for surfaces (quaternary ammonia compounds).[36] Aldehydes and quaternary ammonia compounds have been identified as some of the most common non-IgE-mediated allergens.[25] In addition, medical gloves containing certain rubber accelerators (thiuram mix and carba mix), and antibacterial hand sanitisers and soaps (chloroxylenol and cocamide diethanolamine), have also been identified as common sources of allergens.[36] The above examples represent some of the most common occupational LMW allergens; however, many other occupationally relevant LMW allergens exist.

High Molecular Weight Allergens

Since the majority of allergies induced by HMW allergens are IgE-mediated, detection and quantification of specific IgE that recognises the responsible protein is used for confirmation of allergy. This can be evidenced through positive skin prick tests or immunoassays.[37] Several challenges

exist in the diagnosis and identification of HMW allergy. The HMW allergens in some compounds, such as wheat and latex, have been better characterised than others. Additionally, while most recombinant proteins are available for testing, multiple proteins may be responsible and individuals may have different sensitivities to different proteins which may present a challenge for the identification of the suspect agent.[37] In addition, LMW chemicals may be a component of the crude allergen (introduced via processing or manufacturing) and may also result in non-IgE-mediated responses.

It is estimated that 6–17% of healthcare workers suffer from latex allergy, with rubber gloves being the most common cause.[38] Latex allergy can manifest as urticaria, rhinitis, conjunctivitis, asthma, anaphylaxis, and ACD. Latex is extracted from the *Hevea brasiliensis* tree (rubber tree) and contains an array of cellular proteins, lipids, and amino acids. The responsible allergens in latex have not been fully characterised but a list of 15 allergens (Hev b 1–Hev b 15) has been established with Hev b 5, Hev b 6.01, and Hev b 6.02 identified as the most common occupational latex allergens.[39] Chemicals such as thiurams, stabilisers, and antioxidants (thiocarbamates, diphenylamine, dihydroquinoline, and phenylenediamine), which may be added to the latex during the manufacturing of rubber, have been recognised to induce ACD.[40]

Flour is another very common HMW occupational allergen and epidemiological reports have revealed that asthma, rhinitis, and ACD are the major health effects due to exposure.[41] Flour is a complex organic dust containing cereals which have been processed by milling. Flour dust usually contains various components which play an important role in dough improvement, such as a variety of enzymes (α-amylase, cellulose, hemicellulose, malt enzymes), additives (baker's yeast, egg powder, milk powder, sugar), flavourings, spices, and chemical ingredients (preservatives, antioxidants, bleaching agents). Wheat is the main flour used in the baking industry and has been found to contain at least 40 allergens which represent about 10–15% of the dry weight of the grain.[42] Baker's asthma is one of the most frequently occurring forms of occupational asthma and most studies indicate that wheat and rye flour proteins are allergens for 60–70% of bakers with workplace-related respiratory problems.[43] The enzyme α-amylase (added to improve baking characteristics), thioredoxin, plain lipid transfer proteins, and serine proteinase inhibitors are among the main factors associated with baker's asthma and studies have found that the highest frequency of specific IgE measurements were identified for α-amylase inhibitors Tri a 28 and Tri a 29.01.[41] Chemical components in flour such as preservative and bleaching agents have also been shown to cause ACD in bakers.[44]

Exposure to laboratory animals has been shown to result in occupational allergy and is commonly observed among technicians, animal caretakers, physicians, and scientists who work in pharmaceutical industries, university laboratories, and animal breeding facilities.[45] Rodents such as mice and rats, that are frequently used in animal research, are the most common causes of occupational allergy to laboratory animals. Mouse sensitisation is increasing in laboratory animal technicians and researchers due to the dramatic increase in the use of mice in experimental models. It is estimated that between 5% and 8% of this population is affected with some estimates suggesting an increase of ≤23% over a 2-year period in the USA. Urine is the main source of the allergenic protein in both mice and rats but allergens can also be found in dander, hair, saliva, and serum.[46] As with most mammals, the major inhaled allergens in mice and rats are lipocalins (Mus m 1 and Rat n 1, respectively). These allergens share 64% homology between their amino acid structures. Mouse urinary protein has shown IgE cross-reactivity with rat urinary protein and Equ c 1 (a major horse allergen).[47]

Metals

Metals are considered to be one of the most common occupational allergens and it is estimated that 10–15% of the population have allergies to at least one species of metal.[48] Occupational exposure to metals can result in varying levels of morbidity and mortality due to the induction of a wide range of allergic diseases including ACD, occupational asthma, and anaphylaxis. Surprisingly, little is known about the immunologic mechanisms driving the reaction behind metal allergy.[26,48] Metals are thought to interact directly with the surface of human lymphocytes to stimulate the adaptive immune response, however the exact mechanism is not fully understood.[49] Recent research also supports a role for the involvement of the innate immune system (specifically toll-like receptors) in the allergic responses to metals.[50] Numerous metals including gold, chromium, cobalt, platinum,

nickel, palladium, and mercury are known to induce allergic responses resulting in ACD and asthma.[19] Following patch testing of 4,454 patients (not all due to occupational exposure), nickel sulphate (19.0%), cobalt chloride (8.4%), and potassium dichromate (4.8%) were among the most common allergens, with nickel being identified as the most frequent positive allergen.[19] Sources of occupational allergen exposure include releases from dental tools and alloys,[51] scissor and nail instruments used by cosmetologists and nail technicians,[52] coin handling operations,[53] and metal processing.[54]

Challenges and Research Needs

Basic research

As new potential allergens are identified, it is critical that we fully understand the immunological mechanisms of occupational allergic disease. Research is needed to fill gaps in basic knowledge about the hazards of these agents. Areas of interest include: i) elucidating the mechanisms of allergic disease, ii) identifying exposure assessment biomarkers, iii) describing the role of genetics and the environment in allergic disease, iv) characterisation of complex exposures leading to allergic diseases, and v) developing predictive testing for the identification of occupational allergens.

The classification of allergens, especially LMW allergens, has often proven to be difficult since studies have identified that exposure to certain chemicals can result in multiple hypersensitivity pathways (i.e. both ACD and asthma). A more complete and thorough understanding of the immune-mediated mechanisms is needed before we will be capable of identifying, preventing, and treating allergic diseases. The need for the identification of potential exposure assessment biomarkers for sensitisation and exposure-sensitisation response relationships of occupational sensitisers is also imperative. Recently, many novel cellular subsets and molecules potentially involved in immunological allergic responses have emerged as potential candidates for biomarkers. The identification of the potential involvement of novel T helper subsets and non-coding RNA elements, such as microRNAs[55] in allergic disease, illustrates the advancement of these research needs. Additional studies are necessary to determine the relative role of individual versus complex workplace exposures in the development of allergic disease. This is of concern because investigations of individual chemicals may not adequately reflect the mixed exposures that often occur in occupational settings. Understanding the role of genetics and the environment on the allergic response is also critical.[56] An example of the importance of genetic factors in susceptibility to allergic disease is the influence of human leukocyte antigen genes on TDI asthma susceptibility. Several studies involving TDI-exposed workers demonstrated that specific human leukocyte antigen Class II genotypes were over-represented in asthmatic workers compared to asymptomatic workers.[56] Characterising the role of exposure route is another substantial challenge. Historically, the focus has been on describing the toxicity associated with the inhalation of hazardous substances. Available evidence clearly demonstrates the role of the skin as an important organ in respiratory disease. Factors such as skin integrity have been shown to influence sensitisation and the development of the respiratory allergic response.[57] However, additional research is needed to fully understand the role of the skin in respiratory allergic disease. Immunological assessment for occupational allergens is limited by the fact that standardised tests are not available for most workplace-relevant allergens. Predictive tests are critical for early identification of the hazard. It is understood that allergens may induce multiple types of allergic reactions. This is especially true for LMW allergens that can induce IgE and non-IgE-mediated responses. Due to the incomplete knowledge regarding mechanisms, predictive tests are lacking for these kind of exposures. Early detection of preclinical biomarkers of sensitisation may prevent development of occupational diseases through the implementation of the proper administrative and engineering controls.

Applied Research

Occupational allergy has significant social and economic implications for workers, their families, their employers, and government agencies. Sensitised workers must avoid exposure to the allergen both at work and outside the workplace in order to have the best chance of improvement or clearing of the allergic manifestations. This may be achieved by altering workplace tasks and duties, implementing engineering controls, or by providing workers with appropriate personal protective equipment. Most often, a sensitised worker would have to move to a completely different area or change to a different workplace or occupation to avoid further exposure to the offending allergen.

However, numerous approaches that integrate risk assessment and risk management strategies have been developed to control workplace exposures to occupational allergens.[58-61] In this context, it is imperative to establish an effective risk management strategy that is designed to prevent the induction of sensitisation in naïve individuals and inhibit elicitation of allergic responses in those that have become sensitised.[2] Such a strategy should include both primary and secondary prevention methods. Primary prevention methods are interventions used to prevent worker sensitisation and may include the following:

- Modification of the allergen to inhibit exposure
- Application of control methods to prevent exposures
- Substitution with a less harmful agent
- Use of personal protective equipment[62]

Secondary prevention methods attempt to characterise workplace exposure, in addition to detecting and limiting the progression of allergic diseases. Examples of secondary preventive methods include medical monitoring[58,62,63] and workplace exposure monitoring.[2,58,61]

Another important tool applied to characterise and aid in controlling workplace exposures to occupational hazards are occupational exposure limits (OELs). Despite their widespread use globally, few OELs are established on the basis of preventing sensitisation. The quantitative risk assessment approaches used to derive OELs have been developed primarily for non-immune-mediated effects, such as portal of entry effects, non-cancer systematic effects, or cancer. Application of these approaches to develop OELs for allergens has been inhibited because of data limitations and a lack of understanding of the biological processes that govern immune-mediated effects. The route of exposure, exposure intensity, and duration/frequency of exposure have also been identified as factors complicating this process.[2] Research addressing these challenges along with a better understanding of allergic disease has direct implications in hazard identification, informing appropriate risk assessment, and management decisions to facilitate interventions and prevention of occupational allergies.

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EXTENSION OF 2016 WORLD HEALTH ORGANIZATION (WHO) CLASSIFICATION INTO A NEW SET OF CLINICAL, LABORATORY, MOLECULAR, AND PATHOLOGICAL CRITERIA FOR THE DIAGNOSIS OF MYELOPROLIFERATIVE NEOPLASMS: FROM DAMESHEK TO VAINCHENKER, GREEN, AND KRALOVICS

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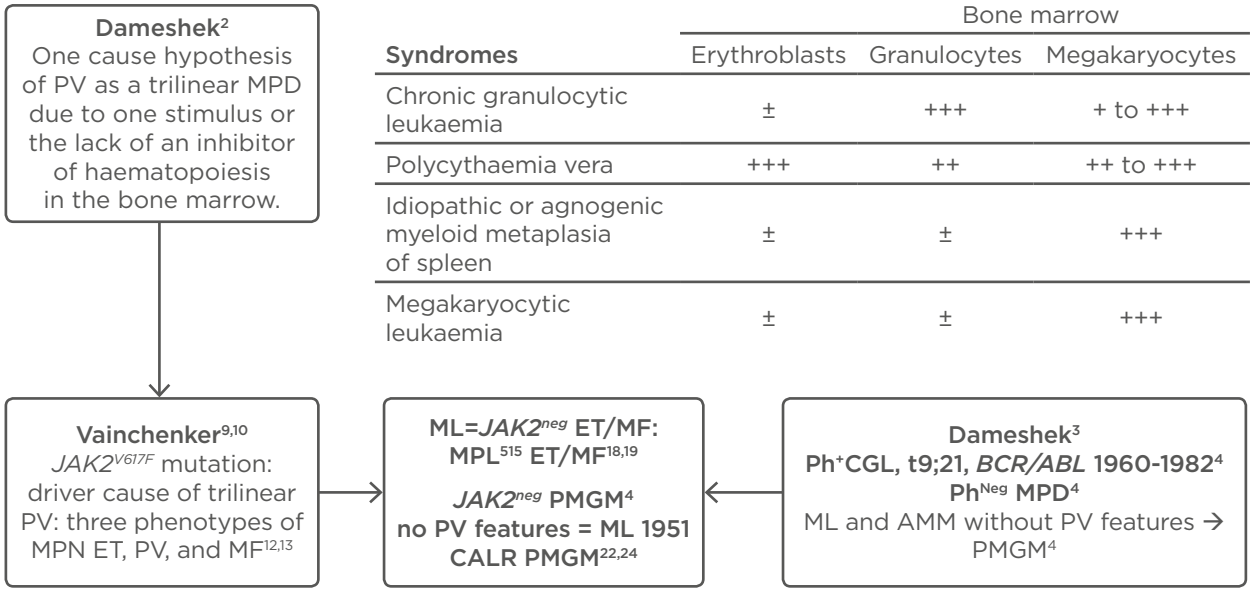
ABSTRACT

Improved Clinical, Laboratory, Molecular, and Pathological (CLMP) 2017 criteria for myeloproliferative neoplasms (MPN) define the *JAK2*^{V617F} trilinear MPNs as a broad continuum of essential thrombocythaemia (ET), polycythaemia vera (PV), masked PV, and post-ET or post-PV myelofibrosis (MF). Normal versus increased erythrocyte counts (5.8x10^{12}/L) on top of bone marrow histology separate *JAK2*^{V617F} ET and prodromal PV from early and classical PV. Bone marrow histology of the *JAK2*^{V617F} trilinear MPNs show variable degrees of normocellular megakaryocytic, erythrocytic megakaryocytic and erythrocytic megakaryocytic granulocytic (EMG) myeloproliferation, peripheral cytoses, and splenomegaly related to *JAK2*^{V617F} allele burden. *MPL*^{515} thrombocythaemia displays predominantly normocellular megakaryocytic proliferation. *CALR* thrombocythaemia intially presents with megakaryocytic followed by dual granulocytic and megakaryocytic myeloproliferation without features of PV. The megakaryocytes are large, mature, and pleomorphic with hyperlobulated nuclei in *JAK2*^{V617F} ET and prodromal, classical, and masked PV. The megakaryocytes are large to giant with hyperlobulated staghorn-like nuclei in *MPL*^{515} thrombocythaemia. The megakaryocytes are densely clustered, large, and immature dysmorphic with bulky (bulbous) hyperchromatic nuclei in *CALR* thrombocythaemia and MF.

Keywords: Myeloproliferative neoplasms (MPN), essential thrombocythaemia (ET), polycythaemia vera (PV), primary megakaryocytic granulocytic myeloproliferation (PMGM), thrombocythaemia, myelofibrosis (MF), *JAK2*^{V617F}, *JAK2* exon 12; *MPL*^{515}, calreticulin, triple negative.



Syndromes	Bone marrow			
	Erythroblasts	Granulocytes	Megakaryocytes	Fibroblasts
Chronic granulocytic leukaemia	±	+++	+ to +++	+
Polycythaemia vera	+++	++	++ to +++	+ to +++
Idiopathic or agnogenic myeloid metaplasia of spleen	±	±	+++	+ to +++
Megakaryocytic leukaemia	±	±	+++	+

Figure 1: Changing concepts of PVSG-WHO classification of myeloproliferative neoplasms into CLMP criteria of *JAK2*^{V617F} mutated essential thrombocythaemia, polycythaemia vera, and myelofibrosis and JAK2 wildtype CALR or MPL mutated thrombocythaemia and myelofibrosis: from Dameshek to Vainchenker, Kralovics and Michiels 1950–2017.

