TSMJ

VOL 16 | 2015

The Trinity Student Medical Journal is intended to provide an inclusive vehicle for students to communicate current medical research, opinions and thoughts to other students, faculty members and faculty of affiliated hospitals and institutions. We publish articles relating to many aspects of medicine including scientific research and clinical experience. Articles are accepted from students in medicine and other related fields, as it is our view that medicine is the meeting point of many disciplines. The aim of the Journal is to provide a medium that is responsive to the rapidly changing face of contemporary medicine, and is able to grow and expand as rapidly as the subject.

All correspondence including orders for advertising space should be addressed to:
Director of Publication, Trinity Student Medical Journal, Trinity Centre,
St. James's Hospital, Dublin 8, Ireland

Alternatively, email can be sent to tsmj@tcd.ie.

Current and previous volumes can be accessed at www.tsmj.ie

ISSN 1393-9572

Abbreviations
TCD: Trinity College Dublin
RCSI: The Royal College Of Surgeons, Ireland
UCC: University College, Cork

TSMJ 2015

CHIEF DIRECTOR
ABDEL SATTAR AL RUBAYAWI

CO-DIRECTORS
KATE RICHARDS
DAVID PARFREY

EDITOR-IN-CHIEF
LAURA MANGAN

PRODUCTION MANAGER
MOHAMED ALSAFFAR

PRODUCTION TEAM
EIMEAR DUFF

MARKETING TEAM
KATE RICHARDS
NAN AFTAB
NEELAM DEVI NATH
TARIQUE HAMID
MICHAEL McCROHAN
JO-YVE WONG
KATHERINE ZAVIN

CONFERENCE COORDINATORS
NAN AFTAB
TARIQUE HAMID
NEELAM DEVI NATH
JO-YVE WONG

PEER EDITORS
MOHAMED ALSAFFAR
NICHOLAS ARROTTA
NEELAM DEVI NATH
ADAM DYER
TURLOUGH HEFFERNAN
CONNOR KELLER
WAQAS KHAN
DIANE KRAJEWSKI
DAVID O'DRISCOLL
ABDEL SATTAR AL RUBAYAWI
ROBERT TA
AMY WORRALL

SPONSORS

THE SCHOOL OF MEDICINE, TRINITY COLLEGE DUBLIN

SWEET CICELY

MEDICAL PROTECTION SOCIETY

DEPT. OF SURGERY, AMNCH,
PROFESSOR KEVIN CONLON

DEPT. OF CLINICAL MEDICINE, ST JAMES'S HOSPITAL, PROFESSOR NASIR MAHMUD

DEPT. OF ANAESTHESIA, AMNCH,
DR GERARD FITZPATRICK

DEPT. OF PAEDIATRICS, TRINITY COLLEGE DUBLIN,
PROFESSOR ELEANOR MOLLOY,
DR NEHAD METWALLY,
DR JUDITH MEEHAN

MR WILLIAM RYAN, DEPT. OF ANATOMY, TRINITY COLLEGE DUBLIN

WE WOULD ALSO LIKE TO THANK OUR ANONYMOUS SPONSORS

DIRECTORS' WELCOME

The Trinity Student Medical Journal (TSMJ) was established in 2000 by a group of forward-thinking students as a platform for their research in the health sciences. In the journal's decade and a half of life, it has blossomed into a forum for ideas, a starting point for discussion, and, to the delight of those involved, a meeting place for like-minded people. The journal would not have reached its current standard without the exceptional talent and capability of past members and leaders and their uncompromised toil to produce the finest work possible. With a continuation of this trend we can be very excited about the future of the TSMJ.

We feel that the articles featured in this year's edition represent the highest standard of submission we have received to date. As a result we had to employ stricter publishing criteria and unfortunately many very high quality articles did not make it to print. However, we sincerely thank all the authors who submitted for their brilliant efforts and for enabling us to showcase the thriving state of student research in Trinity.

We are privileged to work such an enthusiastic and talented team. We appreciate their commitment and hard work in the face of such narrow deadlines. We would like to thank our Chief Editor, Laura Mangan, for ensuring such a high standard of content of this year's edition. In addition, we are convinced that this year's peer editors are some of the most capable members of health science community. We are glad to have them onboard and we are looking forward to more talented individuals joining us in the coming years.

This edition sees a complete re-invigoration of the journal's layout. We wish to extend our thanks to our new Production Manager, Mohamed Alsaffar, for his work on this re-design, one we feel reflects our continued effort to keep the look of the TSMJ as modern and elegant as its content. We are indebted to our Marketing and Conference teams, who work tirelessly to make the journal a reality. We would like to thank Neelam Devi Nath for her participation in the Marketing, Conference and Editing teams.

We would also like to take this opportunity to thank the School of Medicine, Trinity College Dublin for their sponsorship and guidance. We would like to extend our thanks to Professor Paul Browne, Head of the School of Medicine, and Professor Martina Hennessy, Director of Undergraduate Teaching and Learning. Their support and encouragement has been indispensible. In addition, we would like to express our gratitude to the hospital departments and individual academics and consultants who have sponsored us, as well as to our anonymous sponsors.

The Professor of Comparative Immunology at Trinity, Professor Cliona O'Farrelly, has once again provided unparalleled guidance in our undertaking. Her supervision has fostered our confidence in our ability to create this student-led production. We also thank Dr. Ronan Mullaney, Clinical Lecturer in Forensic Psychiatry at Trinity, for his expert opinion and advice regarding the journal's content.

We must extend our thanks to the previous Co-Director of the TSMJ, Dr Peter Tsakkos, for his continued assistance. Indeed, Peter's mentoring will have a long-lasting impact on the journal. We wish him all the best in his career.

In keeping with tradition, this edition is of no charge to our readers. We are grateful for your continuous feedback and contribution – your input has ensured the success of the journal.

Welcome to Volume 16 of the TSMJ.

Sincerely,

Abdel Sattar Al Rubayawi, Chief Director
Kate Richards and David Parfrey, Co-Directors

CONTENTS

EDITORIAL

ORIGINAL ARTICLES

CASE STUDIES

REVIEWS

EDITORIAL

Personalised Medicine - Bespoke Healthcare is the Latest Trend

Laura Mangan, Editor-in-Chief

Personalised medicine is one of the most rapidly expanding and arguably the most exciting paradigm in medicine. It promises dramatic reductions in healthcare expenditure in conjunction with greater efficacy and safety of therapies that are tailored to the individual needs of each patient. It involves molecular profiling of patients with subsequent tailoring of treatments to offer timely, targeted prevention of disease. The completion of the Human Genome Project (HGP) 12 years ago catalysed much of the genetic profiling utilised in personalised medicine today[1]. Although it was thought that once the human genome was sequenced, we would find 'the gene' causing each disease, it is now acknowledged that many genetic and environmental factors often synergistically cause disease.

It is hoped that future identification of susceptibility variants with significant disease associations will allow for the appraisal of individual disease risk. Encouragingly, genome-wide association studies (GWAS) have linked numerous polymorphic DNA sequence variants to many common diseases[2]. Moreover, advances in next generation sequencing following on from the HGP contribute greatly to translational research in genomics. Specifically, panels of genes and biomarkers are being catalogued, facilitating the individualisation of modern medical interventions. Molecular markers for genetic profiles will guide targeted treatment while genetic loci for disease susceptibility will determine those who would benefit from prophylactic intervention[3].

Personalised Medicine and Cancer

Current cancer treatment involves surgery, chemotherapy and radiotherapy depending on the type, site and stage of the tumour. Nonetheless, not all patients with the same type or stage of tumour will respond to the standard treatment regimen. This disparate response is due to genetic heterogeneity. Consequently, the input of personalised medicine in cancer prevention, diagnosis and treatment is imperative as it has the potential to negate such disparity. Molecular diagnostic and pharmacogenetic intervention in cancer allows for the specific targeting of genes or proteins which are essential for cancer growth and survival. Personalised medicine allows for the stratification of cancer patients into low- and high-risk groups according to specific genetic signatures and thus directs therapeutic interventions appropriately[4]. In breast cancer, molecular diagnostic tools such as MammaPrintTM (70-gene prognostic signature of breast cancer) can determine treatment protocol[5]. Cusumano and colleagues[6] found that MammaPrintTM classification (low- vs. high-risk) influenced adjuvant chemotherapy recommendations and decreased inter-institutional and international variation in adjuvant treatment guidance for patients.

Holistic Healthcare

There is much debate as to whether or not personalised medicine is a more patient-centric paradigm. Notably, personalised medicine utilises a combination of individual genetic, clinical, familial, and demographic variables to inform decision making on disease prognosis, prevention, diagnosis and treatment[7]. The integration of these variables, forming models of disease prognosis and progression is arguably more holistic than any other medical field to date. Personalised medicine is attempting to form a 'picture' of the patient which assimilates factors from their genes to their lifestyle and everything in between. This is no mean feat.

Personalised medicine heralds the new era of proactive healthcare which endeavours to predict and prevent disease. This is in stark contrast to the reactive, one-size-fits-all medical model where patients with the same disease are given the same drugs at the same dose. The benefits of a proactive approach as exemplified by BRCA1 and BRCA2 gene detection include regular mammography, chemoprevention and prophylactic surgery thus minimising or eliminating disease risk[8]. The shortcomings of a reactive approach include increased adverse side effects, poor adherence, and trial-and-error prescribing with accompanying cost implications. Thus by using targeted therapies, personalised medicine aims to minimise adverse effects while maximising therapeutic benefit, getting ever closer to Paul Ehrlich's "magic bullet"[9].

Ivacaftor Controversy

The individualisation of healthcare has in some cases incurred substantial costs. For instance, the drug Ivacaftor (KalydecoTM) was developed as a targeted therapy for a subset of cystic fibrosis (CF) patients with a rare, functional G551D mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) protein[10]. While Ivacaftor offers substantial benefits to this subgroup of patients it incurs an equally substantial cost to the Irish Exchequer. An estimated 121 patients benefitted from Ivacaftor in 2013, nevertheless it cost the Exchequer in excess of €28 million in that year alone[11]. In a socialised healthcare system such as Ireland's this creates an ethical dilemma in terms of fair allocation of resources in the context of a finite budget. While products of pharmacogenetics such as Ivacaftor have remarkable potential, future endeavours in personalised medicine will no doubt spark many more such debates.