Left) The Dameshek (1950) one cause hypothesis of PV as a trilinear MPD[2] has been confirmed by Vainchenker's discovery in 2005 of the *JAK2*^{V617F} mutation[8] as the driver of the trilinear MPNs, ET, PV, and MF.[9,10,11,33] Upper) Dameshek (1951)[3] speculated on the possible causal interrelationship among MPS showing trilinear bone marrow features in PV, dual increase of megakaryocytes and fibroblasts in AMM and unilinear megakaryopoiesis in ML. Right middle) The Hannover and Rotterdam Bone Marrow Classifications of the MPD recognised prefibrotic ML and AMM as a distinct entity of prefibrotic and fibrotic stages of PMGM without features of PV.[4] Bottom) ML defined by Dameshek in 1951 can readily be translated into JAK2-negative *MPL* mutated ET and MF and *CALR* ET associated with PMGM without features of PV.

ET: essential thrombocythaemia; MF: myelofibrosis; PV: polycythaemia vera; MPN: myeloproliferative neoplasms; MPD: myeloproliferative disorders; ML: megakaryocytic leukaemia; MPS: myeloproliferative syndromes; AMM: agnogenic myeloid metaplasia; PMGM: primary megakaryocytic granulocytic myeloproliferation.

INTRODUCTION

The diagnostic clinical and bone marrow criteria of polycythaemia vera (PV) between 1940 and 1950 were plethoric appearance, splenomegaly, elevated erythrocyte count >6x10^{12}/L, elevated platelet count, elevated haematocrit (Ht), and pathognomonic bone marrow features showing a panmyelosis with increased erythrocytic megakaryocytic granulocytic (EMG) trilinear haematopoiesis.[1] About one-third of PV patients develop splenomegaly and myelofibrosis (MF) after follow-up of 15–30 years.[2,3] The combination of a persistent increase of platelet counts (>350x10^9/L) and a monolinear proliferation of large mature megakaryocytes in the bone marrow is diagnostic for essential thrombocythaemia (ET).[4]

Dameshek[3] speculated on the possible causal interrelation among myeloproliferative disorders (MPD) showing trilinear bone marrow features in PV, the dual increase of megakaryopoiesis in agnogenic myeloid metaplasia (AMM), and unilinear megakaryopoiesis in megakaryocytic leukaemia (ML) without PV features in the bone marrow (Figure 1).[3] The Polycythemia Vera Study Group (PVSG) criteria for the clinical diagnosis of PV do not include a bone marrow biopsy to differentiate between PV and erythrocytosis and overlook idiopathic erythrocythaemia by definition.[5,6] Idiopathic erythrocythaemia is distinguished by increased red cell mass (RCM), normal platelet count and leukocyte count, and no splenomegaly on palpation.[5,6] Lamy et al.[7] measured RCM in 85 patients meeting the PVSG criteria for PV

(haemoglobin [Hb] >18 g/dL, Ht >0.52 in males and Hb >16 g/dL, Ht >0.47 in females) and in 18 patients with masked PV (inapparent PV) with normal erythrocyte, Hb, and Ht values. RCM was increased in patients with classical PV with no or minor splenomegaly. RCM was increased in masked or inapparent PV patients due to significant splenomegaly and hypersplenism.[7] The PVSG defined idiopathic or AMM of spleen and bone marrow as primary MF (PMF). As MF is a secondary event in all variants of MPD, the Hannover and Rotterdam Bone Marrow Classification discovered prefibrotic ML and fibrotic stages of AMM as the third distinct MPD entity of primary megakaryoctic granulocytic myeloproliferation (PMGM) without features of PV (Figure 1).[4]

MOLECULAR AETIOLOGY OF *JAK2*^{V617F} TRILINEAR MYELOPROLIFERATIVE DISORDERS

The one cause hypothesis of Dameshek[2] that PV is a trilinear MPD has been confirmed by Vainchenker's discovery in 2005 that the acquired *JAK2*^{V617F} mutation is the driver cause of three MPD phenotypes: ET, PV, and MF (Figure 1).[8-10]

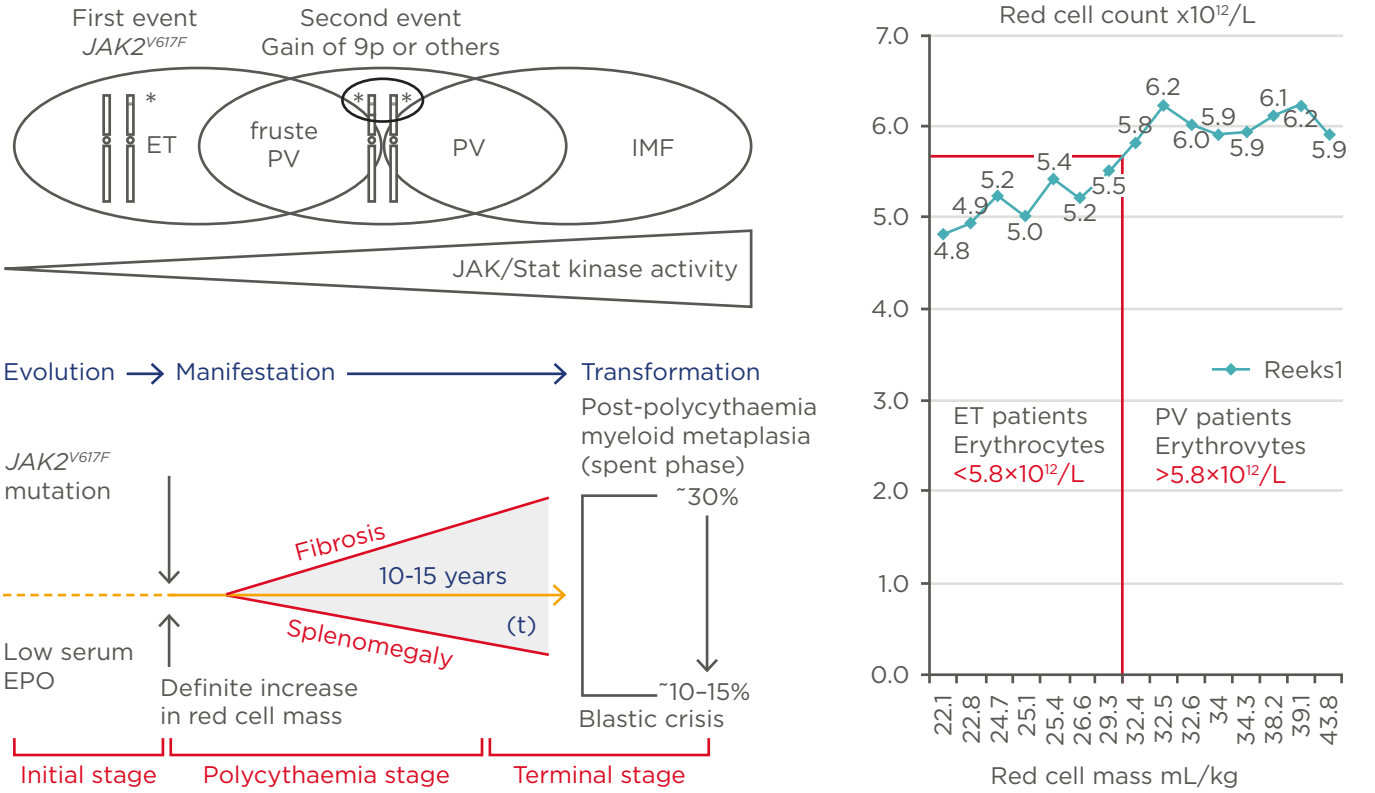


Figure 2: The sequential occurence of CLMP defined essential thrombocythaemia, polycythaemia vera, and myelofibrosis related to *JAK2* allele burden in *JAK2*^{V617F} mutated trilinear myeloproliferative neoplasms.

Upper) The discovery of the somatic *JAK2*^{V617F} gain mutation can explain the three sequential phenotypes of ET, PV, and MF. A slight increase (changes) in the *JAK2*^{V617F} kinase activity in heterozygous mutated MPN is enough to produce the clinical phenotype of ET. Increasing levels of *JAK2*^{V617F} kinase activity in trilinear MPN due to mitotic recombination resulting in heterozygous/homozygous and predominantly homozygous mutated MPN is associated with early, overt, and advanced PV, respectively (Vainchenker and Constantinescu 2005,[9] Villeval et al. 2006.[10] Lower) Dynamics of the *JAK2*^{V617F} disease processes in PV as a broad spectrum (Tables 1 and 2) ranging from normocellular ET, prodromal PV mimicking ET and the defintive increase in red cells (>5.8x10^{12}/L) followed by masked PV, PV complicated by fibrosis and splenomegaly, spent phase PV and blastic transformation. Designed by Michiels et al. 2006–2016. Right) Initial stage of *JAK2*^{V617F} mutated ET and prodromal PV with normal RCM and erythrocytes <5.7x1012/L versus manifest PV with definitive increase of RCM and erythrocytes >5.7x10^{12}/L.[4,37]

EPO: erythropoietin; ET: essential thrombocythaemia; RCM: red cell mass; PV: polycythaemia vera; MF: myelofibrosis; MPN: myeloproliferative neoplasms.

Vainchenker and Constantinescu[9] proposed the concept that low *V617F* constitutional kinase activity in heterozygous mutated *JAK2*^{V617F} mutated patients is enough to produce the ET phenotype and that higher *V617F* constitutional kinase activity in *JAK2*^{V617F} mutated heterozygous/homozygous or homozygous mutated patients is needed to produce the PV phenotype (Figure 2).[9,10] The *JAK2*^{V617F} dosage hypothesis has been confirmed at the bone marrow haematopoietic stem cell level by the demonstration that endogenous erythroid colonies (EEC) from ET patients are mainly heterozygous for the *JAK2*^{V617F} mutation, whereas all PV patients are either hetero/homozygous or mainly homozygous for the *JAK2*^{V617F} mutation (Figure 2).[11]

Michiels and Medinger[12] studied RCM in relation to erythrocyte count in World Health Organization (WHO)-defined ET patients (24 patients) and PV patients (46 patients) with no or minor splenomegaly. The *JAK2*^{V617F} mutation load in 24 ET patients was zero in 10 patients and positive in 14 patients; this mutation load ranged from 3–20%, 20–42%, and >50% in six, five, and two cases, respectively. The *JAK2*^{V617F} mutation load in 36 evaluable PV patients ranged from 3–20% and from 20–50% and was >50% in 5, 12, and 19 PV cases, respectively. Increased erythrocyte counts above normal levels (>5.8x10^{12}/L in males and >5.6x10^{12}/L in females) correlated with increased RCM in PV patients whereas ET patients had normal erythrocyte counts and RCM (Figure 2).[12] Increased RCM and erythrocytes >5.8/5.6×10^{12}/L in PV were associated with Hb values from 14.6–18.9 g/L and Ht values from 0.46–0.57. Normal RCM in ET patients were related to erythrocyte counts of 4.6–5.4×10^{12}/L, Hb from 14.0–16.1 g/L, and Ht from 0.39–0.47,[13] consistent with the diagnosis of ET or prodromal PV (Tables 1 and 2).

The mutation load in percentages of *JAK2* mutated granulocytes in a large retrospective Italian study of *JAK2*^{V617F} trilinear MPNs was low in 250 ET patients (median: 18%), significantly higher in 212 PV patients (median: 42%) and 18 post-ET MF patients (median: 42%), and predominantly high (>50%) in post-PV MF (median: 93%) patients.[14] A JAK2 allele burden >50% (homozygous) was recorded in 2% of 250 ET patients, in 41% of 212 PV patients, in 72% of 18 post-ET patients, and in 93% of 55 post-PV patients.[14] The correctness of the *JAK2* dosage hypothesis has been confirmed in patients with hereditary ET caused by the heterozygous germline gain of function mutations *JAK2*^{V617I} and *JAK2*^{R564Q} in the JAK2 gene.[15-17] Affected hereditary ET patients heterozygous for the *JAK2*^{V617I} and *JAK2*^{R564Q} germline mutations have a clinical ET phenotype with normal values for Hb, Ht, erythrocytes, thrombopoietine (TPO), and erythropoietin (EPO) levels. The response to EPO in the EEC assay was normal in congenital *JAK*^{V617I} and *JAK2*^{R564Q} but increased in acquired *JAK2*^{V617F}.[15-17]

JAK2 WILDTYPE *MPL*^{515} MUTATED MEGAKARYOCYTIC LEUKAEMIA OR ESSENTIAL THROMBOCYTHAEMIA (FIGURE 1)

With the advent of the *JAK2*^{V617F} discovery, two variants of *JAK2*^{neg} MPN have been discovered: *MPL*^{515} mutated ET and MF[4,18,19] and *CALR* mutated ET and MF in PMGM patients.[19-23] (Figure 1). *MPL*^{W515L} and *MPL*^{W515K} as the driving cause of MPN in large series of ML, or ET and MF patients occurred with a frequency rate of approximately 1% and 5%, respectively.[18,19] In a European study[19] of 176 cases with the *MPL*^{515} mutation, the *MPL*^{W515L} mutation occurred in 110 cases, and the *MPL*^{W515K} mutation in 58 cases. The overall mutation levels were lower (25%) in *MPL*^{W515L} (n=106) compared with the level of 37% in cases with *MPL*^{W515K} (n=32). Of the 138 *MPL*^{515} cases (ET, n=99; MF, n=36; ratio of ET versus MF: 2:1), the median *MPL*^{W515L} mutation levels were significantly lower (21%) in ET than those (46%) in MF patients. The 29 homozygous *MPL*^{515} positive cases had a diagnosis of MF in 15 patients and ET in 12 patients.

The presence of clustered small and giant megakaryocytes with deeply lobulated staghorn like nuclei in *MPL*^{515} mutated ET are not seen in *JAK2*^{V617F} positive normocellular ET, prodromal PV, masked PV, and PV.[20,21] The pleomorphic megakaryocytes in *JAK2*^{V617F} mutated ET in bone marrow biopsy were not larger but similar in size to medium to large megakaryocytes (pleomorphic) in prodromal and overt PV. Erythropoiesis in *MPL* thrombocythaemia is reduced whereas a local increase of erythropoiesis in areas of loose clustered pleiomorphic megakaryoctyes is present in *JAK2*^{V617F} normocelluar ET and prodromal PV. LAF score, serum EPO, and ferritin levels are normal in *MPL* MPN cases and increased in *JAK2*^{V617F} MPN cases.[20,21]

Table 1: International Clinical, Laboratory, Molecular, and Pathobiological (2017 CLMP) criteria for diagnosis of *JAK2*^{V617F} mutated essential thrombocythaemia, prodromal polycythaemia vera, masked polycythaemia vera due to splenomegaly, and post essential thrombocythaemia myelofibrosis.