Conclusion

Personalised medicine is arguably a more patient-centric and holistic paradigm in healthcare, bringing with it the potential for great innovation and abundant benefits for patients into the future. By tailoring interventions therapeutic effectiveness will increase, while adverse effects and misdirected expenditure will decrease, thus heralding more effective and cheaper healthcare[1]. As molecular markers and genetic signatures are akin to a unique disease fingerprint treatments will be tailored according to the needs of the patient. With these weapons in its armoury, personalised medicine can generate pathway-directed genetic patient profiles which for example, could curtail exposure to costly therapies in poor-responders[3].

Notably, personalised medicine may lead to ethical quandaries in which fair allocation of resources must balance individual patient benefits. Reassuringly, the costs of high-throughput genotyping and DNA sequencing are continually decreasing while there is an ever increasing volume of genetic data being deciphered to amass genetic patient profiles[3]. In sum, it appears that personalised medicine has the capacity to shape the future of healthcare and offer much hope and innovation for the benefit of patients. There is however, much work that remains to be done if patients are to reap the potentially immense advantages of such practices.

References

1 European Science Foundation. Personalised Medicine for the European Citizen - towards more precise medicine for the diagnosis, treatment and prevention of disease. (2015). Available at: http://www.esf.org/index.php?eID=tx_nawsecuredl&u=0&file=fileadmin/be_user/CEO_Unit/Forward_Look/iPM.pdf&t=1426213045&hash=ce6cb2ebd43e2a11bb16071b12110514325c5b08 (Acessed: 12th March 2015).

2 Welter D, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res. 2014;42:1001–1006.

3 Ortega VE, Meyers DA, Bleecker ER. Asthma pharmacogenetics and the development
of genetic profiles for personalized medicine. Pharmgenomics Pers. Med. 2015;8:9-22.

4 Shukla S, Bhargava S, Somasundaram K. Cancer gene signatures in risk stratification: use in personalized medicine. Curr. Sci. 2014;107(5):815-823.

5 Piccart-Gebhart MJ, Sotiriou C, Adjuvant chemotherapy – yes or no? Prognostic markers in early breast cancer. Ann. Oncol. 2007;18(12):2–7.

6 Cusumano PG, et al. European inter-institutional impact study of MammaPrint. Breast. 2014;23:423-428

7 Abul-Husn NS, et al. Implementation and utilization of genetic testing in personalized medicine. Pharmgenomics Pers. Med. 2014;7:227–240.

8 Personalised Medicine Coalition. The Case for Personalized Medicine (4th Edition; 2014) Available at: http://www.personalizedmedicinecoalition.org/Userfiles/PMC-Corporate/file/pmc_the_case_for_personalized_medicine.pdf (Accessed: 12th March 2015).

9 Bosch R, Rosich L, The Contributions of Paul Ehrlich to Pharmacology: A Tribute on the Occasion of the Centenary of His Nobel Prize. Pharmacology. 2008;82(3):171–179.

10 Ramsey BW, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med. 2011;365(18):1663–1672.

11 National Centre for Pharmacoeconomics. Cost-effectiveness of Ivacaftor (Kalydeco™) for the
treatment of cystic fibrosis in patients age 6 years and older who have the G551D mutation. (2013) Available at: http://www.ncpe.ie/wp-content/uploads/2012/08/Ivacaftor-Summary.pdf (Accessed: 11th March 2015).

ORIGINAL ARTICLES

Matrix Metalloproteinases 2 & 9: novel biomarkers for sepsis severity?

Niamh Buckley and Patrick Collins
Third year Medicine, Trinity College Dublin

Abstract

Sepsis is a common clinical syndrome and a significant cause of death worldwide. The development of thrombocytopenia is a determinant prognostic factor in these patients. Matrix metalloproteinase (MMP) -2 and -9 are released from platelets during aggregation, and leukocytes during inflammation respectively. Upregulation of MMP-9 in plasma of septic patients has been demonstrated but little is known regarding MMP-2 levels. We aimed to identify if MMP-2 activity in the plasma and platelet membranes of septic patients correlated with increasing disease severity.

A platelet poor plasma fraction and a platelet pellet fraction were separated by differential centrifugation from blood samples of healthy controls (n=9), septic non-thrombocytopaenic (n=6) and septic thrombocytopaenic (n=3) patients. Following protein concentration standardisation, MMP -2 and -9 activity in each of the two donor fractions was determined using gelatin zymography and quantified by densitometry. MMP-2 and MMP-9 activity was upregulated in the plasma of septic patients compared to healthy controls as was MMP-9 activity in the platelet pellet of septic patient samples compared to healthy controls. There was a trend whereby MMP-2 activity was further upregulated in the thrombocytopaenic patients.We conclude that plasma MMP-2 activity may provide a novel non-specific marker for increasing severity of systemic inflammation.

Introduction

Sepsis describes a clinical syndrome whereby there is a non-specific systemic inflammatory response to an infection of suspected microbial origin. It may progress to severe sepsis, in which case the illness is accompanied by tissue hypoperfusion or dysfunction of at least one organ system. When further complicated by hyperlactataemia or hypotension requiring vasopressor therapy despite fluid resuscitation, it is termed "septic shock"[1].

Sepsis is the most common cause of death in non-coronary Intensive Care Units (ICU) worldwide and among the leading causes of death in North America[2].

Thrombocytopaenia is frequently seen among septic patients in ICU and is associated with poorer prognosis[3], prolonged treatment in the ICU, and increased mortality[4,5]. It is a marker of severity of the underlying pathological process and occurs due to increased consumption, (e.g. disseminated intravascular coagulation) reduced production (e.g. bone-marrow depression), or sequestration of platelets.

Activation of platelets is often seen in sepsis. Once activated, platelets release granules containing a number of pro-inflammatory substances that modulate the function of adjacent cells. They also react with neutrophils to form platelet-neutrophil complexes, which are elevated in sepsis[6].

Matrix Metalloproteinases (MMP)

are a group of calcium and zinc-dependent endopeptidases that are synthesised as pro-enzymes, requiring cleavage of their pro-peptide domain for activation[7]. They regulate extracellular matrix turnover through degradation of its components[8]. They are also heavily involved in the inflammatory process, being responsible for the release of CD40 ligand and various chemokines[9].

MMP-2 is constitutively expressed and found in the cytosol of platelets. Upon platelet activation, it is recruited to the membrane in endosome-like structures and released (schematic 1)[10,11]. MMP-2 mediates platelet aggregation independent of the classical thromboxane A2 and ADP-dependent pathways[10].

The concentration of the gelatinase MMP-9 is elevated in septic patients[12]. MMP-9 has been found within resting human platelets. MMP-9 inhibits platelet aggregation in a concentration-dependent manner, opposing the effects of MMP-2[13]. However, some controversy exists as to whether platelets are in fact a source of MMP-9, or if its detection may be due to contamination of the sample with leukocytes, which are known to release the enzyme (schematic 2)[14].

Objectives

It has been demonstrated that patients suffering from sepsis have elevated MMP-9 levelsand this is associated with increased mortality among critically ill patients[15,16]. Equally, numerous studies have shown that there are higher mortality rates in cohorts of septic thrombocytopaenic patients[4, 5]. Our aim was to measure MMP-2 and MMP-9 activity in plasma fractions from healthy, septic non-thrombocytopaenic and septic thrombocytopaenic patients. Furthermore, we sought to identify if there was differential activity of MMP-2 in the plasma of non-thrombocytopaenic and thrombocytopaenic septic patient cohorts.

CLINICAL POINTS

Sepsis is a common clinical syndrome caused by a systemic inflammatory response to microbial components in the body. Thrombocytopenia, a feature of a more severe sepsis, is associated with increased mortality

Matrix metalloproteinase (MMP) -2 and -9, which are stored in platelets and leukocytes, are upregulated in the plasma of septic patients

MMP-2 may be a novel biomarker for disease progression in sepsis as activity rises in the sicker, or thrombocytopenic septic patient cohort

MMP-2 activity can be measured in the blood sample drawn from the septic patients as part of the 'sepsis six' diagnostic protocol, meaning that no additional invasive test is required to quantify this

MMP-2 could potentially be incorporated into a sepsis screen that also considers other inflammatory markers such as C-Reactive Protein or Erythrocyte Sedimentation Rate for a more comprehensive overview of disease state

As CRP and ESR are elevated in a number of pathological processes, we believe that MMP-2 up regulation in septic patients requires further investigation to determine if it is a more sensitive and specific marker of inflammation

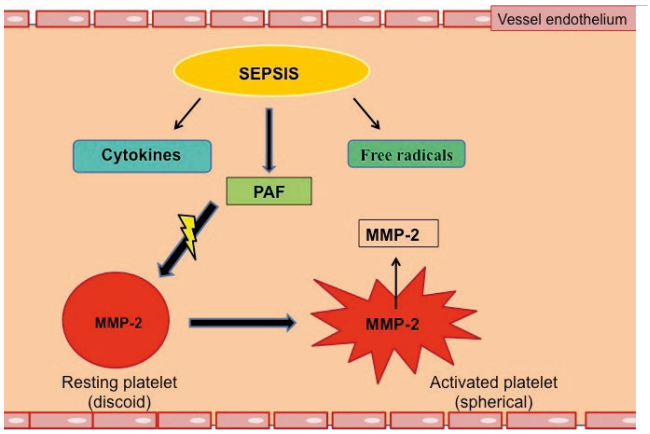
Materials and Methods

Recruitment of donors

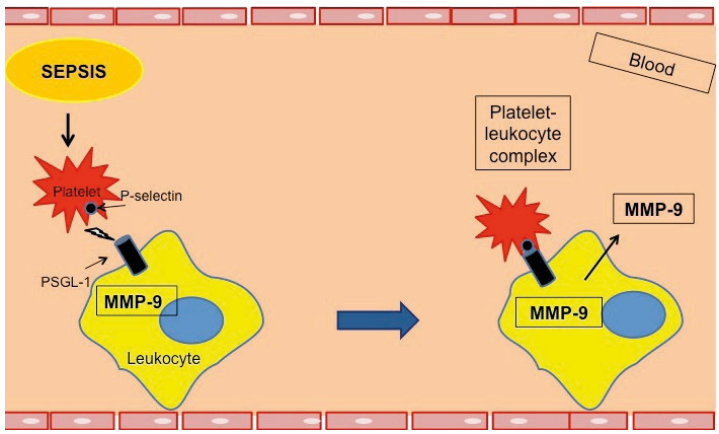
Three distinct groups were recruited for participation in this study – healthy controls (*n*=9), septic patients without thrombocytopaenia (*n*=6) and septic patients with thrombocytopaenia (*n*=3). The septic patients included were those admitted to the Intensive Care Unit (ICU) of St James' Hospital, Dublin from the 27th of February 2013 to the 10th of March 2014. The demographic and laboratory data relating to the patients is displayed in table 1.

Sepsis was defined as patients having at least one positive blood culture and/or an identified focus of infection. Severe sepsis, or septic shock, was defined as the presence of at least one organ failure attributable to sepsis necessitating the use of vasopressor therapy. Thrombocytopaenia was diagnosed when platelet count was below 100,000/ µL of blood.

The exclusion criteria used in this



schematic 1 Sepsis manifests as a number of inflammatory responses in the body including cytokine and PAF release and free radical generation. PAF stimulates the resting platelet, which is discoid in shape, to become activated and hence, spherical in shape. Platelet activation is the stimulus for the translocation of MMP-2 from the platelet cytosol to the membrane and its subsequent release into the plasma



schematic 2 During sepsis, platelets and leukocytes interact to form platelet-leukocyte complexes via P-selectin on the platelet and PSGL-1 on the leukocyte membrane. It is hypothesised that MMP-9 translocates from the cytosol of the leukocyte to the plasma, accounting for the increased activity in the septic state

table 1 Septic-patient data

Diagnosis	Culture	Sex	APACHE II score (% mortality)	SOFA score	Age	Platelets (number/µl blood)	WCC
LRTI following lobectomy	Klebsiella in sputum	M	22 - 42.4	10	63	174	7.9
Necrotizing Fasciitis in right hand	Coagulose-negative staphylococcus	M	32 - 76	12	64	355	14.5
Intra-abdominal sepsis following appendicectomy	Enterococcus avium + Escherichia coli	M	22 - 42.4	9	65	395	12.2
Colectomy	Clostridium difficile colitis	F	36 - 85.1	11	80	151	15.7
Abdominal sepsis - perforated colon	Staphylococcus hominis	M	27 - 60.5	10	75	143	17.1
Influenza A H1N1		M	19 - 32.2	9	48	261	9.8
Mediastinits	Saccharmyces cerevisiae in chest drain fluid	M	19 - 32.2	9	50	84	8
Cellulitis + parastomal abscess	Escherichia coli	F	28 - 76.4	11	67	84	10.5
Abdominal sepsis	Candida albicans	M	23 - 48.9	15	46	59	15.5

Abbreviations: LRTI - lower respiratory tract infection, WCC - white cell count, APACHE II - acute physiology and chronic health evaluation, SOFA - sequential organ failure assessment

study were age below 18 years, presence of a haematological malignancy, patients post-cardiopulmonary bypass, history of thrombocytopaenia thrombotic purpura, history of idiopathic thrombocytopaenia purpura, massive haemorrhage, and current antiplatelet agent use (e.g. clopidogrel/aspirin, GPIIb/IIIa inhibitors).

Reagents

Human HT 1080 fibrosarcoma cells were purchased from American Type Culture Collection (ATCC). They release MMP-2 and MMP-9 after stimulation with phorbol 12 myristate 13-acetate (PMA) and their conditioned media was used as a positive control for MMP activity. All other reagents were purchased from Sigma-Aldrich.

Preparation of samples

Blood was collected from subjects in the three study groups and platelet suspensions washed in prostacyclin were prepared as previously described[17] to yield a platelet poor plasma (PPP) fraction and a platelet pellet. Blood from septic patients was collected on admission to the ICU. The PPP and the platelet pellet samples were stored at -20 °C. The platelet pellets were lysed using 0.2% NaCl and centrifuged at 13,000 RPM to yield a pellet containing the platelet membrane only. Homogenising buffer containing 1% Triton X-100 was also added to each sample to solubilise the proteins.

Protein assay

Protein concentrations in each of the samples was determined using the Bradford Protein Assay as previously described[18].

Gelatin zymography

The standardised PPP and platelet membrane pellet samples were subject to gel electrophoresis at 150V for 2 hours and 20 minutes in an 8% sodium dodecyl sulphate (SDS) polyacrylamide gel co-polymerised with 2 mg ml-1 gelatin. The gels were then washed with 2.5% Triton X-100 to remove SDS. They were subsequently incubated with zymography buffer (50 mL of 2 M Tris HCl, 18 g NaCl, 1.47 g CaCl2, 1 g NaN3 made up to 2000 mL) at 37°C for 17 hours. Following incubation, the gels were stained with a solution of 0.05% Comassie Brilliant Blue G-250, 25% methanol and 10% acetic acid for one hour and destained with a solution of 4% methanol, 8% acetic acid. Gels were visualised using the gel documentation system from Biorad. The software "Quantity One" used densitometric analysis to measure the intensity of the bands which reflected MMP-2 and -9 activity.

Statistical analysis

Unpaired two-tailed t-tests and ANOVA and Tukey's post tests were performed to analyse the data using the programme GraphPad Prism 6 (GraphPad Software, San Diego, CA). The data are shown as mean ± standard deviation (SD). A *p*-value of less than 0.05 was considered statistically significant.

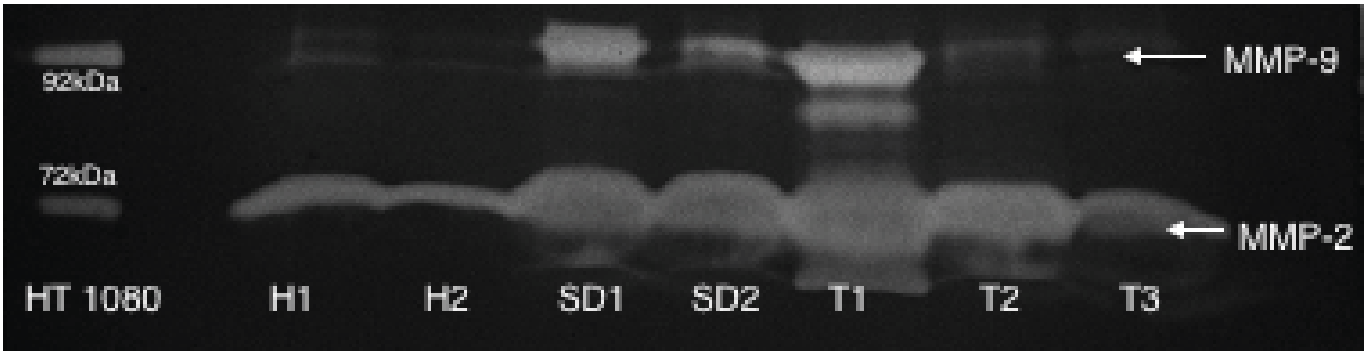
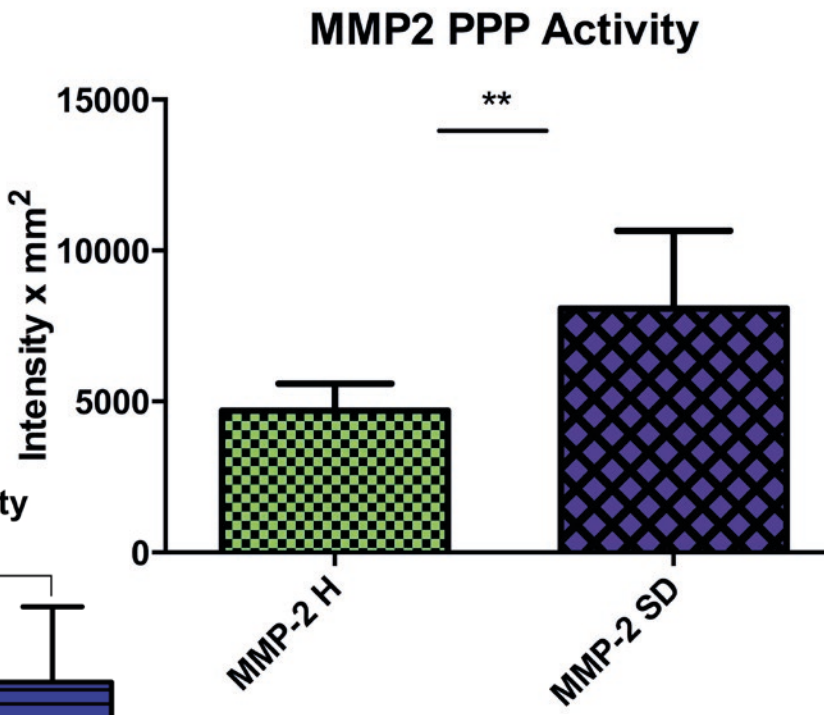
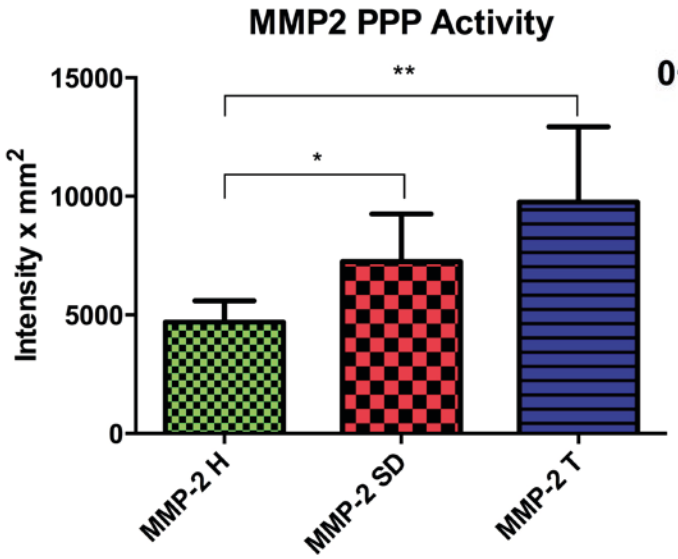


image 1 Gel under UV light showing MMP-2 and MMP-9 activity in the PPP. Gelatin is dark. Areas where gelatin has been digested appear light. These are areas of MMP activity. Quantification of MMP activity is achieved through absorbance measurements, indicating degree of gel digestion and therefore activity of enzyme