CLM criteria	Bone marrow cellularity and pathology
ET	**Normocellular megakaryocytic**
1. Platelet count of >350x10^9/L 2. Heterozygous *JAK2*^{V617F} low *JAK2* mutation load 3. Normal erythrocytes <5.8x10^{12}/L males; <5.6x10^{12}/L females 4. Normal haemoglobin and hematocrit	Normocellular bone marrow (<60%), M proliferation and clustering of medium sized to large (pleomorphic) mature megakaryocytes No proliferation of granulopoiesis and no or some increase of erythropoiesis. RF 0 or 1
Prodromal PV	**Hypercellular EM**
1. Platelet count of ≥350x10^9/L; Normal erythrocytes. <5.8x10^{12}/L males; <5.6x10^{12}/L females. 2. *JAK2*^{V617F} intermediate to high *JAK2* mutation load 3. Low EPO, increased LAP score 4. Spontaneous EEC	Increased cellularity (60–80%) due to variable degrees of EM proliferation and no increase of granulopioesis. Proliferation and clustering of medium sized to large (pleomorphic) mature megakaryocytes. RF 0 or 1
Prefibrotic hypercellular ET Masked PV or myelofirosis	**Hypercellular trilinear EMG = masked PV[7,37] Hypercellular megakaryocytic granulocytic (MG=ET-MF)**
1. Platelet count of ≥350x10^9/L 2. Hb >12g/dL 3. *JAK2*^{V617F}; high *JAK2* mutation load 4. Slight or moderate splenomegaly 5. No preceding or allied CML, PV, PMGM, RARS-T, or MDS	EMG or MG proliferation with relative reduced erythroid precursors. Loose to dense clustering of pleiomorphic megakaryocytes with hyperploid or clumpsy nuclei. **Grading of RF and MF:**[4,37] Prefibrotic RF 0/1 = MF 0, Early fibrotic RF 2 = MF 1, Fibrotic: RF3/4, RCF = MF2/3

CLM: Clinical, Laboratory, and Molecular; ET: essential thrombocythaemia; PV: polycythaemia vera; EPO: erythropoietin; EEC: endogenous erythroid colony formation; Hb: haemoglobin; CML: chronic myeloid leukaemia; PMGM: primary megakaryocytic granulocytic myeloproliferation; MDS: myelodysplastic syndrome; RARS-T: refractory anaemia with ringed sideroblasts associated with marked thrombocytosis; EMG: erythrocytic megakaryocytic granulocytic; RF: reticuline fibrosis; MF: myelofibrosis.

MEGAKARYOCYTIC LEUKAEMIA AND *CALR* THROMBOCYTHAEMIA WITHOUT POLYCYTHAEMIA FEATURES

CALR as the driving cause of ML[3] or PMGM[4] (Figure 1) has been detected in the majority of *JAK2*^{neg} WHO-defined ET and PMF cases by Kralovics;[22] this was the second groundbreaking event in MPN molecular research that prompted us to revise and simplify the 2016 WHO and European Clinical, Molecular and Pathological (ECMP) MPN classifications[4,13,20,21] into a new set of Clinical Laboratory, Molecular and Pathologic (CLMP) criteria for *JAK2*, *MPL*, and *CALR* mutated MPNs (Tables 1, 2, and 3). The MPN research laboratory of Kralovics[4] discovered somatic mutations of 52-bp deletion in one patient, of 1-bp deletion in one patient, and recurrent 5-bp insertion in four PMF patients.[22]

Following sequencing and mutation screening in a cohort of 896 MPN patients, *CALR* mutations were detected in 78 of 311 (25%) ET patients, in 72 of 203 (35%) PMF patients, and in none of 382 PV patients. A total of 36 types of somatic *CALR* mutations (insertions and deletions) caused a frameshift reading frame with the resulting mutant CALR protein that shares a novel sequence in exon 9 with the C-terminal becoming positively charged amino acids, whereas the C-terminal of non-mutant CALR protein is negatively charged. Mutations of Type 1 (52-bp deletion) and mutations of Type 2 (5bp-insertions) accounted for 53% and 31.7% of all *CALR* cases. Other *CALR* variant mutations were observed at low frequencies or only in a single *JAK2* wildtype ET or MF patient.

A large cohort of 1,235 ET and PMF patients carried the *JAK2*^{V617F}, *MPL*^{515}, and *CALR* exon 9 mutation in 63.4%, 4.4%, and 23.5% of cases, respectively, and 8.8% were triple negative for these clonal markers.[22] *CALR* mutations mutually excluded both *JAK2*^{V617F} and *MPL*^{515} mutations since all *CALR* mutated ET and MF patients were negative for *JAK2*^{V617F}, exon 12 *JAK2*, and *MPL* mutations. The *CALR* mutation was detected in 195 of 289 (67%) *JAK2/MPL* wildtype ET, and in 105 of 120 (80%) *JAK2/MPL* wildtype MF.

Table 2: International Clinical Molecular and Pathological criteria for the diagnosis of *JAK2* mutated classical polycythaemia vera, masked polycythaemia vera due to splenomegaly or exon 12 PV versus primary or secondary erythrocytoses.

CLM criteria (A: major; B: minor)	Bone marrow pathology
A1) Erythrocytes >5.8x10^{12}/L in males; >5.6x10^{12}/L in females. Increased RCM optional **A2)** *JAK2*^{V617F} intermediate high mutation load **A3)** Low serum EPO level. Increased LAP score **B1)** Platelets >350 x10^{9}/L **B2)** Leukocytes >10 x10^{9}/L and raised LAP-score or increased CD11b expression **B3)** Splenomegaly on echogram (>12 cm). **Masked PV is defined by *JAK2*^{V617F} mutation, normal Hb, Ht, and erythrocytes <5.6x10^{12}/L, splenomegaly, and increased RCM due to splenomegaly and EMG bone marrow pathology.**[7,31,37]	**PV:** Increased cellularity (60–100%) due to increased EM in early stage and trilinear EMG proliferation (panmyelosis) **Endogenous erythroid colony formation** **Grading of reticuline fibrosis/myelofibrosis**[4,37] **Prefibrotic: RF-0/1 = MF-0** **Early fibrotic: RF-2 = MF-1** **Fibrotic: RCF 3/4 = MF-2/3** *JAK2* exon 12 mutated PV is a distinct entity

CLM: Clinical, Laboratory, and Molecular; PV: polycythaemia vera; Hb: haemoglobin; Ht: haematocrit; RCM: red cell mass; EMG: erthrocytic megakaryocytic granulocytic; RF: grading fibrosis; MF: myelofibrosis.

The *CALR* mutation was found in none of the 45 chronic myeloid leukaemia patients, 73 of myelodysplastic syndrome patients, 64 of chronic myelomonocytic leukaemia patients, and in 3 of 24 refractory anaemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) patients. The 24 RARS-T patients carried the *JAK2*^{V617F} in 10 cases, *MPL* in 2 cases, *CALR* in 3 cases, and *SF3B1* in 16 cases.[22] The UK MPN study Group of Dr Tony Green and co-workers detected the *CALR* somatic mutation in 110 of 158 *JAK2/MPL* wildtype MPN samples (80 of 112 ET, and 18 of 32 MF samples) in none of 511 *JAK2*^{V617F} or exon 12 *JAK2* mutated MPNs and in 10 of 120 myelodysplastic syndrome samples: RA in 5 of 53 cases, RARS in 3 of 27 cases, RA with excess blasts in 2 of 17 cases, CMML in 1 of 33 cases, and atypical CML in 1 of 29 cases.[23] The somatic *CALR* mutation was not found in 502 solid tumours, 1,015 cell lines, and 505 controls.[23]

The 52-bp deletions (*CALR* Type 1) eliminate almost all negatively charged amino acids, whereas the 5-bp insertions (*CALR* Type 2) retain aproximately half of the negatively charged amino acids.[22] Such genetic differences in Type 1 and Type 2 *CALR* mutations predict different clinical phenotypes. *CALR* Type 1 deletions occur more frequently in MF than in ET.[22] The USA–Italian study of Tefferi and Vanucchi[24] divided 1,027 ET patients into a test (n=402) and validation cohort (n=625). Among 402 ET patients, 227 (57%), 11 (3%), and 114 (28%) harboured *JAK2*, *MPL*, and *CALR* mutations, respectively and 12% were triple negative.[24] The 114 *CALR* ET patients were Type 1 in 51 (45%) and Type 2 in 44 (39%). Male sex was associated with Type 1, younger age with Type 2 variants, and platelet counts were significantly higher in Type 2 versus Type 1 *CALR* ET in the test and validation (n=111) cohorts of *CALR* ET patients.[24] A large French study by Cabagnols et al.[25] of 368 *CALR* MPN patients analysed the association of *CALR* Type 1 and Type 2 in ET (n=251) and MF (n=64) patients. The ratio of *CALR* ET to MF patients was 3.9.[25] The relative frequency of *CALR* Type 1 versus *CALR* Type 2 in 251 ET patients was 51% versus 39% and in 64 MF patients it was 70% versus 13%; the median age was 61 years in Type 1 and 52.5 years in Type 2 patients; and the mean platelet count was 731 x10^{9}/L in *CALR* Type 1 and 870x10^{9}/L in Type 2 *CALR* MPN patients.[25] A higher allelic burden was more frequent in CALR MF (5/35=14.3%) than in ET (6/158=3.8%). *CALR* MPN patients with a low allelic burden (<25%) were only observed in ET (19/158= 11.9%). *CALR* ET and MF patients were younger and had higher platelet counts than *JAK2* ET patients in several studies.[22,23-26] Leukocyte alkaline phospatase scores in the recent study of Kondo et al.[27] was normal to decreased in *CALR* MPN. Increased LAP scores are a prominent feature of *JAK2*^{V617F}, ET, PV, and masked PV.[4,21,28]

THROMBOSIS FREE AND OVERALL SURVIVAL IN *JAK2*, *CALR*, AND *MPL* MUTATED MYELOPROLIFERATIVE NEOPLASMS

In the original Austrian study by Kralovics of 186 CALR, 576 *JAK2*, and 35 *MPL* mutated ET

patients, the overall survival (OS) at 10 years was 96.9% for *CALR* ET patients and 91.1% in *JAK2*^{V617F} ET patients.[29] In the Italian ET study of 89 *CALR*, 369 *JAK2*^{V617F}, and 25 *MPL*^{515} mutated and 93 wildtype ET patients, the frequencies of microvessel symptoms were 24.7%, 27.4%, 56%, and 21.5%, respectively,[30] whereas the frequencies of major thrombosis at diagnosis, in the preceding 2 years and during follow-up were 13.5%, 30.1%, 40.0%, and 16% in *CALR*, *JAK2*, *MPL*, and wildtype ET patients, respectively.[30] The major thrombosis free survival was significantly longer in 89 *CALR* and 93 wildtype patients as compared with 369 *JAK2* and 25 *MPL* ET patients. The cumulative incidence of major thrombosis at 10 years were 5.1%, 14.5%, 19.5%, and 8.1% in *CALR*, *JAK2*, *MPL*, and wildtype thrombocythaemia patients, respectively.[30] In contrast, the Kaplan-Meier OS curves did not show significant differences between *JAK2*, *MPL*, and *CALR* ET patients, which indicated that the high frequency of major thrombosis patients in *JAK2* and *MPL* ET as compared to *CALR* ET patients has no prognostic significance in terms of OS in ET.[30]

CALR MF patients had a better OS than *JAK2* MF patients than in *CALR* MF (p=0.049) in several studies.[22-26] In the Austrian study, 98 *CALR* MF patients had a longer OS (median: 21 years) as compared with 189 *JAK2* MF (median: 11.0 years) and 18 *MPL* MF patients (median: 8.2 years).[29] The OS curves from a large Italian PMF study)[29] in 72 *CALR*, 396 *JAK2*, 25 *MPL*, and 53 triple negative MF patients show that *CALR* MF patients had a better median OS than *JAK2* MF patients (hazard ratio: HR *CALR/JAK2* 2.3, p<0.001), MPL-MF patients (HR *MPL/JAK2* 2.6, p=0.009) and triple-negative MF patients (HR wildtype/*JAK2* 6.2, p<0.001).[29] The median ages at time of diagnosis were 50, 63, 64, and 67 years for *CALR*, *JAK2*, *MPL*, and triple negative MF patients, respectively, indicating that the ultimate median age reached at time of death from MF will be near to 75 years and quite similar in PMF patients caused by the driver mutations *CALR*, *JAK2*, and *MPL* for MPN.[22,29]

CLINICAL, LABORATORY, MOLECULAR, AND PATHOLOGICAL FEATURES OF MYELOPROLIFERATIVE NEOPLASMS

The large cross-sectional Korean study of 407 WHO-defined MPN patients (111 PV, 179 ET, and 117 MF)[31] translated the 2016 WHO classes of ET, PV, and MF patients into four distinct CLMP classes of *JAK2*^{V617F}, exon 12 *JAK2*, *MPL*^{515}, and *CALR* myeloneoproliferations. The three driver mutations were detected in 82.6% of 407 MPN patients and showed a distribution frequency of three distinct MPNs: *JAK2* in 275 patients (67.5%), *CALR* in 55 patients (13.7%), and *MPL* in 6 patients (1.5%). The clinical phenotypes in 275 *JAK2* mutated MNP were PV in 101 cases, ET in 95 cases, and MF in 79 cases. The clinical phenotypes in 56 *CALR* mutated MPN were PV in no cases, ET in 40 cases, and MF in 16 cases.[31] The clinical phenotypes in six MPL cases were ET in three and MF in three. The seven cases of exon 12 *JAK2* were diagnosed as PV in its purity and none as ET or MF.[31]

The mean age of *CALR* mutated MPN patients (57.5 years) was 8.5 years younger than in *JAK2* mutated MPN patients (66 years).[31] Exon 12 *JAK2* mutated MPN patients presented with increased erythrocyte counts >5.8x10^{12}/L, normal platelet counts of <350x10^{9}/L, and no anaemia consistent with the diagnosis of erythrocythemic PV (Figure 2).[31] *CALR* mutated MPN (ET and MF) patients presented with normal to decreased values for Hb, Ht, and erythrocytes (upper limit <5.8/5.6x10^{12}/L) (Figure 2). Erythropoiesis in bone marrow histology studies was normal or reduced in all cases of *CALR* and *MPL* mutated MPN.[32]

The values for Hb, Ht, and erythrocyte counts in 2016 WHO-defined *JAK2*^{V617F} mutated MPN cases ranged from anaemic in MF, normal in ET, and increased in PV when the CLMP criteria are applied (Tables 1 and 2). Bone marrow lineage proliferation profile in 265 WHO-defined *JAK2* mutated MPN revealed monolinear megakaryocytic proliferation in 29.1% of cases; dual proliferation of erythropoiesis and megakaryopoiesis (EM, prodromal PV) in 13.5% of cases; trilinear proliferation of erythropoiesis, megakaryopoiesis, granulopoiesis (EMG, classical PV) in 31.3% of cases; and granulopoiesis megakaryopoiesis (*JAK2* MF) in 26.2% of cases when the simplified and improved EuroAsian CLMP criteria in Tables 1, 2, and 3 were applied.[31] Bone marrow lineage proliferation profile in 56 *CALR* mutated MPN cases revealed E and EG in zero, monolinear megakaryocytic in 66%, and dual GM in *JAK2*/MPL wildtype, but *CALR* mutated MPN in 34%[31] (formerly diagnosed as ML by Dameshek[3] or PMGM myeloneoproliferation [MNP] by Michiels et al.[4]).

The *JAK2* allele burden in WHO defined *JAK2*^{V617F} mutated MPN (ET, PV, MF) from the Korean cross-sectional MPN study[31] was widely distributed from 1.8–98.6%. The allele burden in exon 12 *JAK2*

mutated MPN remained <50%, which is completely in line with the heterozygosity of mutated exon 12 at the EEC level.[33] *JAK2*^{V617F} mutated 'forme fruste' PV (prodromal PV), early PV, and exon 12 PV patients presented with EM bone marrow neoproliferation without fibrosis (MF 0/1).[31] The allele burden in EM and EGM of the two JAK2 mutated MPNs was significantly higher in *JAK2*^{V617F} (84.9%) than in exon 12 *JAK2* (44.5%) MPN. The mean values of the *JAK2*^{V617F} allele burden in megakaryocyte (=ET), GM (=MF), EM (=PV), and EGM (=PV) bone marrow proliferations were 37.5%, 68.9%, 76%, and 89.2%, respectively. The *JAK2*^{V617F} EM and EGM molecular pathologic (MP) groups are associated with high allele burden and increased erythrocytes (>5.8x10^{12}/L, Figure 2), which is consistent with the diagnosis of classical PV. The normocellular megakaryocytic molecular genetic-pathologic (GP) groups in various clonal MPNs are associated with normal erythrocytes and leukocytes consistent with the diagnosis *JAK2* or *CALR* mutated thrombocythaemia and have the lowest allele mutation burden.[31] *JAK2*^{V617F} EGM and GM molecular GP groups have the highest allele burden and most pronounced leukocytosis, whereas allele burden and leukocytosis are much less pronounced in the *CALR* GM GP group. The grade of fibrosis in the Korean study[31] was divided into minimal (MF 0/1) and overt (MF 2/3), according to standardised criteria.[4,29,34] The frequency of overt fibrosis in *JAK2*^{V617F} and *CALR*-mutated and triple-negative MPN patients were 22.2%, 27.1%, and 29.3%, respectively. *JAK2*^{V617F}-GM and *CALR*-GM bone marrow histology showed a high rate of overt fibrosis (46.0 and 42.1%), followed by *JAK2*^{V616F}-M (17.5%), *CALR*-M (17.2%), and *JAK2*^{V617F} EGM (10.4%; p<0.001). None of the *JAK2*-EM ('forme fruste' and early PV and exon 12 PV) patients presented overt fibrosis. Bone Marrow Fibrosis (BMF) Grade MF 0/1 versus Grade 2/3 appeared to be a main adverse prognostic factor when associated with *JAK2*^{V617F} and triple negative MPN disease.[31]

MOLECULAR PATHOBIOLOGY *CALR* THROMBOCYTHAEMIA AND MYELOFIBROSIS

All *JAK2* and *MPL* thrombocythaemias are driven by indirect cytokine activation (*JAK2*^{V617F}→STAT5 or TPO→TpoR=*MPL*), or direct cytokine activation (*MPL*^{515} and *MPL*^{505}) cytokine receptor activation.

Table 3: Clinical Laboratory, Molecular and Pathological criteria for hypercellular essential thrombocythaemia associated with primary megakaryocytic, granulocytic myeloproliferation caused by calreticulin mutations.

CM criteria PMGM or CALR thrombocythaemia A1) No preceding or allied other subtype of myeloproliferative neoplasm PV, CML, MDS. A2) Presence of CALR mutation **Clinical stages of CALR thrombocythaemia** C1) Early clinical normocellular prefibrotic **M** stage: Hb >12g/dL, slight-to-moderate splenomegaly Normal or decreased LAP score C2) Intermediate clinical **MG** hypercellular pre/early fibrotic stage: slight anaemia Hb <12 to >10 g/dL, decreasing platelet count, splenomegaly, increased LDH C3) Advanced **MG MF** stage: anaemia Hb <10 g/dL, tear drop erythrocytes, increased LDH, increased CD34+ cells, pronounced splenomegaly, normal or decreased platelet counts, leucocytosis, or leukopaenia.	**Pathological criteria of PMGM or CALR MGM** Normocellular M proliferation stage in a normocellular bone marrow, no increase of granulopoiesis. Hypercellular MG proliferation stage with no increase or relative or absolute reduction of erythropoiesis and erythroid precursors. Abnormal dense clustering and increase in atypical medium sized, large to giant immature megakaryocytes containing bulbous (cloud-like) hypolobulated nuclei and definitive maturation defects. **Grading RF and MF**[4,37] MF 0 Prefibrotic *CALR* MGM, no reticulin fibrosis RF 0/1 MF 1 Early fibrotic *CALR* MGM slight reticulin fibrosis RF 2 MF 2 Fibrotic *CALR* MGM increase RF Grade 3 and slight to moderate collagen fibrosis MF 3 Advanced fibrotic *CALR* MGM with collagen fibrosis-osteosclerosis

The combination of A1 + A2 and P1 establishes *JAK2* wildtype PMGM or *CALR* thrombocythaemia and various clinical stages (C1, C2, C3) with sequential stages of normocellular *CALR* thrombocythaemia (M) and hypercellular thrombocythaemia (MG) related to the degree of myelofibrosis.
CLM: Clinical, Laboratory, and Molecular; Hb: haemoglobin; PV: polycythaemia vera; CML: chronic myeloid leukaemia; PMGM: primary megakaryocytic granulocytic myeloproliferation; MDS: myelodysplastic syndrome; EMG: erthrocytic megakaryocytic granulocytic; RF: reticuline fibrosis; MF: myelofibrosis; LDH: lactate dehydrogenase.

Constantinescu, Kralovics, and Vainchenker measured STAT5 transcritional activity in *CALR* mutated and wildtype Ba/F3 cells along with a cytokine receptor TpoR, EpoR, and GCSFR.[32] *CALR* mutants Type 1 and 2, but not wildtype *CALR*, did induce STAT5 activation via TpoR (*MPL*) and GCSFR, but not via EpoR, and not by *CALR* mutants lacking exon 9. The STAT5 activation via GSCFR was much weaker than via TpoR (*MPL*). *CALR* mutants Type 1 and 2 could not induce TpoR (*MPL*) activation in the absence of the JAK2 gene. In transiently HEK293 cells, *CALR* mutants induced dimerisation of JAK2 in the presence of TpoR (MPL), but not of EpoR. The extracelllar domain of TpoR (MPL), but not of EpoR, was indispensible for *CALR* mutant induced activity and the D1D2 distal part of the extracelluar TpoR domain and its associated N-glycosylation sites but not the TPO binding site in the D3D4 domain of TpoR control *CALR* mutant activity, which was more pronounced for *CALR* del52 (Type 1) than for *CALR* ins5 (Type 2) mutants. The Asn residues N117 and N178 present in D1D2 are key players in TpoR (MPL) activation by *CALR* mutants. Knocking down either MPL/TpoR or *JAK2* in megakaryocytic progenitors from *CALR* thrombocythaemia patients inhibited cytokine-independent (spontaneous) megakaryocyte colony formation. Using a retrovirus mouse bone marrow transplant model clearly showed the induction of an *MPL*-mediated thrombocythaemia in *CALR* mutated mice.[35] *CALR* del52 Type 1 mutation and, to a lesser extent, *CALR* ins5 Type 2 mutation induced thrombocythaemia due to megakaryocytic myeloneoproliferation in the early post-bone marrow transplant period. The *CALR*-thrombocythaemia disease was transpantable into secondary recipients. After 6 months, *CALR* del2 Type 1 thrombocythaemia mice, in contrast to rare in *CALR* ins5 transduced mice, developed a MF phenotype associated with splenomegaly and marked osteosclerosis mimicking the natural history of *CALR* thrombocythaemia into MF, myeloid metaplasia of the spleen, and hypocellular MF in patients with *JAK2/MPL* wildtype PMGM (Table 3).[20,21]

Araki et al.[36] found that expression of *CALR* mutants in UT-7/TPO and YT-7/EPO cells induces TPO independent growth of UT-7/TPO cells but not of UT-7/EPO cells. C-MPL (TpoR) is required for this TPO-independent growth of UT-7/TPO cells. The *CALR* mutant specific carboxyterminal terminus portion (D1D2) binds to the P-domain of the *CALR* mutant to allow the N-domain of the mutant *CALR* to interact with c-MPL (TpoR), thereby explaining the gain-of function activity of CALR mutants Type 1 and 2. *CALR* mutants activate the *JAK2* downstream pathway via binding to c=MPL (TpoR) in UT-7/TPO cells and in TOP-independent megakaryopoiesis in induced pluripotent stem cells and this induction was blocked by *JAK2* inhibitors.

CONCLUSION

The cross sectional Korean MPN research study on GP characteristics[31] could translate the 2016 WHO classification[13] into a new set of improved EuroAsian CLMP criteria for the diagnosis and staging of MPN (Tables 1, 2, and 3).[37] The 2017 CLMP criteria will pick up asymptomatic latent, masked, early stage, and symptomatic overt stages of thrombocythemias and polycythaemias 5–10 years earlier compared to the 2008—2016 WHO classifications. Prefibrotic *JAK2*^{V617F} normocellular thrombocythaemia, prodromal PV, and the sequential stage of classical PV and masked advanced PV as well as prefibotic normocellular MPL thrombocythaemia and *CALR* thrombocythaemia ET in the complete absence of any signs of PV are poorly or not defined by the 2016 WHO classification.[13,37] The EuroAsian CLMP criteria in Tables 1, 2, and 3 are based on detailed analysis and interpretation of recent advances in the molecular aetiology and pathobiology of *JAK2* trilinear MPN, exon 12 PV, MPL thrombocythaemia, and CALR thrombocythaemia and MF, which have important prognostic and therapeutic implications.[37] The diagnostic differentiation staging related to the natural history of prefibrotic (MF 0/1) and fibrotic (MF 2/3) *JAK2*, *MPL*, and *CALR* mutated MPNs should be based on bone marrow megakaryocyte morphology, bone marrow cellularity due to increased erythropoiesis and/or granulopoiesis, *JAK2*, *MPL*, and *CALR* mutation load, and the degree of anaemia, bone marrow fibrosis, and splenomegaly.[32,37]

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THE 'NUCLEOLUS' HYPOTHESIS OF AUTOIMMUNE DISEASES AND ITS IMPLICATIONS

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ABSTRACT

Many autoimmune diseases, such as lupus and Sjögren's syndrome, have a female bias and adult onset. One possible explanation for this bias is disruption of the inactive X chromosome, which is a major epigenetic feature in female cells. Indeed, only one X chromosome is needed in male and female somatic cells because most X-linked genes are not sex-specific. Therefore, one of the two X chromosomes in each female cell is inactivated and appears as a heterochromatic body near the nuclear membrane. It has also been reported that the inactive X is often in close association with a nucleolus, as if nucleoli help maintain the inactive state. The main function of nucleoli is to assemble ribonucleoprotein complexes (RNPs) such as ribosomal subunits and splicing components. For that purpose, nucleoli have high levels of polyamines which assist with the folding and assembly of RNPs. However, as observed under abnormal circumstances such as cellular stress, the nucleolus is very active and can expand dramatically, potentially engulfing the inactive X, which is sandwiched between the nuclear membrane and the nucleolus. As a consequence, polyamines present in the nucleolus could stabilise autoantigenic complexes including those arising from disruption of the inactive X, or autosomes that contain nucleolar organising regions that keep those chromosomes near nucleoli. This suggests that a variety of seemingly unrelated autoantigens can occur in autoimmune diseases through this scenario. In fact, many autoantigens are, at least transiently, components of the nucleolus. Here, with particular emphasis on the inactive X chromosome, we discuss the 'nucleolus' hypothesis in which disruption of chromatin due to abnormal nucleolar exposure can lead to autoimmune diseases.

Keywords: Epigenetics, nucleolus, autoimmune diseases, X chromosome, polyamines, lupus, rheumatoid arthritis (RA), multiple sclerosis (MS), Sjögren's syndrome (SjS).

INTRODUCTION

More than 80 autoimmune diseases have been identified, affecting 5–8% of the population as a whole, but many of these diseases share common triggers, symptoms, and patterns making differential diagnosis difficult.[1] For example, both systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) exhibit autoantibodies targeting histones and Z-DNA, which is an infrequent conformation compared with B-DNA.[2-4] As another example, an increase in citrullinated proteins occurs in RA (citrullinated collagen) and multiple sclerosis (MS) (citrullinated myelin basic protein), suggesting possible common abnormalities in the functioning of peptidylarginine deiminase enzymes which convert arginine residues to citrulline in special circumstances such as NETosis.[5,6] These common aspects of autoimmune diseases can provide hints about their underlying mechanisms.[7] The fact that the majority of patients with SLE, RA, MS, and Sjögren's syndrome (SjS) are female suggests involvement of the X chromosome (Table 1).

Table 1: Arguments supporting the nucleolus hypothesis with regards to the inactive X chromosome in autoimmune diseases.

	SLE	SjS	RA
Sex ratio (female:male)	9:1	9:1	3:1
Elevated levels of polyamine metabolism[54,55]			yes
Effect of polyamine autoantibodies[56-58]	Anti-dsDNA		*PADI*
X chromosome			
PAR1 neg[59]	Dysregulation		
LINE, Alu neg[60]	Dysregulation		
HERV	Dysregulation		
CD40L	Dysregulation		
Xi reactivation[61]	Yes		
X gene overexpression[62]	*CD40L, TLR7, CXCR3*		

SLE: systemic lupus erythematosus; SjS: Sjögren's syndrome; RA: rheumatoid arthritis; HERV: human endogenous retroviruses; Anti-dsDNA: anti-double stranded DNA; TLR7: toll-like receptor 7; CXCR3: C-X-C motif chemokine receptor 3; PADI: peptidylarginine deiminase.

The fact that SjS can be a primary disease or secondary to SLE, RA, or MS, suggests commonalities among these four diseases.[8] Another common aspect is that they have adult onset and episodes can be triggered by stressful events, such as infections.[9,10] Adult onset suggests manifestation may arise from accumulation of damage by repeated stresses to cells of the immune system (immune-driven) or cells targeted by the immune system (antigen-driven). The diseases could have a similar mechanism at the cellular level, but differ in symptoms due to occurrence in different tissues or sites with more or less access to the immune response and to systemic circulation of autoantigen-autoantibody complexes, for example, behind the blood-brain barrier versus circulating in the blood. Although a genetic basis is suspected and some statistically significant genetic associations have been reported, in most cases the specific mutations considered to be the main causes of autoimmune diseases have not been found; this suggests that genetic differences may be contributing factors.[11,12] As a result, the scope of hypotheses and research have recently expanded to include possible epigenetic involvement, because epigenetic changes can accumulate under environmental influence and lead to altered gene expression without there necessarily being associated genetic mutations.[13-19]

THE INACTIVE X CHROMOSOME

Most genes on the X chromosome are not sex-specific. Therefore, female cells with two (or more) X chromosomes only need one active X to give the same expression levels of X-linked genes as seen from the one X in male cells. Attaining this equivalency of X-linked expression is referred to as X-linked dosage compensation. Early in embryogenesis, each female cell randomly chooses to inactivate either the paternally-derived or the maternally-derived X. Then each daughter cell will inherit this choice so that the adult is a mosaic of areas where all cells have the paternally-derived X inactivated and, in a neighbouring area, all cells will have the maternally-derived X inactivated. Expression and accumulation on the inactive X of the X-inactive specific-transcript RNA is a major step in establishing X inactivation;[20] it recruits epigenetic silencing factors such as histone deacetylases and DNA methyltransferases to the inactive X. The end result is that the inactive X is a dense heterochromatic structure with 75–85% of its genes epigenetically silenced.[21,22] The inactive X localises to the nuclear periphery against the nuclear membrane, as if it has been pushed aside by more active chromosomes. This puts the inactive X in a vulnerable situation as the periphery is less accessible for DNA repair, replication, and methylation. The inactive X is the last chromosome to complete replication in late S phase, but requires more effort for DNA

repair and methylation, scaffold attachment, and repackaging at a time when the methyl donor, S-adenosylmethionine (SAM), may be at low levels due to recent methylation of other chromosomes.

Adding to these difficulties, the X has fragile sites that can contain chromosome breakpoints or unresolved alternate DNA conformations that impede progression of repair and replication. Completion of replication can be delayed in some regions of the inactive X until late S phase or early Gap 2.[23] This can lead to loss of genes (from chromosome breaks), unequal distribution of chromatin to daughter cells, or reactivation of previously silenced DNA when epigenetic control cannot be properly re-established. With age and repeated episodes of stress, there can be chromatin opening and loss of dosage compensation, i.e. gene reactivation on the inactive X.