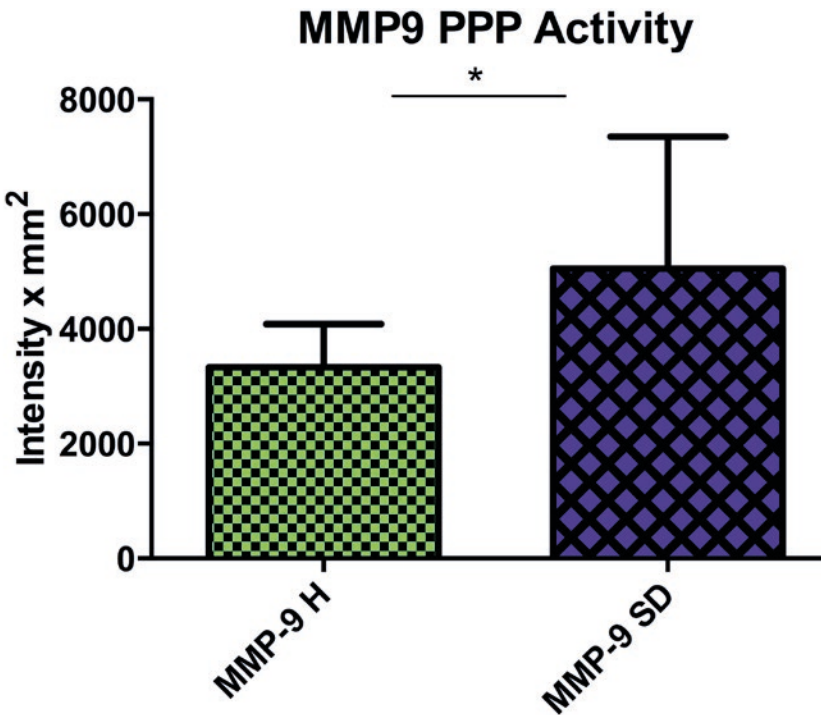


▶ figure 1 Comparison of MMP-2 activity in the platelet poor plasma of septic patients versus healthy subjects. MMP-2 activity, detected by densitometric analysis of the gelatin zymography, was found to be significantly increased in septic subjects (**p=0.0018, healthy n=9 and septic n=9) when the results were analysed using an unpaired t-test. Bars represent mean + standard deviation



▲ figure 2 Comparison of MMP-2 activity in the platelet poor plasma of healthy (n=9), septic (n=9) and septic thrombocytopaenic (n=3) subjects. MMP-2 activity, detected by densitometric analysis of the gelatin zymography, was found to be significantly increased in septic non-thrombocytopaenic compared with healthy subjects (* - $p \leq 0.05$). MMP-2 activity was also significantly increased in septic thrombocytopaenic patients compared with healthy subjects (** - $p \leq 0.01$). Non-significantly elevated activity levels of MMP-2 were observed in septic thrombocytopaenic patients compared with septic subjects. The results were analysed using one way ANOVA. A Tukey post-test was applied to generate the p-value. Bars represent mean + standard deviation

▼ figure 3 Comparison of MMP-9 activity in the platelet poor plasma of healthy (n=9) versus septic subjects (n=9). MMP-9 activity, detected by densitometric analysis of the gelatin zymography, was found to be significantly increased (* - p=0.0495) in septic versus healthy subjects. The results were analysed using an unpaired t-test. Bars represent mean + standard deviation



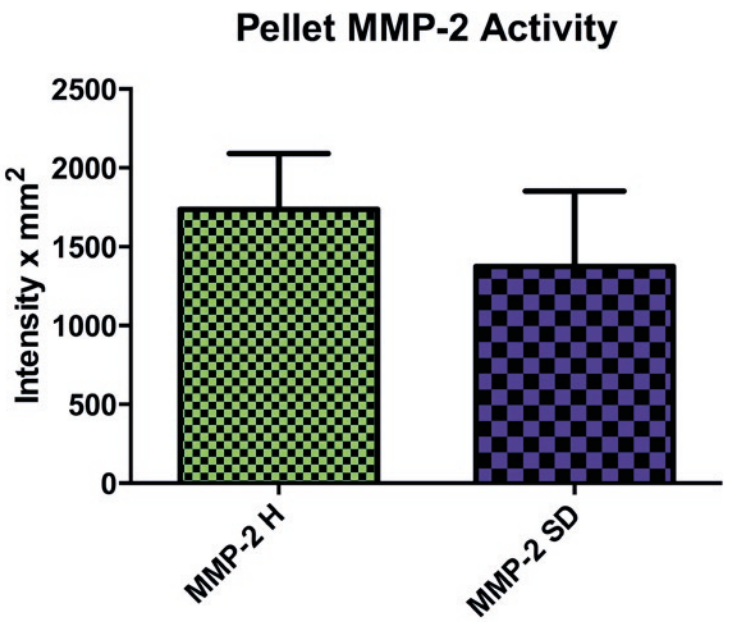


figure 4 Comparison of MMP-2 activity in the platelet pellet of septic patients versus healthy subjects. No statistically significant difference in MMP-2 activity, detected by densitometric analysis of the gelatin zymography, was found between septic and healthy subjects (p=0.1836, healthy n=6 and septic n=5) when the results were analysed using an unpaired t-test. Bars represent mean + standard deviation

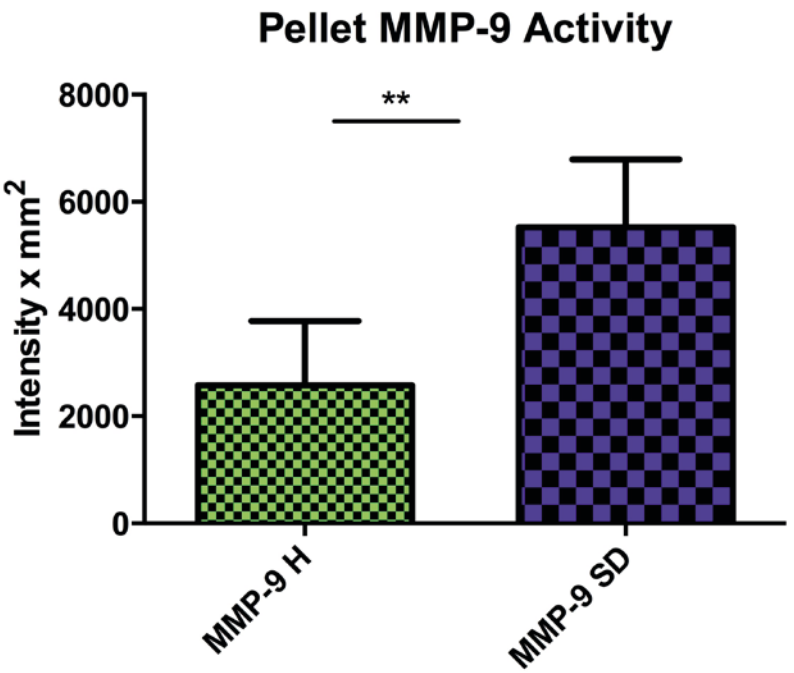


figure 5 Comparison of MMP-9 activity in the platelet pellet of healthy (n=6) versus septic subjects (n=5). MMP-9 activity, detected by densitometric analysis of the gelatin zymography, was found to be significantly increased in septic compared with healthy patients (**p=0.0034). The results were analysed using an unpaired t-test. Bars represent mean + standard deviation

Results

Platelet-poor plasma

Nine healthy, six septic non-thrombocytopaenic and three septic thrombocytopaenic samples were analysed. Following standardisation, zymography was performed on the samples to determine the activity of MMP-2 and MMP-9 (image 1) in the plasma of septic and healthy donors.

MMP-2 activity was found to be significantly upregulated in septic donors (n=9) compared with healthy ones (n=9) (p=0.018 (figure 1). ANOVA was used to compare MMP-2 activity across all three groups. MMP-2 activity was significantly increased (p≤0.01) in septic thrombocytopaenic patients (n=3) compared to healthy donors (n=9) as was MMP-2 activity in septic non-thrombocytopaenic patients (n=6) compared to healthy subjects (p≤0.05) (figure 1). No statistically significant difference in MMP-2 activity between thrombocytopaenic and non-thrombocytopaenic septic patients was found (figure 2).

MMP-9 activity was also significantly upregulated in septic donors (n=9) compared with healthy donors (n=9) (p=0.0495) (figure

3). No difference in MMP-9 activity between thrombocytopaenic (*n*=3) and non-thrombocytopaenic septic donors (*n*=9) was found.

Platelet pellet

There were six healthy, four septic non-thrombocytopaenic and two septic thrombocytopaenic samples of sufficient size to use in the investigation. As before, the samples were standardised and zymography performed. Due to the very small concentration of protein in some samples, certain septic and septic thrombocytopaenic samples were not of sufficient quantity for zymography. Only one thrombocytopaenic sample was suitable for use in zymography, therefore statistical analysis could not be performed on septic non-thrombocytopaenic versus septic thrombocytopaenic activity levels.

There were no significant differences in MMP-2 levels between septic (*n*=5) and healthy samples (*n*=6) (*p*=0.1836) (figure 4). MMP-9 levels were significantly increased in septic donors (*n*=6) compared to healthy donors (*n*=5) (*p*=0.0034) (figure 5).

Discussion

In this study, the activity of the enzymes MMP-2 and MMP-9 were compared in the plasma and the platelet membranes of septic and healthy donors. Furthermore, the MMP-2 and MMP-9 activities in the plasma were compared between two subsets of septic donors – thrombocytopaenic and non-thrombocytopaenic.

Under normal physiological circumstances, MMP-2 is released into the blood by activated platelets, as one of the mechanisms by which other platelets are recruited to the site of injury[13]. In sepsis, due to the systemic inflammatory response to infection, leukocytes release many cytokines, which in turn may stimulate platelet activation. It would appear that due to increased platelet activation, more MMP-2 is released by platelets. Ongoing functional studies using a quartz crystal microbalance have further confirmed that platelets are more labile in sepsis. We have demonstrated that MMP-2 is upregulated in sepsis and are the first, to our knowledge, to discuss this finding. Upregulation of MMP-2 may also be a contributing factor in the common septic condition of disseminated intravascular coagulation, as it is already known that MMP-2 is a pro-aggregatory agent[13].

Although this study could not confirm that MMP-2 activity is further upregulated in thrombocytopaenic patients compared to the non-thrombocytopaenic septic cohort due to the low number of donors available, we hypothesise that with a higher powered study, this may be demonstrated. Thrombocytopaenic donors have fewer platelets, but whether they are sequestered within organs, or have been activated and have aggregated, is unclear. Although thrombocytopaenic patients have fewer circulating platelets, we believe those remaining are more labile and prone to aggregation as they are very active in terms of MMP-2 release. It is known that thrombocytopaenia is indicative of poorer prognosis among the critically ill[5]. Therefore the fact that MMP-2 is likely to be upregulated among thrombocytopaenic patients indicates that upregulation may be indicative of illness severity and independent of platelet count.

Although it has previously been shown that MMP-9 is upregulated in septic cohorts[19], our findings further confirmed those results.

Our investigation yielded a number of interesting results with regard to MMP activity in the platelet membrane. We noticed a trend in which MMP-2 activity was upregulated in the platelet membrane of healthy donors compared to the septic sample. As previously mentioned, MMP-2 is a constitutively expressed enzyme, and platelet activation results in the translocation of its inactive pro- form to the membrane, where it is cleaved and activated prior to its release into the plasma[11]. Platelets are anucleate

cells and do not contain mRNA for MMP-2[20]. Therefore they cannot synthesise new MMP-2, so when MMP-2 is recruited to the platelet membrane, the stores in the cytosol are irreversibly depleted, reflecting this translocation. Our observation may be explained by such a depletion of the MMP-2 stores within the platelet cytosol, meaning that little remains to be translocated to the membrane and subsequently released into the plasma. This would be consistent with our findings of MMP-2 activity upregulation in the plasma of septic patients. There may be greater MMP-2 activity in the platelet membrane of septic patients than we detected should the investigation be carried out prior to this huge release of MMP-2 into the blood.

As we found less MMP-2 activity in the plasma of healthy donors we hypothesise that the MMP-2 is still located in the cytosol awaiting platelet activation and subsequent release. The platelet lysate could be analysed to confirm this.

Our results also revealed that MMP-9 activity in the platelet membrane is significantly upregulated in septic patients compared to healthy controls. However, there is continuing debate regarding the expression of MMP-9 in platelets and their capacity to release this enzyme. Certain studies have found evidence of MMP-9 in human platelets and its translocation to the membrane upon stimulation, similar to the mechanism of MMP-2 release[13,21]. A number of others dispute this and believe that platelets play a central role in leukocyte recruitment to the site of vascular damage, linking the processes of thrombosis and inflammation. Platelet-leukocyte complexes are formed when P-selectin glycoprotein ligand-1 on the surface of the leukocyte interacts with P-selectin expressed on the surface of activated platelets[14]. The presence of these complexes is significantly increased in the septic process[6] due to the inflammatory mechanisms at play in the pathogenesis of the disorder.

Interaction of platelets is thought to regulate the synthesis of the inducible MMP-9 in leukocytes in a paracrine manner. Platelet binding stimulates the leukocytes to release MMP-9 among other inflammatory mediators[22]. No method exists to isolate the separate components of the complexes and so, the debate continues as to whether it is the platelet or the leukocyte that releases the MMP-9. We believe that as platelets are more labile in septic patients, there may be increased likelihood of such platelet-leukocyte interactions occurring. This could possibly account for the upregulation in MMP-9 activity seen in the platelet membrane of septic patients.

As previously mentioned, we believe a higher powered study is required to confirm if the trend in MMP-2 activity that we have identified is of significance. An unfortunate limitation in our investigation was inadequate protein concentrations for analysis in four of the septic thrombocytopaenic samples.

Conclusion

In conclusion, we have shown that MMP-2 and MMP-9 activity is upregulated in the plasma of septic patients. Our finding of MMP-9 upregulation is in accordance with previous studies. However, the finding of upregulation in the activity of MMP-2 in septic patients has not previously been discussed. Furthermore, we postulate that with a larger study, increasing upregulation of MMP-2 activity in the thrombocytopaenic cohort of septic patients would be found. Thrombocytopaenia is an indicator of sepsis severity. In this study, the transition from non-thrombocytopaenic to thrombocytopaenic was paralleled by an increase in MMP-2 activity. We hypothesise that the progressive upregulation of MMP-2 with increasing severity of sepsis could be a novel marker for disease progression.

Acknowledgements

We would like to acknowledge Dr. Maria Santos-Martinez for her invaluable support and guidance throughout our project and thank the School of Pharmacy & Pharmaceutical Sciences, Trinity College Dublin. We also extend our gratitude to Dr. Caroline Larkin of the ICU in St. James' Hospital.

References

1 Levy, M. M. et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 31, 1250-1256, doi:10.1097/01.CCM.0000050454.01978.3B (2003).

2 Mayr, F. B., Yende, S. & Angus, D. C. Epidemiology of severe sepsis. Virulence 5, 4-11, doi:10.4161/viru.27372 (2014).

3 Sharma, B. et al. Thrombocytopenia in septic shock patients--a prospective observational study of incidence, risk factors and correlation with clinical outcome. Anaesth Intensive Care 35, 874-880 (2007).

4 Baughman, R. P., Lower, E. E., Flessa, H. C. & Tollerud, D. J. Thrombocytopenia in the intensive care unit. Chest 104, 1243-1247 (1993).

5 Hui, P., Cook, D. J., Lim, W., Fraser, G. A. & Arnold, D. M. The frequency and clinical significance of thrombocytopenia complicating critical illness: a systematic review. Chest 139, 271-278, doi:10.1378/chest.10-2243 (2011).

6 Clark, S. R. et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med 13, 463-469, doi:10.1038/nm1565 (2007).

7 Birkedal-Hansen, H. Proteolytic remodeling of extracellular matrix. Curr Opin Cell Biol 7, 728-735 (1995).

8 Santos-Martínez, M. J., Medina, C., Jurasz, P. & Radomski, M. W. Role of metalloproteinases in platelet function. Thrombosis research 121, 535-542, doi:10.1016/j.thromres.2007.06.002 (2008).

9 Rahman, M. et al. Metalloproteinases regulate CD40L shedding from platelets and pulmonary recruitment of neutrophils in abdominal sepsis. Inflamm Res 61, 571-579, doi:10.1007/s00011-012-0446-6 (2012).

10 Sawicki, G., Salas, E., Murat, J., Miszta-Lane, H. & Radomski, M. W. Release of gelatinase A during platelet activation mediates aggregation. Nature 386, 616-619, doi:10.1038/386616a0 (1997).

11 Sawicki, G. et al. Localization and translocation of MMP-2 during aggregation of human platelets. Thrombosis and haemostasis 80, 836-839 (1998).

12 Dal-Pizzol, F. et al. Matrix metalloproteinase-2 and metalloproteinase-9 activities are associated with blood-brain barrier dysfunction in an animal model of severe sepsis. Mol Neurobiol 48, 62-70, doi:10.1007/s12035-013-8433-7 (2013).

13 Fernandez-Patron, C. et al. Differential regulation of platelet aggregation by matrix metalloproteinases-9 and -2. Thromb Haemost 82, 1730-1735 (1999).

14 Ghasemzadeh, M. & Hosseini, E. Platelet-leukocyte crosstalk: Linking proinflammatory responses to procoagulant state. Thromb Res 131, 191-197, doi:10.1016/j.thromres.2012.11.028 (2013).

15 Yassen, K. A., Galley, H. F. & Webster, N. R. Matrix metalloproteinase-9 concentrations in critically ill patients. Anaesthesia 56, 729-732 (2001).

16 Nakamura, T., Ebihara, I., Shimada, N., Shoji, H. & Koide, H. Modulation of plasma metalloproteinase-9 concentrations and peripheral blood monocyte mRNA levels in patients with septic shock: effect of fiber-immobilized polymyxin B treatment. Am J Med Sci 316, 355-360 (1998).

17 Radomski, M. & Moncada, S. An improved method for washing of human platelets with prostacyclin. Thromb Res 30, 383-389 (1983).

18 Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-254 (1976).

19 Yazdan-Ashoori, P. et al. Elevated plasma matrix metalloproteinases and their tissue inhibitors in patients with severe sepsis. Journal of critical care 26, 556-565, doi:10.1016/j.jcrc.2011.01.008 (2011).

20 Cecchetti, L. et al. Megakaryocytes differentially sort mRNAs for matrix metalloproteinases and their inhibitors into platelets: a mechanism for regulating synthetic events. Blood 118, 1903-1911, doi:10.1182/blood-2010-12-324517 (2011).

21 Sheu, J. R. et al. Expression of matrix metalloproteinase-9 in human platelets: regulation of platelet activation in in vitro and in vivo studies. Br J Pharmacol 143, 193-201, doi:10.1038/sj.bjp.0705917 (2004).

22 Galt, S. W. et al. Differential regulation of matrix metalloproteinase-9 by monocytes adherent to collagen and platelets. Circ Res 89, 509-516 (2001).

Niamh Buckley

Patrick Collins

CASE STUDIES

A Multifactorial Assessment: falls in the older population within the emergency department

Elizabeth Gialanze' and Nicole Grech
Fifth year Medicine, University of Malta

Introduction

Falling in the elderly is associated with an increase in morbidity, mortality, reduced functioning and premature admission to nursing homes[1, 2]. The factors responsible for falling can often be corrected depending on the age of the patient, any underlying medical conditions, and the presence of any hazards within their home environment. As age increases, both the incidence of falls and the severity of the related injuries worsen and it is suggested that in geriatric populations, the mechanism of injury is often not proportional to the severity of resulting complications[3]. The elderly also often fail to report an episode of a fall to their doctor[4]. This results in any prophylactic intervention for falls taking place after injury, where disability may already have resulted. The patient is often brought to a clinic by another family member or an eye witness to the fall.

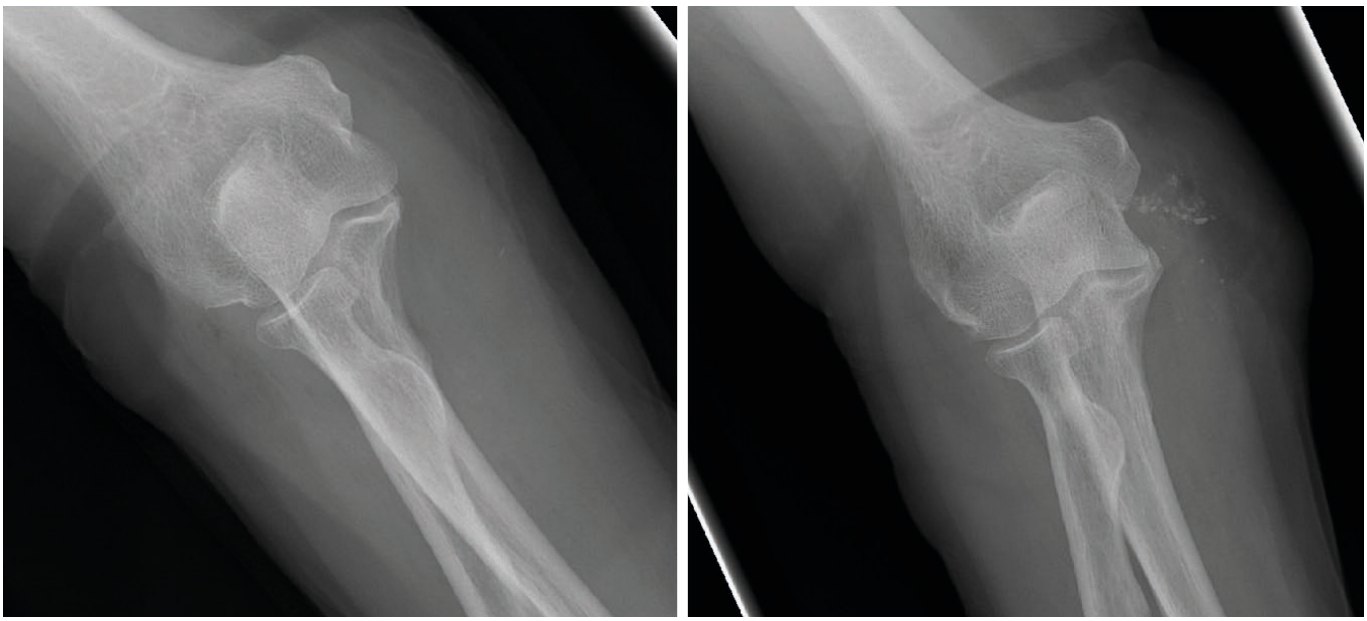


figure 1 The patient's soft tissue injury over the olecranon of the posterior right upper limb