THE NUCLEOLUS AND POLYAMINES

The nucleolus is a prominent nuclear component, appearing in stained cells as an area mostly devoid of DNA in the nucleus, but the nucleolus does not have a membrane to act as a strict selective barrier. The nucleolus is one of the most active, dynamic, and multifunctional cellular structures, frequently changing its contents and size depending on the cell's demands for RNA processing and protein synthesis.[24] The main function of the nucleolus is to fold RNA transcripts and assemble ribonucleoprotein complexes (RNPs), such as ribosomal subunits, splicing components, transfer RNA (tRNA), signal recognition particles, and small nuclear RNAs.[25,26] Some RNA transcripts are expressed in the nucleolus while others are transported into the nucleolus. Folding and macromolecular complex assembly that occurs in the nucleolus requires sufficient open space free of most chromatin, so that large linear RNA transcripts can be folded properly and bind specific proteins required in the RNP.

The Sicca syndrome (SS)-A/Ro and SS-B/La antigen proteins are found in the nucleolus where they act as chaperones that assist in movement and maturation of RNA transcripts and assist in identifying and refolding misfolded transcripts. Nucleolin is a multifunctional protein comprising 10% of the nucleolar proteins. Among its functions, nucleolin assists in chromatin decondensation and assists in expression and assembly of ribosomal subunits. SS-A/Ro, SS-B/La, and nucleolin are frequently targeted as autoantigens in SLE, SjS, and scleroderma.[27-29]

The nucleolus also contains high levels of the polyamines spermidine and spermine compared to the rest of the cell (Figure 1a, top), which assist in RNA folding in the nucleolus. Polyamines are suspected of having a role in autoimmune diseases.[30] RNA transcripts in the nucleolus have an abundance of negatively charged phosphate groups along their length such that self-repulsion makes the RNA incapable of tight folding on its own. Therefore, there is a need for polyamines as counter ions to assist in the folding. Polyamines have unique combinations of length, flexibility, and high positive charge that are beneficial in many cellular functions.[31,32] This allows a polyamine to bind and neutralise several neighbouring phosphates in RNA to facilitate bending and intrastrand hybridisation of bases to form helices. These polyamine and RNA interactions are usually transient but folding can persist long enough for further folding or protein binding to occur. In some cases polyamines remain bound in the final tertiary structure, such as spermine bound in the bend of tRNA between the acceptor stem and the anti-codon arm.[33,34] The importance of polyamines in folding and assembly in the nucleolus is supported by the facts that polyamines are at their highest levels in the nucleolus and dramatic increases in the size of nucleoli during cell growth and stress response correspond directly to increased polyamine synthesis.[35-37] Cells need to synthesise proteins and RNPs that will help in recovery from stress or support growth, thus there is increased demand for nucleolar activity requiring polyamines. Viruses also want to take over a host cell's nucleoli to produce viral components which require nucleolar products. Therefore, active viruses induce increased polyamine synthesis as an initial step in taking over control of the nucleoli.

Although polyamines are essential in many functions, polyamine synthesis and recycling must be tightly controlled to avoid potential detrimental effects (Figure 1b). Putrescine, the precursor of spermidine, is normally kept only in trace amounts in the cell to suppress polyamine synthesis as putrescine can bind an allosteric site in S-adenosylmethionine decarboxylase (AMD1) increasing AMD1 activity.[38] AMD1 converts SAM to decarboxylated S-adenosylmethionine (dcSAM). SAM is the cellular methyl donor whereas dcSAM cannot be used as a methyl donor. Polyamine synthesis therefore competes directly with cellular

methylation, which is important in epigenetic control of gene expression. Overexpression of polyamines could potentially lower SAM levels sufficiently such that some epigenetic silencing could be lost, allowing reactivation of genes with unpredictable consequences. Putrescine must also be kept low to avoid formation of nuclear aggregates of polyamines (NAPs) (Figure 1a, bottom). NAPs are complexes of phosphate ions with putrescine, spermidine, and spermine. NAPs have been observed *in vivo* and are suspected of binding cellular macromolecular complexes, possibly stabilising them in autoantigenic forms which may be more protected from nucleases and proteases by NAPs compared with individual polyamines (Figure 2).[39]

Two polyamine genes, *spermine synthase* (*SMS*) and *spermidine/spermine N1 acetyltransferase* (*SAT1*), are located on the X chromosome at Xp22.1. Normally *SMS* and *SAT1* are silenced on the inactive X.[21,40] However, loss of dosage compensation could lead to *SMS* and *SAT1* overexpression. This could lead to wasteful synthesis and recycling of polyamines, wasting SAM needed for methylation. *SAT1* can undergo superinduction, (i.e. expression increased several hundred fold).[41] In polyamine recycling, SAT1 acetylates spermine to acetylated spermine, which is oxidised to spermidine, and spermidine can be acetylated and oxidised to putrescine, thereby providing an abnormal increase in putrescine that can allosterically increase AMD1 activity to support new polyamine synthesis. This alternate route of putrescine production via polyamine recycling could support NAP formation. In addition, further oxidation of putrescine can create acrolein, a very toxic compound, the appearance of which shows a strong correlation with the intensity of SjS.[42] Therefore, maintenance of the inactive X is important to avoid consequences of abnormal polyamine synthesis and recycling.

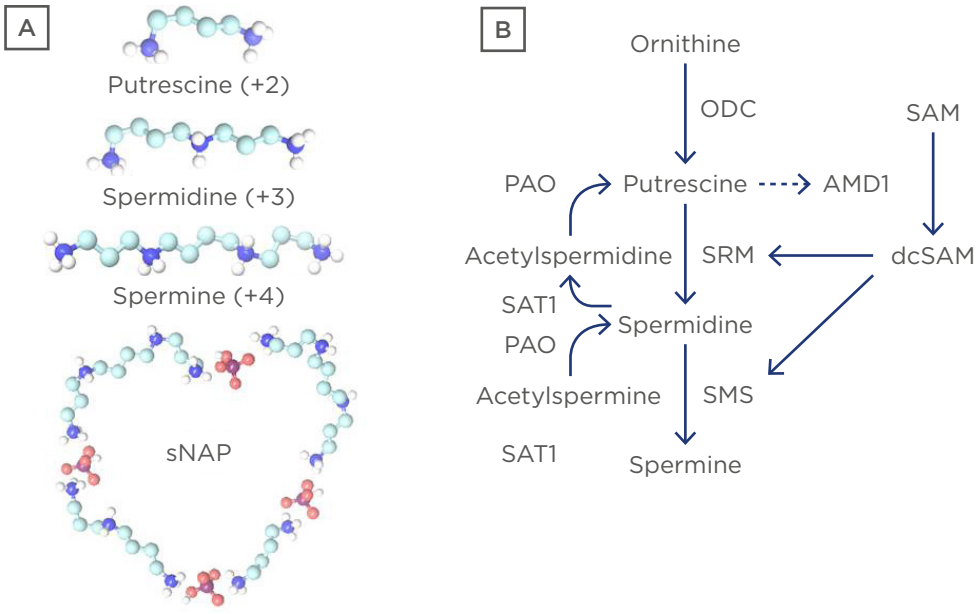

Figure 1: Polyamines.

A) Polyamines: spermidine (+3), spermine (+4), the precursor putrescine (+2), and depiction of a small nuclear aggregate of polyamines (sNAP) containing phosphate ions (believed to be HPO_4^{-2}), putrescine, spermidine and spermine. NAPs have been observed *in vitro* and *in vivo*;[39] B) Polyamine synthesis and recycling pathways. Ornithine is converted to putrescine by ornithine decarboxylase (ODC). Putrescine can allosterically increase S-adenosylmethionine decarboxylase (AMD1) activity to convert S-adenosylmethionine (SAM) to decarboxylated SAM (dcSAM) competing with cellular methylation for SAM. dcSAM provides aminopropyl groups for polyamine synthesis by SRM (spermidine synthase) and SMS (spermine synthase). Polyamines can be recycled with acetylation by SAT1 (spermidine/spermine N1 acetyltransferase) followed by oxidation by polyamine oxidase (PAO). Therefore, two routes exist for generating putrescine, which is normally at only trace amounts to control polyamines. ODC is one of the most tightly controlled enzymes in cells to control polyamine synthesis. Note: *SMS* (synthesis) and *SAT1* (recycling) genes are at Xp22.1 and are normally inactive on the inactive X.[21] Reactivation of *SMS* or *SAT1* from the inactive X could lead to detrimental consequences, such as formation of NAPs.

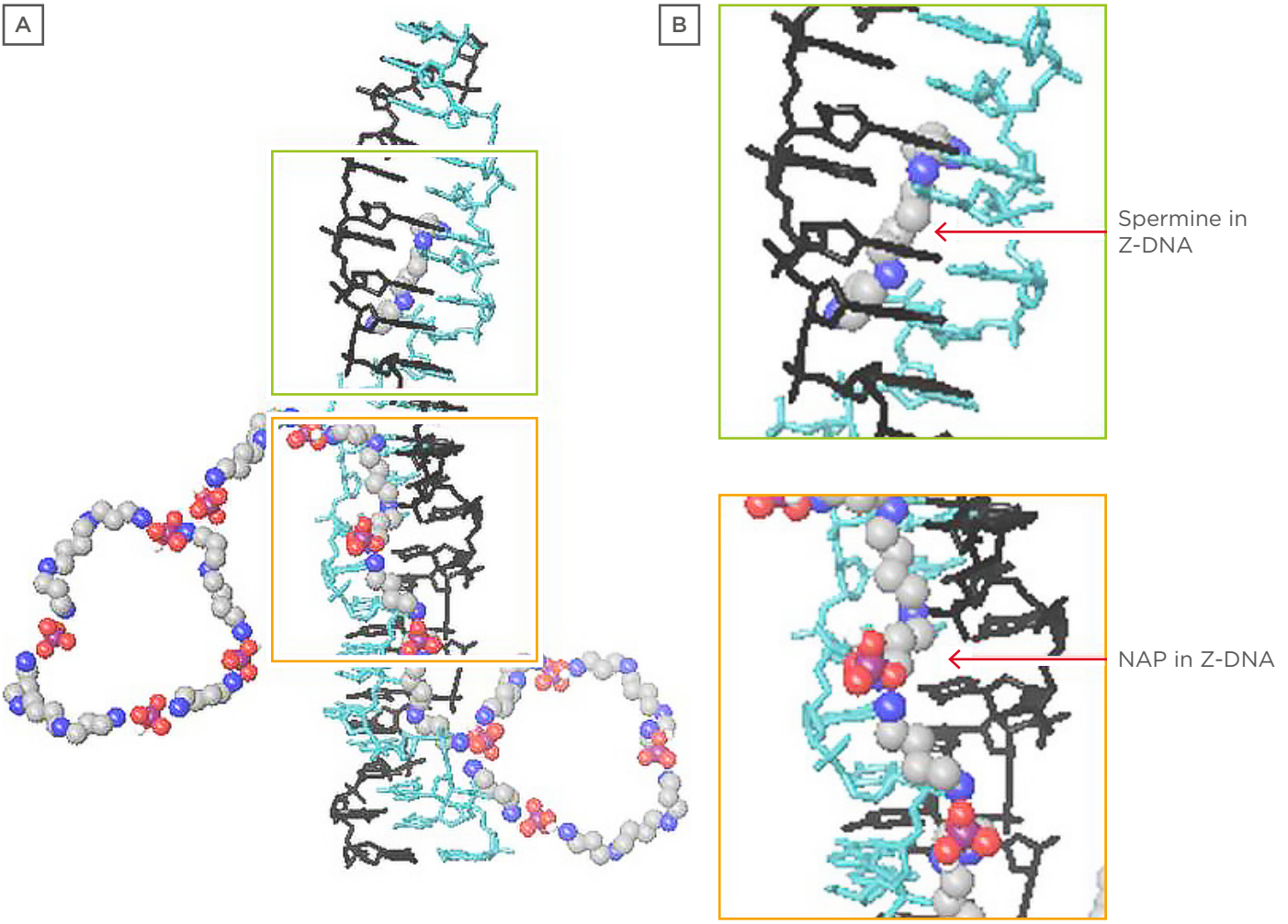

Figure 2: Z-DNA with spermine and nuclear aggregates of polyamines.
A) Depiction of Z-DNA with spermine and NAPs; B) Close-up. Note how NAPs could bind in the Z-DNA minor groove with polyamines, assisting each other and providing more resistance to nucleases and stability to potentially autoantigenic conformations.
NAPs: nuclear aggregates of polyamines.

Increased polyamines and acetylated polyamines could throw off efficient nucleolar functioning and cause misfolding, misassembly, and stabilisation of abnormal conformations of DNA, RNA transcripts, and associated proteins. Persistence of these structures in potentially autoantigenic forms could lead to an autoimmune response. For example, the SS-A/Ro and SS-B/La proteins and nucleolin chaperone RNA transcripts and associated proteins through nucleolar processing. These proteins also help identify abnormal folding and assembly. Recently, the SS-B/La autoantigen has been demonstrated to be dysregulated at the epigenetic level in salivary gland epithelial cells from patients with primary SjS.[43-45] As such, they could be frequent common epitopes within larger complexes that contain autoantigenic epitopes. SS-A/Ro, SS-B/La and nucleolin could be targeted because of 'guilt by association' with misfolded RNAs stabilised by an overabundance of polyamines.

THE 'NUCLEOLUS' HYPOTHESIS

The inactive X has been reported to be in close association with nucleoli in 90% of cells in S phase and about one-third of cells in the overall cell cycle, except for mitosis during which the nucleoli disappear.[46] Expansion of the nucleolus due to cellular stress could potentially involve engulfment of some or all of the inactive X (Figure 3).[34] This would expose the inactive X to high levels of polyamines in the nucleolus, and there could be putrescine and NAPs from abnormal polyamine synthesis and recycling. Some of the inactive X is euchromatic, such as the *PAR1* region that escapes inactivation.[47] Inactive peptidylarginine deiminase 4

(PAD4), a calcium-activated enzyme that converts arginine residues into neutral citrulline, localises to euchromatin. When PAD4 is activated such as during NETosis, citrullinated histones lose their hold on DNA resulting in chromatin opening. We have proposed previously that polyamines could activate PAD4 in place of calcium.[48] For example, two calcium binding sites are 13.5 Å apart, which could potentially bind spermine. The other three calcium binding sites are in alignment spanning 11.0 Å which is similar to the length of spermidine. Thus, PAD4 associated with the inactive X could become active when exposed to high nucleolar polyamine levels. This would open more of the inactive X, allowing reactivation of genes. As the nucleolus returns to normal, newly opened X-linked chromatin could bind transcription factors and polymerases. Of particular concern is a high concentration of Alu elements in *PAR1*. Alu elements comprise ~10% of the human genome but 28.8% the *PAR1* region of the X.[49] Normally these ≥2,500 Alu elements would be silenced by a positioned nucleosome but exposure to high levels of polyamines and polyamine-activated PAD4 could lead to expression of these Alu elements by RNA polymerase III (Pol III). The SS-A/Ro and SS-B/La proteins associate with Pol III transcripts to assist maturation, including the refolding of misfolded transcripts. This sudden appearance of Alu transcripts could take up the available SS-A/Ro and SS-B/La, hampering maturation and refolding of other Pol III transcripts. In addition, Alu transcripts can form intrastrand hybridisation that can be stabilised by polyamines in potentially autoantigenic forms or they can compete with the Alu domain of the signal recognition particles for proteins.[34]

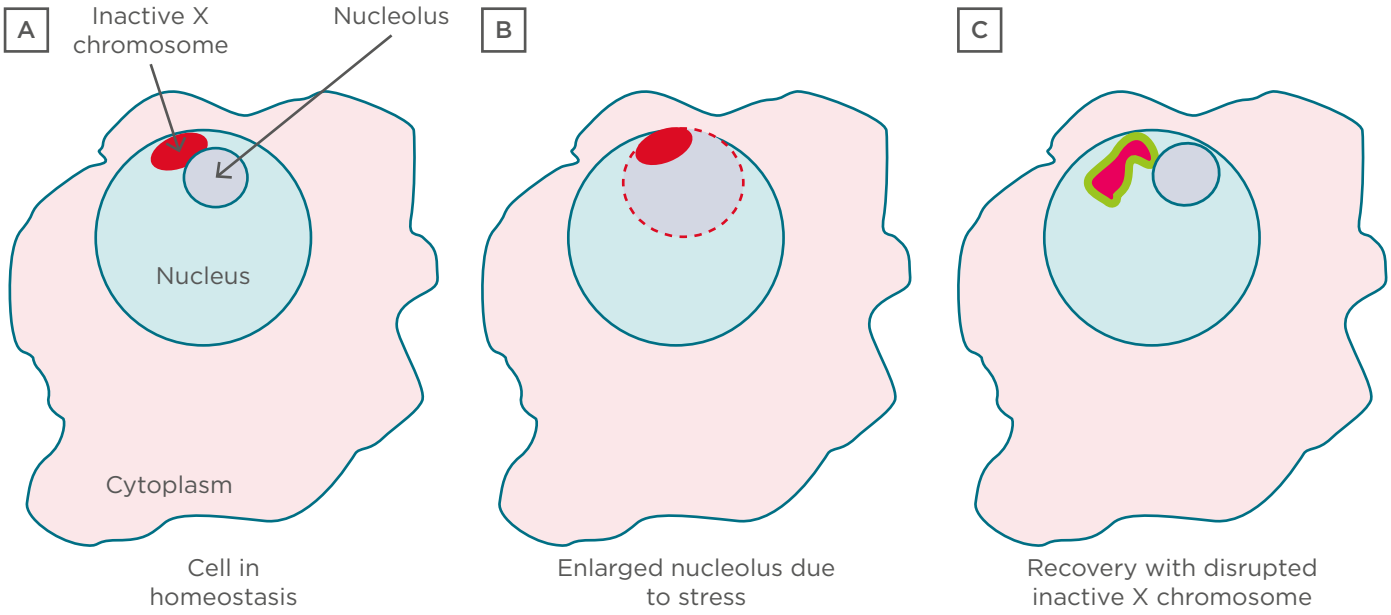

Figure 3: The 'nucleolus' hypothesis.

A) The inactive X (red) is in close association with a nucleolus (grey) in 90% of cells in S phase and one-third of cells throughout the cell cycle.[46] This puts one of the most inactive components of the nucleus, the inactive X, next to one of the most active and dynamic components, the nucleolus. Normally the inactive X is located at the nuclear membrane. B) Stress can cause a rapid increase in the size of the nucleolus as it is called on to produce more ribonucleoproteins needed for recovery. Increased nucleolar size is directly related to increased polyamines which assist in folding and assembly of ribonucleoproteins in the nucleolus.[35] The enlarged nucleolus could engulf the inactive X, exposing it to high levels of polyamines and NAPs. Inactive PAD4 localised in euchromatin (such as *PAR1* of the inactive X[47]) could be abnormally activated by binding polyamines instead of calcium.[48] PAD4 could open previously sequestered genes. C) With cell recovery, X-linked genes may be overexpressed including reactivated genes on the inactive X (green border). Abnormally high levels of polyamines and NAPs, along with reduced cellular methylation, could lead to generation of abnormal DNA, RNA, and RNP conformations stabilised by polyamines and NAPs in autoantigenic forms.

NAPs: nuclear aggregates of polyamines; PAD4: peptidylarginine deiminase 4; RNP: ribonucleoprotein complex.

When released, negative supercoiling stress previously stored in nucleosomes on the Alu elements would flux through the DNA and potentially form Z-DNA that could be stabilised by polyamines and NAPs. In addition, there is a fully functional *LINE1* reverse transcriptase in the inactive X short arm.[34] If this is expressed, it could reverse transcribe Alu transcripts to create hypomethylated Alu DNA. Li and Steinman[50] reported a high percentage of Alu DNA in the free DNA of lupus patients' sera and proposed that it could be reverse-transcribed DNA.

IMPLICATIONS

This discussion has focussed on the inactive X and nucleolus association, which could explain female predominance of some autoimmune diseases, yet we should keep in mind that chromosomes 13, 14, 15, 21, and 22 have nucleolar organising regions that keep them in close association with the nucleolus. They could also be disrupted by a nucleolar stress response and result in similar consequences as described for the inactive X chromosome.

Alu elements and other repetitive elements such as human endogenous retroviruses are important in the hypothesis,[51-53] particularly the high Alu concentration in *PAR1* of the X. Mice do not have any significant amounts of Alu elements; the mouse X consists of only a long arm and no short arm, so inactivation is established relatively consistently on the mouse X. As such, it has been difficult to study partial X reactivation in mice. The human X has both a short and long arm separated by a centromere, making the X inactivation state less consistent. These differences between human and mouse X chromosomes explain why studies on X-linked epigenetics in autoimmune diseases are problematic when using mouse models.

Finally, the close proximity of a functional *LINE1* reverse-transcriptase to a high concentration of Alu elements in the X short arm has very interesting possibilities with regards to retrotransposition events and how stress could trigger such events.

CONCLUSIONS

The 'nucleolus' hypothesis provides a comprehensive explanation for: i) the female bias of many autoimmune diseases, ii) generation of a broad range of autoantigens many of which are at least transiently in the nucleolus, iii) a common mechanism that can relate to different autoimmune diseases, and iv) major involvement of epigenetics in autoimmune diseases.

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PAIN DURING ILLUSORY OWN ARM MOVEMENT: A STUDY IN IMMERSIVE VIRTUAL REALITY

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ABSTRACT

Previous studies have demonstrated that the vision of one's own body, or of external embodied limbs, can lead to pain relieving outcomes. Analgesic effects have also been related to the vision of illusory limb movements. Nonetheless, whether these two processes can be put together to obtain a summatory analgesic effect is not yet clear. The aim of this work was to investigate if it is possible to combine the analgesic effects of looking at one's own body with those deriving from the illusion that one's own limb is moving. Thirty-eight healthy participants underwent four visual conditions in immersive virtual reality while their heat pain thresholds were measured. In different conditions the subject watched from a first-person perspective: i) a still virtual arm, ii) a moving virtual arm, iii) a still non-corporeal object, and iv) a moving non-corporeal object. All participants were asked to keep their arms completely still during the visual exposures. After each condition, participants answered questions about their illusory experience. Our results show that the vision of the 'own' body significantly increased participants' pain threshold as compared to the vision of the non-corporeal object. However, no statistically significant analgesic effect of vision of the virtual arm movement was found. The implications and limitations of this study are discussed.

Keywords: Virtual arm, virtual reality (VR), body ownership, pain threshold, pain modulation, multisensory integration, illusory kinaesthesia.

INTRODUCTION

The relationship between pain perception and body-part movement is not new. Many studies have shown how strong the bidirectional relationship between the two can be.[1] However, what is relatively new are studies relating limb pain and movement representation techniques such as mirror therapy, motor imagery, and movement observation.[2,3] For instance, in the classic mirror therapy technique, patients with phantom limb pain move the healthy limb while watching its reflection on a mirror positioned in front of them, namely between the healthy limb and the residual limb. In this way, the patient has the illusion that the missing limb is back on its site and is fully functioning. The visual exposure to the illusory movement allows the reduction of the patient's perceived pain.[4,5] This is because of a modification of the body representation, which embodies the reflected limb, replacing the missing paralysed phantom one.[6] At a neural level, studies making use of brain stimulation techniques have shown a link between pain states and motor neural networks, finding that a stimulation of the latter may positively translate into effective pain management.[7-10] In one study combining transcranial direct current stimulation of the motor cortex and visual illusion on patients with spinal cord injuries, Soler et al.[11] found that, despite transcranial direct current stimulation and visual illusion being both valid analgesic techniques, the combination of the two yielded the greatest and longest lasting pain relief. In a successive work, Villiger et al.[12] found that the exposure to illusory own limb movements via a non-immersive virtual reality (VR)-augmented technique improved

neuropathic pain in patients with incomplete spinal cord injury. Similarly, watching a video of hand movements led to a body site-specific increase of the pressure pain threshold in a group of healthy participants.[13]

Interestingly, not only the vision of limb movement, but also the vision of the own body has been shown to yield analgesic benefits. Although some studies failed to find such an effect, the majority of studies have found a reduction of pain at the sight of the own body that holds true even during the observation of prosthetic or virtual body parts provided that these were perceived as belonging to the participants' own body.[14]

In support of this body-related visually induced analgesia, studies have pointed at an activation of the inhibitory GABAergic interneurons in the somatosensory areas in response to the vision of the own body.[15-17] After all, a strong connection between internal body representation and pain conditions has been highlighted in several studies taking into account chronic pain patients that reported a distorted body image.[18]

VR is a tool that has been effectively used in neuro-rehabilitation and neuroscience, including pain management. For instance, it has been shown that VR can be effectively used as an adjunctive treatment in patients with severe burns, and that its effects are comparable to opioids.[19-21] Interestingly, immersive VR ("a typical immersive VR system delivers stereo vision that is updated as a function of head tracking, possibly directional audio, and sometimes some type of limited haptic interface")[22] gives the possibility to display digital characters (avatars) that can be seen from a first-person perspective (1pp). In this way, it is possible to deceive the brain and challenge the user's normal body representation, generating body ownership illusions (BOIs) over the avatar's body.[23] Based on these premises, in the current study we wanted to investigate whether the analgesic effects of vision of the own body could be added to the analgesic effect of the vision of illusory limb movement. To do so, participants were exposed to four different visual conditions in immersive VR, where either an avatar's arm or a non-corporeal object (i.e. a purple tube) were displayed in place of their real arm. Both the avatar's arm and the tube either performed a regular movement or kept still, while participants' arms were always still. Importantly, in a previous experiment (unpublished data) we found that it is possible to induce BOI over a moving avatar's arm seen from a 1pp, despite the visuo-motor mismatch between the participant's still limb and the avatar's moving limb. Increasing ramps of heat stimuli were applied to the participants' wrists during the visual exposures. Our hypotheses were that participants' pain thresholds would be higher during the conditions where the avatar's arm was displayed, and highest during the vision of the moving avatar's arm. We also expected to find stronger BOIs in the body conditions, which were likely to be highest during the vision of the still avatar's arm (because of multisensory correspondence with the real arm). The strongest feeling of own arm movement illusion was predicted to be found in the avatar's arm moving condition.

MATERIAL AND METHODS

Participants

Forty-two subjects were initially recruited for the experiment, a sample size based on previous similar studies.[24-28] Four of these were discarded because their pain threshold was <40˚C,[29] one because of an instructions breach, and another one because they were ambidextrous. Therefore, 36 healthy participants (20 females, mean±standard deviation [SD] age: 24.9±4.7 years, age range: 20–45) were included in the final analysis. They were all right-handed (mean±SD: 91.42±11.65, range: 62.5–100) according to the short version of the Edinburgh Handedness Inventory.[30] This was important because stimuli induction and visual representation involved the use of the right arm; therefore, only right-handed participants were considered in order to rule out possible confounding factors due to laterality, and to increase homogeneity within the sample. They had normal or corrected-to-normal vision, no history of neurological disorders, and no other condition potentially interfering with pain sensitivity (e.g. drug intake). Upon arrival at the laboratory they were asked to read and sign a consent form. The experiment was approved by the local ethics committee and was in accordance with the Declaration of Helsinki.

Virtual Reality System

The stereoscopic head-mounted display (HMD) was an Oculus Rift DK2 (Oculus VR, Irvine, California, USA) with a resolution of 960x1080 per eye and a field of view of 100˚, displayed at 60 Hz. The virtual environment was programmed using the Unity platform (Unity Technologies, San Francisco,

California, USA). Noise isolation was ensured by the administration of pink noise via headphones, with a constant volume set at 70 dB sound pressure level.

Thermal Stimulation

Thermal heat stimuli were delivered by means of a TSA-II Neuro Sensory Analyzer (Medoc Ltd., Ramat Yishai, Israel), with a 30x30 mm thermode tied with a Velcro® strap on the palmar side of the right wrist. The probe temperature was increased from normal skin temperature (constant baseline temperature=32°C) at 2°C/s. Participants were asked to press a button with their left hand as soon as they perceived the stimulation as being painful. Immediately after pushing the kill-switch button the temperature reached was recorded as the pain threshold and the probe temperature rapidly decreased to the baseline level (32°C). A fixed maximal temperature of 51°C was set for safety reasons.

Procedure

Upon arrival, participants were invited to sit on a chair and to read and sign the consent form. Then an experimenter secured the thermode on the participant's right volar forearm close to the wrist, with a Velcro strap, ensuring the surface of the thermode remained in contact with the skin evenly. Before donning the HMD, participants were familiarised with the heat stimuli.

As the participants donned the HMD the room lights were turned off and the pink noise played. Regardless of the visual condition they were asked to visually explore the virtual scenario, and in particular to look down as if they were looking at their real body. All subjects went through the same four visual conditions (within subject design): i) a virtual body (avatar) seen from the 1pp, with its right arm still, ii) as in 'i' but the avatar's right arm moved, iii) no virtual body, only a virtual tube in replacement of the virtual right arm, and iv) as in 'iii' but the tube moved (see Figure 1 for all visual conditions). The conditions differed on the movement because the virtual object could be still or in motion, and on the nature of the virtual object, which could be similar to a real human arm or a simple purple tube. All participants completed the four conditions, with the order of the conditions being balanced across participants. The movement consisted of a constant angular speed (ω) of 5.0, and the object moved 22.5° towards the right and 22.5° towards the left horizontally, using the elbow as a pivot.

To match participants' physical features as much as possible, both the sex (male or female) and the avatar's skin colour (black or white) was pre-selected. Care was taken to match the position of the real participant's body to the virtual body as well as the real right arm and the right virtual arm/tube; the participants were asked to mimic the avatar's body posture as much as possible, particularly for the right arm position, until they felt perfectly co-located with the virtual body. Participants were asked to keep their arms still during all VR conditions and to focus their attention on the right virtual arm/tube. Before the visualisation of the virtual scenario, participants were asked to lay their right elbow on top of a small box, rendered in VR below the avatar's right elbow. This was done to limit the sensorial mismatch between the still real arm and the moving avatar's arm/tube (in the movement conditions) to the movement itself, and to limit the expected tactile sensation that would have been produced by the avatar's arm rubbing the table during the movement. To match the avatar's arms position, participants' lower arms were kept perpendicular to the shoulder-shoulder axis and at ~20 cm distance from the torso.

During each visual condition four heat stimuli were provided, with an inter-stimulus interval of 30 seconds; since the avatar's arm movement was kept constant and the end of the thermal stimulation was under the participants' control, the recording of the specific avatar's arm position by the time the heat stimulation was stopped was not possible, nor within the scope of this study. Each observational condition lasted for about 2 minutes. At the end of each condition, the thermal pain threshold was obtained by averaging the four participants' thresholds. A total of 16 heat ramps were administered by the end of the experiment. After the fourth heat pain threshold had been recorded the visual condition terminated with the removal of the HMD and the subject responded to the questionnaire.