Presentation of case

An 82 year old Caucasian woman presented to the Emergency Department (ED) for the treatment of a soft tissue injury over the olecranon on the posterior part of her right upper limb. This wound was sustained following a fall on the street. The injury was a deep flap wound measuring 5 cm by 5 cm with the tip of the flap positioned distally, with a degree of

muscular exposure.

Management

On physical examination, the patient was systemically well and her vitals were normal with blood pressure values within normal range. Normal pulse rate and rhythm were recorded and there were normal heart sounds with no added sounds.

A history was taken with particular attention being given to whether she lost consciousness before or after the fall. The patient however claimed to remember tripping over loose gravel in the street and declared not to have lost consciousness. This was confirmed by an accompanying eye-witness. It was noted in the patient's history that this was the third fall in the past year. Therefore, the patient was referred to a Falls and Blackouts Unit (FABU).

She was initially sent for a radiograph of her right arm, taken in the antero-posterior and lateral views. This showed no fractures and no oedema of soft tissues, however small particles of a radio-opaque nature were present lateral to the site of injury. Adequate cleaning and irrigation of the wound was then conducted, six continuous subcuticular absorbable 4/0 sutures and thirteen interrupted non-absorbable 4/0 sutures were used. The patient was subsequently sent for a repeat radiograph of the same site, and it was noted that the former radio-opaque particles were no longer present, confirming that debris was introduced during the sustainment of the injury.

Outcome and follow-up

Given that the patient had not received Tetanus immunisation, apart from the first dose of the passive immunisation, 250IU Tetanus Immunoglobulin was administered to provide immediate protection. Amoxicillin/Clavulanic acid three times daily for five days was prescribed and an appointment was made for the sutures to be removed in ten days.

Discussion

Falls are the most common cause of accidental injury in older people and a frequent cause of accidental death in those who are over 75 years of age[3]. About 20-30% of those who endure a fall sustain a moderate or severe injury – falls are the most common cause of fractures and traumatic brain injury[3, 6], with falls due to

table 1 Intrinsic causes of falls in the elderly

Cause	Neurological	Cardiovascular	Musculoskeletal	Metabolic	Psychological
Examples	Syncope • Due to carotid hypersensitivity • Simple vasovagal syncope • Situational syncope e.g. triggered by micturition or coughing	Orthostatic hypotension	Osteoarthritis	Hypoglycemia	Anxiety
		Cardiac arrhythmias	Rheumatoid arthritis	Hypothyroidism	Munchhausen syndrome
		Stokes Adams attacks	Fragility fractures (e.g. neck of femur)		
	Cognitive impairment (e.g. dementia or delirium)		Muscle weakness (e.g. due to vitamin D deficiency)		
	Labyrinthine problems				
	Peripheral neuropathy				
	Drop attacks				
	Epilepsy				
	Reduced vision (e.g. presbyopia)				

syncope likely to result in facial bruising[5]. Presentation after a fall is the most common cause for older people to attend the ED. This results in subsequent admission to hospital for further medical or surgical care. Injury occurs more commonly in frail persons and the nature of the fall affects the risk and type of injury[7]. In the more active and younger generation, wrist fractures are most common whereas in the older population, hip fractures are most common[5].

Falls in the elderly can have a negative effect on morbidity and activities of daily living, and this impact on their activities of daily living contributes towards a loss of confidence in leaving the house[3].

A prospective study targeting sixty-five elderly patients (mean age of 78 years) in a FABU was carried out over a period of six months. Initial patient evaluations included ambulatory electrocardiography, carotid sinus massage before and after atropine and prolonged tilt-head testing[8]. The study concluded that a diagnosis was attributed to symptoms in 92% of patients. These included the following: carotid sinus syndrome (CSS; 45%), postural hypotension (32%), vasovagal syncope (11%), cardiac arrhythmia (21%), epileptic seizures (9%), cerebrovascular disease (6%) and others (12.5%)[8].

To identify the risk factors contributing to a fall, there is a four step process which includes; (i) Focused history taking (ii) Physical examination (iii) Functional assessment of the Activities of Daily Living (ADLs), and an (iv) Environmental Assessment including home safety[9, 10]. Focused history taking is of great importance in the management of the older patient, since this would delineate the probable cause for the fall. The aetiology of falls can be classified into intrinsic causes and extrinsic causes. Extrinsic causes of falls in the elderly would include objects in their way causing tripping (e.g. appliance wires, carpets, incontinence, unsafe footwear, confusion, poor lighting and an uneven walking surface)[11]. The identification of any underlying risk factors is also of great importance since this would contribute towards the patient's overall morbidity. A further subcategory is whether the cause was mechanical (affecting the patient's mobility) or non-mechanical (not affecting the patient's mobility). The intrinsic causes of falls are vast and apart from being attributed to lethargy, an increasing problem in the elderly (anemia, infections or hypothermia are frequent causes of increased lethargy), they can also be grouped as following in a systematic manner[11].

Medication is a common aetiological factor for falls in the elderly. Common pharmacological culprits are anti-hypertensives, sedatives, and drugs which cause parkinsonism as a side effect, such as prochlorperazine and metoclopramide[11]. There is a strong link between psychotropic medication and falls in the older population, especially with use of benzodiazepines[12]. The patient who is the subject of this case study reported having been prescribed benzodiazepines a few months previously for anxiety. These and other psychotropic medications have been associated with increased incidence of falls in the elderly[13]. Moreover, they

CLINICAL POINTS

Falls are a common cause of presentation to the Emergency Department in the elderly and can lead to loss of confidence and independence

Causes are often multifactorial in origin and there is evidence for a linear relationship between frailty and an increase frequency of falling among the elderly

The incidence of falls in the elderly contributes towards increased psychosocial morbidity, and can result in patients being fearful of leaving their home

This causes a restriction in overall mobility which may lead to increased hospitalisation resulting in negative health sequelae and increased mortality

A patient-centred management and referral to appropriate clinics for follow-up is especially essential within the older population

have been found to be independent risk factors. Positively they are a modifiable risk factor, signifying that a detailed drug history and appropriate changes to the patient's drug regimen may thus prevent further episodes[14].

An issue which is not always fully respected in geriatrics is the issue of polypharmacy. Polypharmacy is defined as four or more drugs prescribed for the patient at any given point in time[15]. Polypharmacy is often described as a risk factor for falls in the elderly, but only when one or more of the prescribed drugs was itself a falls-risk associated drug[16]. The 82-year-old patient in this study was on other medication for other conditions at the time of her fall. Review of the patient's medication is essential, and if this is found to be a contributory factor, modification of drug therapy is warranted[12].

It is important to determine whether the patient lost consciousness, before or after the fall, if at all. The patient often reports finding him or herself on the ground, or it may have been a result of the fall due to sustaining a head injury. A collateral history from an eye witness is an important contributing factor in the determination of the exact sequence of events and is always of great benefit as it may influence the management of a patient. Any preceding symptoms or signs must also be investigated – these may include clouding of vision, diplopia or vertigo.

A physical examination should then follow history-taking, where specific attention should be given to the cardiovascular and neurological status of the patient, as well as other specific investigations such as blood pressure parameters (when supine and standing upright), glucose levels and imaging modalities. It is important to rule out any other affecting chemicals (including use of sedatives or abuse of alcohol) and the possibility of any metastatic or non-metastatic manifestation of malignancy, as well as cerebellar lesions[17]. The aim of the physical examination is to support the history and identify which therapeutic group the patient belongs to.

An assessment of the living facilities and the patient's ability to perform any activities of daily living is of great importance especially when dealing with frail patients or those affected by the geriatric giants (instability, incontinence, immobility, intellectual impairment, and iatrogenic problems)[18]. Stairs might manifest as a physical barrier impeding movement and toileting for the patient. It is also important to assess the patient's support framework; whether he or she lives alone or has any live-in help, how close his or her next of kin live and how supportive they are[19].

An integration of the findings of the multifactorial assessment of the home environment must always be acknowledged by clinicians in order to make it safer for the patient. This can potentially offer home help and mobility aids. Appropriate referral to physiotherapy may be warranted, and often occupational therapy within the community is also indicated.

Conclusion

Falls as a presenting complaint often result in hospital admissions in the elderly. These patients must be assessed as to whether they will benefit from rehabilitation and inpatient care. Intervention will therefore benefit from referral to an appropriate clinic.

Acknowledgements

This case report was written during a four week elective within the Emergency Medicine Department at St James' Hospital in Dublin Ireland, with the help and supervision of Dr. C Geraldine McMahon, and additional input by Ms Gabrielle Dunne.

References

1 McMahon, C.G., Kenny, R.A., Bennett, K., Bouamra, O., & Lecky, F. Diurnal variation in mortality in older nocturnal

fallers. Age and Ageing. 41, 29–35 (2012).

2 Tinetti, M.E. & Williams, C.S. Falls, injuries due to falls, and the risk of admission to a nursing home. N Engl J Med. 337, 1279–84 (1997).

3 Sterling, D.A., O' Connor, J.A. and Bonadies, J. Geriatric falls: Injury severity is high and disproportionate to mechanism. Journal of Trauma – Infection & Critical Care. 50, 116-119 (2001).

4 Blake, A.J., Morgan, K., Bendall, M.J., Dallosso, H., Ebrahim, S.B.J., Arie, T.H.D., Fentem, P.H. & Bassey, E.J. Falls by elderly people at home: prevalence and associated factors. Age and Ageing. 17, 365-372 (1988).

5 Speechley, M. & Tinetti, M. Falls and injuries in frail and vigorous community elderly persons. Journal of the American Geriatrics Society. 39, 46-52 (1991).

6 Alexander, B.H., Rivara, F.P. & Wolf, M.E. The cost and frequency of hospitalisation for fall-related injuries in older adults. American Journal of Public Health. 82,; 1020-1023 (1992).

7 Finbarr M. 'Falls', British Guidelines Society Good Practice Guide (2007).

8 McIntosh, S., Da Costa, D. & Kenny R.A. 'Outcome of an Integrated Approach to the Investigation of Dizziness, Falls and Syncope, in Elderly Patients referred to a 'Syncope' Clinic'. Age and Ageing. 22, .53-58 (1993).

9 Davies, A.J., & Kenny, R.A. 'Falls presenting to the Emergency and Accident Department: Types of Presentation and Risk Factor Profile. Age and Ageing. 25, 362-366 (1996).

10 Shaw, F.E. & Kenny, R.A. The overlap between syncope and falls in the elderly. Postgrad Med J. 73, 635-639 (1997).

11 Bueno-Cavanillas, A., Padilla-Ruiz, F., Jimenes-Moleon, J.J., Peinado-Alonso, C.A. & Galvez-Vargas, R. Risk factors in falls among the elderly according to extrinsic and intrinsic precipitating causes. European Journal of Epidemiology. 16, 849-859 (2000).

12 McMahon, C.G., Cahir, C.A., Kenny, R.A., Bennett, K. Inappropriate prescribing in older fallers presenting to an Irish emergency department. Age and Ageing. 43, 44-50 (2013).

13 Huang, A.R., Mallet, L., Rochefort, C.M., Eguale, T,, Buckeridge, D.L. & Tamblyn, R. Medication-related falls in the elderly: causative factors and preventive strategies. Drugs Aging. 29, 359–76 (2012).

14 National Institute of Clinical Excellence. Clinical Guideline 21. The Assessment and Prevention of Falls in Older People. National Institute of Clinical Excellence: London: http://www.nice.org.uk (2014).

15 Patterson, S.M., Hughes, C., Kerse, N., Cardwell, C.R. & Bradley, M.C. Interventions to improve the appropriate use of polypharmacy for older people. Cochrane Database Syst Rev. 5, CD008165 (2012).

16 Ziere, G., Dieleman, J.P., Hofman, A., Pols, H.A.P., Van der Cammen, T.J.M. & Stricker, B.H.CH. Polypharmacy and falls in the middle aged and elderly population. British Journal of Clinical Pharmacology. 61, 218-223 (2006).

17 Sharma, J.C., Mac Lennan, W.J. Causes of ataxia in patients attending a falls laboratory. Age and Ageing. 17, 94-102 (1988).

18 Close, J.C.T. Interdisciplinary practice in the prevention of falls - areview of working models of care. Age and Ageing. 30, 8-12 (2001).

19 Sattin, R.W., Rodriguez, J.G., DeVito, C.A. & Wingo, P.A. Home environmental hazards and the risk of fall injury events among community-dwelling older persons. Study to Assess Falls among the Elderly (SAFE) Group. Journal of the American Geriatrics Society. 46, 669-676 (1998).

Elizabeth Gialanze'

Nicole Grech

Should Psoriasis Patients be Screened for Heart Disease?

Michelle De Deyn, Ciara Guerin, Kevin Moloney, Yasmine Roden, Neelam Nath
Third year Medicine, Trinity College Dublin

Abstract

Psoriasis is a systemic, immune-mediated disorder that manifests as chronic skin and joint inflammation. While the prevalence of psoriasis worldwide is 2%, its prevalence in developed countries is on average about 4.6%. Psoriasis is associated with cardiovascular disease (CVD). For this literature review, Pubmed and Medline were used to source articles using the keywords 'psoriasis' and 'cardiovascular disease'. The inclusion criteria were studies with sample sizes of 100 or more published in English from 2000 onwards. Out of 1,657 papers retrieved, 37 were deemed relevant. Of these, 36 papers contained evidence in support of screening for CVD while one paper had evidence against screening for CVD. Substantial evidence suggested a higher CVD prevalence in psoriasis patients compared to populations without psoriasis. Furthermore, the risk of developing CVD correlated highly with increased psoriasis severity and duration in addition to other CVD risk factors. The pathophysiological link between psoriasis and CVD is a common immunological pro-inflammatory state. In conclusion, psoriasis is an independent risk factor for CVD, particularly in younger patients with severe psoriasis, and is associated with increased mortality. Further research is required to better understand the relationship between psoriasis, traditional risk factors and development of CVD.

Introduction

Psoriasis is a systemic, immune-mediated disorder that manifests as a chronic skin and joint inflammation[1]. While the prevalence of psoriasis worldwide is 2%, its prevalence in developed countries is on average about 4.6%[2]. The gold standard for measuring psoriasis is the Psoriasis Area and Severity Index (PASI)[3]. Nearly two thirds of people with psoriasis have a mild form of the disease, classified as less than 3% of the skin surface affected[4].

The pathogenesis of psoriasis shows an association with certain types of human leukocyte antigen (HLA), suggesting a genetic component[5]. Furthermore, the genesis of new lesions at sites of trauma suggests a role for exogenous stimuli. Sensitized CD4+ T-helper (Th)-1 and Th-17 cells, and activated cytotoxic T lymphocytes that accumulate in the epidermis may drive keratinocyte proliferation by elaborating cytokines[5]. In particular, Th-1, Th-17 and Th-22 cell populations are expanded and stimulated to release inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-17 and IL-22[6, 7, 8]. The inflammation that drives psoriatic pathology is systemic and there is evidence to suggest that it contributes to immunological and metabolic changes that enhance and perpetuate psoriasis, as well as to the development of co-morbidities[9].

CLINICAL POINTS

Psoriasis is a systemic, immune-mediated disorder

Psoriasis is an independent risk factor for CVD, particularly in younger patients with severe disease

Psoriasis is associated with increased mortality

The NICE guidelines recommend that patients with severe psoriasis be offered screening for any potential CVD risk

The literature shows that moderate to severe psoriasis is associated with diseases such as ischaemic heart disease, stroke, hypertension, dyslipidaemia and diabetes[4].

This paper will review the evidence for CVD screening in patients with psoriasis of all severities, evaluate the strength of the association and deliberate

table 1 Vascular disease and mortality - psoriasis and traditional cardiovascular risk factors[13]

Risk factor	Peripheral vascular disease	Ischaemic heart disease	Cerebrovascular Disease	Any Vascular Disease	Mortality
Hypertension	3.52 (2.44-5.07)	2.92 (2.48-3.44)	3.32 (2.59-4.25)	3.21 (2.76-3.73)	1.13 (0.95-1.34)
Diabetes	2.40 (1.84-3.13)	2.30 (1.96-2.70)	2.08 (1.70-2.54)	2.38 (2.04-2.79)	1.27 (1.05-1.52)
Dyslipidaemia	1.81 (1.39-2.36)	2.39 (2.05-2.80)	1.56 (1.27-1.90)	2.16 (1.86-2.52)	0.35 (0.28-0.43)
Tobacco use	1.78 (1.20-2.63)	1.83 (1.42-2.37)	1.78 (1.32-2.40)	2.14 (1.67-2.74)	0.80 (0.59-1.09)
Psoriasis	1.98 (1.38-2.82)	1.78 (1.51-2.11)	1.70 (1.33-2.17)	1.91 (1.64-2.24)	1.86 (1.56-2.21)

Patient group	Mortality (% total patients)
Patients with psoriasis (n=3236)	19.6
Controls (n=2500)	9.9

when CVD screening may become necessary for psoriasis patients. It also discusses whether earlier and more stringent control of the disease-driving inflammatory processes could potentially prevent the development and worsening of CVD. This has important implications in terms of risk stratifying patients with psoriasis for CVD risk and eventually improving overall patient outcomes.

Currently various CVD risk scores are being used. Equations derived from the American Framingham cohort study are the most widely used in the United Kingdom (UK)[10]. A 2007 study by Hippisley-Cox et al. derived a new CVD risk score (QRISK) for the UK and its performance validated against the Framingham algorithm and ASSIGN (used in Scotland). The Framingham algorithm over-predicted CVD risk at 10 years by 35%, ASSIGN by 36%, and QRISK by 0.4%. The study concluded that QRISK, which includes additional variables of positive family history and antihypertensive treatment, provides a more appropriate risk estimate for UK patients on the basis of age, sex, and social deprivation[11].

Methods

For this literature review, Pubmed and Medline were used to source articles using the keywords 'psoriasis' and 'cardiovascular disease'. The inclusion criteria were studies with sample sizes of 100 or more, published in English from 2000 onwards. Out of 1,657 papers retrieved, 37 were deemed relevant. Of these, 36 papers contained evidence in support of screening for CVD while one paper had evidence against it.

Results

36 papers with evidence supporting screening psoriasis patients for CVD were relevant. Of these,

- 26 papers concluded that psoriasis causes a higher incidence of CVD
- 4 papers suggested that pharmacological treatment of psoriasis increases the risk of CVD
- 3 papers assessed the risk of patients with

psoriasis and psoriatic arthritis developing CAD, peripheral vascular disease and cerebrovascular accidents

- 3 papers showed a causal link between psoriasis and metabolic disease, predisposing to CVD

A systematic literature review (1980 – 2011) by Horreau C et al[12]. assessed cardiovascular morbidity and mortality in psoriasis and psoriatic arthritis patients. This research identified 33 observational studies, which compared psoriasis and psoriatic arthritis patients with a control group. A common link, immunological and inflammatory pathways, was observed between psoriasis and psoriatic arthritis patients who developed stroke, coronary artery disease (CAD), or peripheral vascular disease (PVD). Psychological stress, sedentary lifestyle and poor compliance to CVD risk factor management contributed to the link. Psoriasis and psoriatic arthritis were shown to be significant risk factors for a cardiovascular event, although, there was no correlation with CVD mortality. The limitations of this study include: heterogeneity in study design, outcome definition and assessment methods.