Subjective Measures

A questionnaire at the end of each experimental condition was administered to measure the subjective feelings experienced by the subjects during exposure to VR. Items were partly selected from a questionnaire used in seminal work on virtual arm ownership.[31] The order of the items was randomised per each condition and participant. Importantly, before the administration of the

questionnaires, participants were told that there were no right or wrong responses and that the only 'right' response was the one that corresponded to their feelings. This was done to ensure that the data reflected the genuine experience of the participants, hence limiting confounding factors such as compliance with 'ideal' expected responses. To ensure that each item was correctly interpreted, the questionnaire was read to the participant by the experimenter. Participants answered verbally using a 7-point Likert scale, with 1 meaning 'totally disagree' and 7 representing 'totally agree'.

Items:

- Q1: During the experiment there were moments in which I had the sensation of having more than one right arm.
- Q2: During the experiment there were moments in which I felt as if the virtual arm/tube was my own arm.
- Q3: During the experiment there were moments in which I felt as if my real arm was becoming virtual.
- Q4: During the experiment there were moments in which the virtual arm/tube started to look like my own arm in some aspects.
- Q5: During the experiment there were moments in which I had the sensation that the heat was coming from the virtual arm/tube.
- Q6: My attention was totally focussed on other things, for example, in what I was watching[1] or totally on the thermal stimulus.[7]
- Q7: During the experiment there were moments in which it seemed that my real arm was moving.

Data Handling

Single trial pain thresholds (in °C) were averaged for each visual condition and subject. Given the high variability between participants, a check of the outliers was carried out. Out of 144 values, 19 values were identified as outliers (>1.5-times the SD from the group's mean) and replaced with the mean scores of the group for the same visual condition.

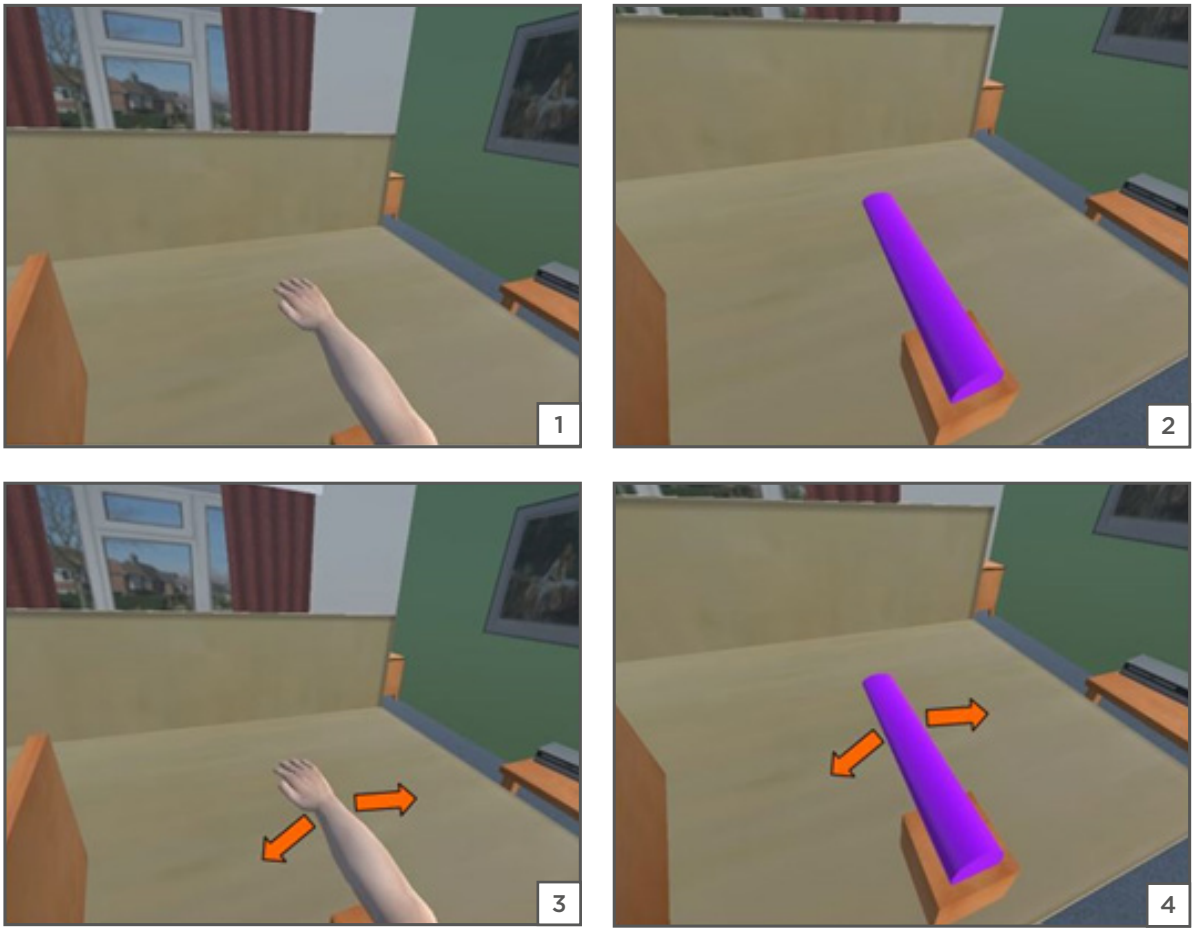

Figure 1: Screenshots of the experimental conditions.
From top left to bottom right: 1) arm still, 2) tube still, 3) arm move, 4) tube move.

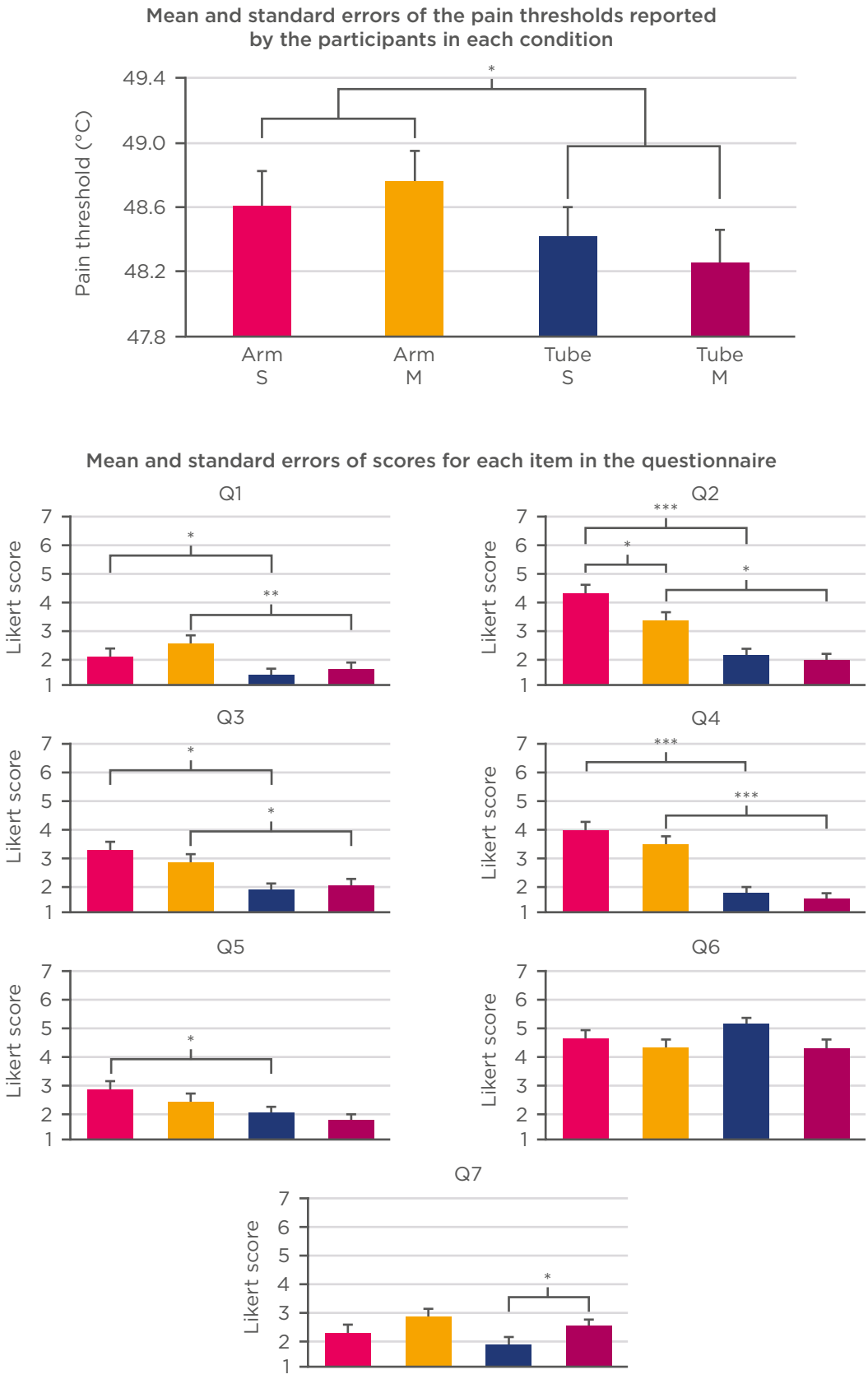

Figure 2: Top row: mean and standard errors of the pain thresholds reported by the participants in each condition (from the left: 'Arm S', 'Arm M', 'Tube S', 'Tube M'). Centre and bottom: mean and standard errors of scores reported at each item of the questionnaire. Colours match the same conditions as in the ones depicted in the pain plot. Only significant contrasts are denoted.

*p<0.05, **p<0.01, ***p<0.001 (Bonferroni p-corrected level=0.0125).

Arm S: arm still; Arm M: arm move; Tube S: tube still; Tube M: tube move.

Resulting data from all conditions were normally distributed according to the Jarque–Bera and the Shapiro–Wilk tests[32] (all p_s>0.05). A 2x2 repeated-measures ANOVA (two factors: 'Body' and 'Movement', both with two levels) was then conducted on mean pain thresholds. The level of significance was set at p<0.05. Questionnaire scores were averaged across subjects per each item and visual condition. The resulting mean scores related to each question were subjected to Friedman ANOVAs. Post-hoc analysis was carried out with Wilcoxon Matched Pairs Tests, with a Bonferroni correction applied for the number of possible comparisons. These comparisons were carried out between 'arm still' versus 'arm move', 'arm still' versus 'tube still', 'arm move' versus 'tube move', and 'tube still' versus 'tube move'. This resulted in a significance level set at p<0.0125.

RESULTS

Pain Threshold

The one-way repeated-measures ANOVA revealed a main effect of the factor 'body' ($F_{1,35}$=5.30, p=0.027, Partial Eta squared=0.13) so that irrespective of the presence of movement, the vision of the avatar's arm was linked to a significantly higher pain threshold as compared with the vision of the object (Figure 2). No main effect of the factor 'movement', nor an interaction between the two factors 'body' and 'movement' were found to be significant (respectively: $F_{1,35}$=0.008 and $F_{1,35}$=0.88, all p_s>0.05). So, our first hypothesis that there would be a higher pain threshold during the vision of the avatar's arm was confirmed, while the second hypothesis that the highest pain threshold would be during the vision of the avatar's arm movement was not statistically supported.

Embodiment Scores

Analysis using Friedman ANOVA of Q1 scores reported a significant p-level (χ^2_3=27.06, p=0.00001). The sensation of having more than one right arm reported in the 'arm still' condition was significantly higher compared with the 'tube still' condition (Wilcoxon post-hoc test, p=0.007) and that in the 'arm move' versus the 'tube move' condition (p=0.0003), despite the mean values from all conditions being very low.

On Q2 scores, the analysis with Friedman ANOVA showed a significant p-level (χ^2_3=44.78, p<0.0001). As hypothesised, the sensation of ownership towards the virtual corporeal or non-corporeal object reported in the 'arm still' condition was significantly higher than in the 'arm move' condition (Wilcoxon post-hoc test, p=0.0018) and also higher than the one in the 'tube still' condition (p=0.00004). Furthermore, the sensation of ownership reported in the 'arm move' condition was significantly higher than the one in the 'tube move' condition (p=0.00084).

The analysis with Friedman ANOVA on Q3 scores reported a significant p-level (χ^2_3=30.50, p<0.00001). The illusion that the real arm was becoming virtual was significantly stronger in the 'arm still' condition compared with the 'tube still' one (Wilcoxon post-hoc test, p=0.001), and it was also significantly stronger in the 'arm move' condition compared with the 'tube move' condition (p=0.003).

Q4 scores, analysed with Friedman ANOVA, showed a significant p-level (χ^2_3=64.14, p<0.00001). As expected, the sensation of similarity between the real arm and the virtual object reported in the 'arm still' condition was higher than the one reported in the 'tube still' condition (Wilcoxon post-hoc test, p<0.00001), and the same pattern is reported between the 'arm move' condition versus the 'tube move' (p=0.00001).

Analysis using Friedman ANOVA on Q5 scores reported a significant p-level (χ^2_3=20.59, p=0.0001). In particular, the sensation that the heat was coming from the virtual arm/tube was stronger in the 'arm still' condition compared with the 'tube still' (Wilcoxon post-hoc test, p=0.0026). The analysis with Friedman ANOVA on Q6 scores failed to report a significant p-level (χ^2_3=7.7, p>0.05). That meant that no significant differences were found in terms of attentional resources drawn by the different conditions.

Lastly, the analysis with Friedman ANOVA on Q7 scores reported a significant p-level (χ^2_3=13.75, p=0.003). A slightly stronger sensation of own arm movement was found in the 'tube move' condition as compared with the 'tube still' condition (p=0.008). Thus, our original prediction that the highest arm movement illusion would be during the vision of the avatar's arm was not statistically supported. No other comparison was found to be significant.

DISCUSSION

The present study aimed at investigating whether it is possible to boost analgesia through the

combination of the vision of the body and limb movement observation. However, with our set-up, we did not find a statistically relevant higher pain threshold during the vision of the avatar's arm movement. What we found is a main effect of the vision of the body, in line with previous studies reporting an analgesic effect by looking either at the real or the dummy body.[33-35] At a subjective level, we found that the conditions where the arm was displayed reported significantly stronger levels of BOI (Q2) compared to the tube ones. If this result could be, on one hand, expected, on the other hand it appears less obvious when we consider that the tube, like the avatar's arm, was co-located with the real arm, and that even in the condition when the avatar's arm is moving the BOI was significantly stronger compared to the tube one. One could argue that the difference in pain threshold reported by this study could be due to a stronger capturing of participants' attention by the arm conditions compared to the tube conditions. Indeed, attention is one of the most important pain modulators,[36,37] and VR-based distraction has been effectively used to reduce the pain felt by patients with severe burns undergoing clinical treatments.[20,21] However, we did not find any significant difference between conditions in terms of attentional resources drawn, in fact the arm and tube conditions followed very similar trends on Q6. Hence, the variation in thermal pain thresholds may be because of the subjective experience related to BOI. In fact, overall, we found a significant difference between the arm conditions and the tube ones in the first four questions. Under BOI paradigms the illusion of having more than one right arm (Q1), the illusion that the real arm is becoming virtual/rubber (Q2), and that the virtual/rubber arm resembles the real one (Q3) are usually considered as control questions, but recently a relation between these illusory feelings and high levels of BOI has been found in some studies.[38-40]

Nonetheless, we failed at finding a significant effect of the movement factor on pain threshold, either alone or in combination with the body factor. This may be because the avatar's arm movement can be considered as an asynchronous feature, and previous studies demonstrated how synchronous and asynchronous conditions can affect the level of BOI.[25,40] So, as shown by the subjective reports for the item on illusory movement (Q7), the feeling that "...my real arm was moving" is likely dampened by the multisensorial mismatch. In any case, although not statistically relevant, we still observed a clear trend in which the arm movement condition reported the highest pain threshold, even higher than in the condition where the avatar's arm is still; that is where the visual and the proprioceptive inputs are coherent. Recently it has been shown how the spatial correspondence between the real and the virtual arm is key to make the body-related visually induced analgesia stand out.[41] In the present work, we found that despite the continuous visuo-spatial mismatch, the arm moving condition reported a slightly higher pain threshold compared to the arm still condition. Thus, despite the non-significant effect of movement, results are encouraging and future studies may want to further investigate the role of illusory own limb movement with slightly different experimental designs and/or set-ups. For instance, future works could investigate if different features of limb movement, such as speed, amplitude, or type of movement, may induce higher level of body ownership and of kinaesthetic illusion in the participants, to see whether and how this translates into pain modulation.

We are aware that the current study presents some limitations: for instance, the evaluation of the body ownership via self-reported questionnaire could have been accompanied by more objective measures, such as galvanic skin responses to a threat menacing the virtual arm. Also, a more realistic virtual arm could have helped improve the BOI during the avatar's arm conditions. Yet, it is notable that our results show that it is possible to generate an illusory sense of ownership over a moving virtual arm despite a strong visuo-motor mismatch between vision and proprioception. This may have therapeutic implications targeting patients with limb movement disorders or immobilised limbs.

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PARADIGM SHIFT FOR THROMBOLYSIS IN PATIENTS WITH ACUTE ISCHAEMIC STROKE, FROM EXTENSION OF THE TIME WINDOW TO RAPID RECANALISATION AFTER SYMPTOM ONSET

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ABSTRACT

Intravenous thrombolysis (IVT) and intra-arterial thrombolysis (IAT) are useful therapeutic tools to improve functional outcomes after recanalisation of occluded vessels in patients with acute ischaemic stroke. IVT could be performed for more patients by extending the time interval to 4.5 hours from onset to IVT initiation; however, this does not significantly improve functional outcomes. Recent studies indicated that IAT, particularly intra-arterial thrombectomy (IA-thrombectomy), significantly improved functional outcomes after recanalisation of occluded vessels, particularly when the recanalisation was performed within 6 hours of symptom onset. The focus of thrombolysis for acute ischaemic stroke patients is changing from extending the time window for IVT to successfully achieving good functional outcomes with IA-thrombectomy, by performing it within the 6-hour time limit. In this review, we discuss the present status of and limitations of extending IA-thrombectomy for improved functional outcomes after thrombolysis.

Keywords: Thrombolysis, intravenous thrombolysis (IVT), intra-arterial thrombolysis (IAT), intra-arterial thrombectomy (IA-thrombectomy).

INTRODUCTION

Thrombolysis, within the appropriate therapeutic window, is an essential tool that should be considered for patients with acute ischaemic stroke. Intravenous thrombolysis (IVT) has been considered as a primary thrombolysis for these patients.[1] Recently, the time frame for which IVT is effective has been extended from <3 to 4.5 hours from symptom onset to the administration of intravenous thrombolytic agent.[2,3] However, when IVT is performed for acute ischaemic stroke within the extended time period, functional outcomes are not always significantly improved.[2,4]

Intra-arterial thrombolysis (IAT) has been used as a bridging therapy after IVT failure or as primary thrombolysis within a 6-hour window for patients with acute ischaemic stroke.[5] A higher recanalisation rate has been reported with IAT than with IVT alone.[5] Furthermore, compared with IVT or IAT using thrombolytic agents, significantly better functional outcomes and recanalisation rates have recently been achieved with the direct removal of the thrombus using intra-arterial thrombectomy (IA-thrombectomy) in acute ischaemic stroke patients.[6-10]

After the recent successes with IA-thrombectomy, it immediately became the thrombolytic tool of choice when performed within the therapeutic window.[11,12] However, IVT is still recommended as the primary thrombolytic method for acute stroke patients, even if endovascular treatments are being considered, as this delivers high recanalisation rates and better clinical outcomes.[13] IAT as the primary or bridging therapeutic option has several barriers to its nationwide or global use, such

as medical cost, limited availability of modern imaging tools, and experienced personnel.[14,15] Here, we review the present status of thrombolysis and the benefits and limitations of IA-thrombectomy to achieve successful functional outcomes after thrombolysis in patients with acute ischaemic stroke.

CHANGES TO GUIDELINES FOR INTRAVENOUS THROMBOLYSIS

IVT can be rapidly initiated after confirmation of the inclusion criteria and time since onset for acute stroke patients.[1,3] Following the publication of the National Institute of Neurological Disorders and Stroke (NINDS) trial for IVT,[1] the appropriate dose and extension of the time window have been sought, primarily to decrease the haemorrhagic side effects and increase the number of patients who benefit from IVT.

Efforts to Change Dose of Thrombolytic Agent for Intravenous Thrombolysis

The recommended standard dose of tissue plasminogen activator (tPA) for IVT is 0.9 mg/kg body weight (maximum 90 mg); administration should be a bolus injection of 10% of the calculated dose, followed by administration of the remaining 90% of the dose at a constant rate over 60 minutes.[1] In previous Asian studies, a lower dose of 0.6 mg/kg tPA was evaluated for safety and to determine if it could achieve the same efficacy as the 0.9 mg/kg dose.[16] This low-dose tPA, when initiated within 4.5 hours, had comparable effectiveness and safety as with the standard dose in South Korean stroke patients.[17] However, the efficacy and safety of low-dose tPA need to be evaluated in a controlled trial.[18] The ENCHANTED trial, which is a randomised controlled trial to evaluate low-dose tPA, recently completed recruitment.[19]

Changes in the Time Window for Intravenous Thrombolysis

Rapid recanalisation of the occluded intracranial artery is an important factor in achieving good clinical outcome after thrombolysis for acute ischaemic stroke patients.[20,21] However, extending the time window for IVT from 3 to 4.5 hours was another important attempt to increase the number of acute ischaemic stroke patients eligible to receive thrombolysis. After the suggestion to extend the time window to >3 hours,[22] the safety and clinical outcomes of IVT performed within 4.5 hours were similar to those within 3 hours in randomised trials including the SITS-ISTR[23] and ECASSIII[3] trials. Extension of the time window to up to 6 hours for IVT was suggested in acute ischaemic stroke patients with penumbral area evaluated diffusion and perfusion mismatch on magnetic resonance imaging.[24,25] Even though subsequent studies showed the functional benefits of extension of the time window up to 4.5 hours for IVT,[26] further studies are necessary to verify the safety and efficacy of this change.[27,28] The 4.5-hour time window for IVT is recommended in the 2015 American Heart Association/American Stroke Association (AHA/ASA) guideline for acute ischaemic stroke patients.[13]

CHANGES TO GUIDELINES FOR INTRA-ARTERIAL THROMBOLYSIS

Compared with IVT, IAT and particularly IA-thrombectomy using advanced interventional devices could achieve recanalisation more reliably and lead to better functional outcomes for acute stroke patients.[5,12] In the updated guidelines for IAT, the primary changes involve the devices used, and, time point for bridging or primary thrombolysis.[13]

Changes in the Thrombolytic Devices for Intra-Arterial Thrombolysis

After a successful randomised trial of direct intra-arterial injection of thrombolytic agents,[29] thrombolytic tools for IAT subsequently advanced to mechanical disruption[30,31] and recently to direct removal of the thrombus using retrievable stents[32,33] and suction devices.[34] With the advances in the IAT tools, the recanalisation rate significantly improved from >60% with direct injection[5] to >80% with direct removal.[35] Ultimately, IA-thrombectomy using stent retrievers was recommended over direct intra-arterial injection as the primary IAT for acute stroke patients in the 2015 AHA/ASA guideline.[13]

Unlike IVT, the recanalisation status of the occluded vessels can be confirmed after IAT. Therefore, the time interval from onset-to-recanalisation (OTR) as well as the onset-to-treatment (OTT) time can be evaluated during IAT, including IA-thrombectomy. In the analysis of the functional outcomes following IAT, the OTT criteria of <6 hours was used. However, OTR is frequently >6 hours because IAT interventions usually take several hours to complete. Although knowledge of OTT is useful

to understand how quickly IAT is initiated, OTR is a better indicator of the start of reperfusion in the infarcted area. Therefore, to evaluate the benefits of IAT, including IA-thrombectomy, OTR might be more useful than OTT.

Three well-known studies that used only OTT to analyse functional outcomes after IAT failed to determine a benefit for these outcomes in acute ischaemic stroke patients.[36-38] However, five pivotal studies that were subsequently conducted reported that good functional outcomes were achieved significantly more often in patients for whom OTR was within 6 hours.[6-10] Another study looking at IA-thrombectomy, showed better functional outcomes (determined by a modified Rankin score 0-2) when OTR was <6 hours.[39] Evidence shows that rapid recanalisation, defined as a 6-hour time limit for OTR, was more important than rapid initiation of IAT, defined as a 6-hour time window for OTT, to achieve a good post-IAT functional outcome. In the 2015 AHA/ASA guidelines, a 6-hour time window for OTT is recommended for IAT, including IA-thrombectomy, as bridging or primary thrombolysis in patients who have a contraindication for IVT.[13] However, the 6-hour time limit for OTR should be considered for the successful achievement of functional outcomes after IAT intervention forfuture acute ischaemic stroke patients.

Bridging Intra-Arterial Thrombolysis After Intravenous Thrombolysis Failure

Although rapid IVT within a 4.5-hour time window is recommended as the primary thrombolysis for acute ischaemic stroke patients with large artery occlusion, the rates of recanalisation and good functional outcome after IVT are <27%[40,41] and <40%, respectively.[5] Bridging IAT within a 6-hour time window has been attempted after a failed initial IVT and achieved a higher recanalisation rate of approximately 60%.[5] Unfortunately, a previous randomised study failed to show clinical benefits in patients who underwent bridging IA-thrombectomy within the 6-hour OTT time window.[36] However, another randomised bridging study that achieved OTR within 6 hours observed a significant improvement in the functional outcome in patients who underwent bridging IA-thrombectomy.[7] Now, IA-thrombectomy is recommended for bridging after unsuccessful IVT or primary therapy within a 6-hour time frame for OTT.[13]

FACTORS INFLUENCING FUNCTIONAL OUTCOMES AFTER INTRA-ARTERIAL THROMBOLYSIS

A 6-hour time limit for OTR, rather than the time frame for OTT, is an important factor for a good functional outcome in patients undergoing IAT. In addition to the OTR time limit, three types of factors are related with a good functional outcome after IAT: patient-related, infarction severity-related, and procedure-related factors.

Patient-Related Factors

Patient-related factors that are associated with prognosis or increased haemorrhagic risk with IVT[42,43] and with functional outcome with IA-thrombectomy[7,44,45] include older age, presence of cardiovascular risk factors, and presence of comorbid diseases before the start of thrombolysis. Atrial fibrillation might be an independent factor for functional outcome with IAT, including IA-thrombectomy.[46] Intra-cerebral haemodynamic alterations[47] caused by systemic haemodynamic changes has been shown in patients with atrial fibrillation.[48] Altered intra-cerebral haemodynamic status in patients with atrial fibrillation could decrease the blood flow in the occluded hemisphere.[47] Ultimately, clinical outcomes following acute ischaemic stroke may be worse in patients with co-morbid atrial fibrillation compared to those without this condition.[46] In addition, age-related vessel changes such as vascular tortuosity, distortion of the aortic arch and aorta, and high atherosclerotic burden inside the vessels delay catheter arrival to the thrombus and cause intervention-related infarctions in elderly patients.[49]

Infarction Severity-Related Factors

Infarction severity-related factors include the initial clinical and radiological status before IAT. A high National Institutes of Health Stroke Score,[45,50] low Alberta Stroke Program Early Computed Tomography score,[45,46] extensive (>8 mm) clot burden,[46] and terminal ICA occlusion[39] on initial imaging are prognostic factors that correlate with poor functional outcome after IAT. The presence of collateral support to the ischaemic lesion on pre-intervention angiography is useful prognostically as it suggests there will be less infarct growth. Good collaterals to the ischaemic lesion are independently related with early improvement in functional outcome after IAT.[46] Furthermore, good collateral circulation to the

ischaemic lesion is related with a higher recanalisation rate, shorter recanalisation time, and fewer haemorrhagic complications after IAT.[52-54]

Procedure-Related Factors

Procedure-related factors include recanalisation grade and development of haemorrhagic complications after IAT as well as the use of advanced intervention tools and shortened time intervals from symptom onset to recanalisation. As already discussed, using retractable stents and/or suction devices markedly increase the recanalisation rate with IAT. General anaesthesia with intubation and conscious sedation has been used for acute ischaemic stroke patients receiving endovascular therapy.[55] Even though the individualised selection of conscious sedation and general anaesthesia was recommended during endovascular therapy in the 2015 AHA/ASA guideline,[13] randomised trial data are still needed to determine the anaesthesia technique for endovascular treatment. In addition, shortened OTRs are related to enhanced functional outcomes after IAT. In previous studies, a successful recanalisation grade was useful for predicting a good functional outcome[5,56] and severe haemorrhagic complications were associated with poor post-IAT outcomes.[57] However, in other previous studies, although the recanalisation grade and haemorrhagic complications were correlated with clinical outcomes after IAT and IA-thrombectomy in univariate analysis, these two factors were not related with functional outcomes in the multivariate analysis.[39,46]

EFFORTS TO EXTEND INTRA-ARTERIAL THROMBECTOMY

Although the five recent pivotal IA-thrombectomy studies showed the importance of a 6-hour OTR time limit to achieve a good functional outcome for patients with acute ischaemic stroke, it is not easy to quickly disseminate the practice of IA-thrombectomy nationwide in many countries.

One primary reason for this difficulty is the need to establish comprehensive stroke centres, in which experienced personnel and the critical pathway, including angiographic tools, are available 24/7. This is necessary to achieve the 6-hour OTR for recanalisation of the occluded vessels using IA-thrombectomy. These centres have been established in the USA and several European countries.[58,59] However, in other, particularly developing countries, the nationwide extension of comprehensive stroke centres might be limited by the availability of local expertise and economic support. Government initiatives could support a model to rapidly establish comprehensive stroke centres, as in South Korea.[60]

Another primary issue is extension of IA-thrombectomy to more rural areas of a country that only have primary stroke centres or local hospitals. In rural primary stroke centres it might only be possible to start IVT for acute ischaemic stroke patients. Therefore, for patients living in these areas, a transfer network for bridging IVT/IA-thrombectomy is needed, with IVT performed first in a primary stroke centre, followed by IA-thrombectomy in a comprehensive stroke centre within the OTR time limit. The transfer network between local hospital and primary and comprehensive stroke centres might be the most important component for extending IA-thrombectomy to rural areas in the near future.

CONCLUSION

For patients with acute ischaemic stroke, IVT is recommended as primary thrombolysis within a 4.5-hour OTT time window.[13] However, the rate of a good functional outcome, namely independent ambulation at discharge, remains at approximately 33% despite an extended time window of 4.5 hours.[4] Therefore IA-thrombectomy is recommended, within a 6-hour time window, as a bridge after IVT failure, and primary thrombolysis in patients who are contraindicated for IVT.[13] IA-thrombectomy increases the overall rate of good functional outcomes to 47% for patients who undergo IAT within the 6-hour OTT time window and to 66% for patients who are re-canalised within the 6-hour OTR time limit after IAT.[39]

To date, there has been a lot of interest in increasing the probability of thrombolysis by extending the time window for acute stroke patients. Given the recent pivotal studies of IA-thrombectomy, attention should be given to increasing the probability of good functional outcomes. Now, we should try to start IVT within the extended 4.5-hour OTT time window. In addition, we need to increase the chance of IA-thrombectomy for acute intracranial large artery occlusion withinthe 6-hour OTR time limit to achieve good post-IAT functional outcomes, despite the barriers that exist in real-life settings.

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