A 2009 observational study by Prodanovich et al[13]. compared 3,236 psoriasis patients with 2,500 control subjects. It was concluded that psoriasis is an independent risk factor for CVD mortality, usually linked to atherosclerosis development. Psoriasis increases the occurrence of diabetes mellitus, hypertension and dyslipidemia, resulting in atherosclerosis. A 2009 literature review by Kimball et al[14]. of articles sourced from Medline (1995 – 2007) reached similar conclusions. Both studies showed evidence of pro-inflammation in psoriasis and CVD. This is consistent with the literature regarding the role of inflammation in vaso-occlusive cardiac disease.

table 2 Cardiovascular risk factors associated with psoriasis

Risk Factor	Mild Psoriasis	Controls (mild)	Odds ratio (95% CI) (mild)*	Severe Psoriasis	Controls (severe)	Odds ratio (95% CI) (severe)*
Diabetes	4.4%	3.3%	1.27 (1.23-1.31)	7.1%	3.3%	1.86 (1.58-2.19)
Hyperlipidaemia	4.7%	3.3%	1.28 (1.24-1.33)	6.0%	3.6%	1.31 (1.11-1.56)
Hypertension	14.7%	11.8%	1.16 (1.14-1.18)	20.0%	13.2%	1.25 (1.13-1.39)
Smoking	28.0%	21.1%	1.40 (1.38-1.43)	30.1%	22.5%	1.31 (1.20-1.44)
BMI = 25 – 30	35.0%	32.9%	1.12 (1.10-1.14)	37.7%	33.4%	1.28 (1.15-1.43)
BMI > 30	15.8%	13.1%	1.29 (1.26-1.32)	20.7%	13.0%	1.84 (1.60-2.11)

*Abbreviations: BMI - body mass index. *after adjustment for age, sex, and person-years, CI - confidence interval*

A 2011 study by Johnsson et al[15]. concluded that a raised BMI and psoriasis could increase risk of diabetes mellitus. Psoriatic patients were shown to have elevated visceral fat, which promotes liver insulin resistance and non-alcoholic fatty liver disease.

A 2013 systematic review and meta-analysis by Samarasekera et al[16]. examined studies regarding the incidence of CVD in psoriasis patients from 1984 to 2013. The systematic review included 14 cohort studies with sample sizes ranging from 130 to 462 subjects (n = 976). Results were classified into two subgroups based on the severity of disease, with those requiring systemic treatment or hospital admission being classified as severe psoriasis. Patients with severe psoriasis were reported to have increased

incidence of CVD mortality in all cohorts, with the absolute increase being 1 in 283 patients per year. However, the evidence for CVD mortality associated with mild psoriasis was inconsistent and hence weak. Inadequate awareness of concomitant CVD risk factors existing in psoriatic patients is a limitation that affects the study outcome preventing a causal relationship from being drawn between psoriasis and CVD. The review concluded that patients with severe psoriasis should be screened for CVD.

Another study by Farajzadeh et al[17]. in 2012 investigated the prevalence of CVD risk factors in psoriasis patients. In the study, 73 psoriatic patients aged 20 to 50 years were age and sex matched to 73 control subjects. The patients included had mild psoriasis according to the PASI scoring system. Individuals with thyroid disease, familial hyperlipidemia, nephrotic syndrome, cholestasis, people on drugs affecting lipid metabolism, and pregnant women were excluded. Relevant risk factors were assessed. Of these, hypertension, diabetes mellitus, hypercholesterolemia, hypertriglyceridemia, impaired fasting glucose and elevated BMI were shown to be more prevalent in the psoriasis patients than in the control group. There was no significant difference in the prevalence of cigarette smoking between both groups. It should be noted that this study is limited by its small sample size and inclusion of patients with mild psoriasis, therefore, the results may not be representative of all psoriasis severity groups.

In 2013, a population-based study by Dowlatshahi et al[18]. included 262 psoriasis patients and 8,009 reference subjects, all above 55 years. They were followed-up for a mean period of 11 years. The results indicated no significant difference in atherosclerosis and cardiovascular events incidence between patients with mild psoriasis and reference subjects. The study was limited by including only subjects above 55 years old; hence it is not representative of all psoriasis patients. The small psoriasis patient sample size may have resulted in the absence of a statistically significant difference observed between the groups. In addition, the prevalence of CVD in patients with severe psoriasis was not addressed in this study.

A 2013 meta-analysis by Gaeta et al[19]. showed a relative risk of 1.24 for CVD in psoriasis patients. Psoriasis patients showed a 25% increased relative risk of CVD. Thirteen studies were included, with sample sizes ranging from 598 to 4,042,257 and subjects aged between 45 and 57.1 years. Duplicate studies, literature reviews, commentaries, poor quality studies and small sample size studies were excluded. The limitation of this study was its heterogeneity in the assessment of psoriasis between studies, thereby inhibiting an association between the effects of psoriasis severity on cardiovascular risk being formed.

NICE guidelines

Current NICE Guidelines recommend: "Offer adults with severe psoriasis of any type a cardiovascular risk assessment at presentation using a validated risk estimation tool. Offer further assessment of cardiovascular risk every 5 years, or more frequently if indicated following assessment."[20]

However, no data was available regarding the adherence to these guidelines.

Discussion

The literature reviewed suggests a significant relationship exists between psoriasis and CVD compared to populations without psoriasis. Specifically, the risk of developing CVD correlated highly with increased psoriasis severity and duration in addition to other CVD risk factors such as smoking, obesity and sedentary lifestyle. The pathophysiological link between psoriasis and heart disease is a common immunological pro-inflammatory state, which occurs with the release of inflammatory cytokines, such as IL1, commonly present in kerati-

nocytes[21]. These act as mediators in initiating and maintaining psoriatic plaques in skin. Co-existence of dyslipidaemia and pro-inflammatory cytokines thus accelerate atherosclerotic lesion and plaque formation, leading to hypertension and CVD due to vaso-occlusive effects. There is also an association between these events and an increase in the inflammatory cytokine IL8. In addition, elevated visceral fat in psoriatic patients can lead to hepatic insulin resistance predisposing to diabetes, which is a significant risk factor of CVD.

Conclusion

There is strong evidence to suggest that psoriasis is an independent risk factor for CVD, particularly in younger patients with severe psoriasis[13], and is associated with increased mortality[14]. However, further research is required to better understand the relationship between psoriasis, traditional risk factors and development of CVD. While no major studies have yet shown whether screening for CVD in psoriasis patients in other countries have been beneficial, we recommend early screening for CVD and risk factors in this patient group be implemented in Irish health systems to improve patient outcomes[17]. In addition, because the study concerning screening of patients with mild psoriasis was not strong, and in view of the underlying common pro-inflammatory state behind CVD and psoriasis, further research is necessary regarding whether screening for CVD is potentially beneficial for all psoriasis patients. This would be beneficial to determine if more stringent control of the disease driven inflammation could potentially prevent the development and worsening of CVD.

Acknowledgements

We would like to thank Dr. Shivashini Jeyarajan of Adelaide and Meath Hospital Dublin incorporating the National Children's Hospital (AMNCH) for her guidance in writing this literature review.

References

1 Reich, K. Review Article - The concept of psoriasis as a systemic inflammation: implications for disease management. Journal of the European Academy of Dermatology and Venereology 26, 3-11 (2012).

2 Lebwohl, M. Psoriasis. The Lancet 361, 1197-1204 (2003).

3 Feldman, S. & Krueger, G. Psoriasis assessment tools in clinical trials. Annals of the Rheumatic Disease 64, 65-68 (2005).

4 WHO. Psoriasis. In Executive Board 133rd session (ed. Secretariat, R.b.t.) (2013).

5 Mitchell, R., Kumar, V., Abbas, A., Fausto, N. & Aster, J. Pocket Companion to Robbins and Cotran Pathological Basis of Disease, (Elsevier Saunders, 2012).

6 Lowes, M., et al. Psoriasis Vulgaris Lesions Contain Discrete Populations of Th1 and Th17 T Cells. Journal of Investigative Dermatology 128, 1207-1211 (2008).

7 Nograles, K., et al. IL-22-producing "T22" T cells account for unregulated IL-22 in atopic dermatitis despite reduced IL-17-produceing TH17 T cells. Journal of Allergy and Clinical Immunology 123, 1244-1252 (2009).

8 Khattri, S., et al. Cyclosporine in patients with atopic dermatitis modulates activated inflammatory pathways and reverses epidermal pathology. Journal of Allergy and Clinical Immunology 133, 1626-1634 (2014).

9 Spah, F. Inflammation in atherosclerosis and psoriasis: common pathogenic mechanisms and the potential for an integrated treatment approach. British Journal of Dermatology 159, 10-17 (2008).

10 Bitton, A. & Gaziano, T. The Framingham Heart Study's Impact on Global Risk Assessment. Progress in Cardiovascular Disease 53, 68-78 (2010).

11 Hippisley-Cox, J., et al. Derivation and validation of QRISK, a new cardiovascular disease risk score for the United Kingdom: prospective open cohort study. British Medical Journal 335(2007).

12 Horreau, C., Pouplard, C. & Brenaut, E. Cardiovascular Morbidity And Mortality In Psoriasis and Psoriatic Arthritis: A Systematic Literature Review. Journal of the European Academy of Dermatology and Venereology, 12-29 (2013).

13 Prodanovich, S., et al. Association of Psoriasis With Coronary Artery, Cerebrovascular, and Peripheral Vascular Diseases and Mortality. Archives of Dermatology 145, 700-703 (2009).

14 Kimball, A.B. & Wu, Y. 2009. International Journal of Dermatology 48, 1147-1156 (Cardiovascular Disease and Classic Cardiovascular Risk Factors in Patients with Psoriasis).

15 Johnsson, H., Mccliness, I.B. & Sataar, N. Cardiovascular and Metabolic Risks in Psoriasis and Psoriatic Arthritis: Pragmatic clinical management based on available evidence. Annals of the Rheumatic Diseases 71, 480-483 (2012).

16 Samarasekera, E.J. & Neilson, J.M. 2013. Journal of Investigative Dermatology 133, 2340-2346 (Incidence of CVD in individuals with Psoriasis: A Systematic review and meta-analysis).

17 Farajzadeh, S., Zandi, S., Panahandeh, L. & Kamyabi, Z. Prevalence of cardiovascular risk factors in psoriasis patients. Journal of Pakistan Association of Dermatologists 22, 86-90 (2012).

18 Dowlatshahi, E.A., et al. Psoriasis Is Not Associated with Atherosclerosis and Incident Cardiovascular Events: The Rotterdam Study. Journal of Investigative Dermatology 133, 2347-2354 (2013).

19 Gaeta, M., et al. Role of psoriasis as independent predictor of cardiovascular disease: A meta-regression analysis. International Journal of Cardiology 168, 2282-2288 (2013).

20 NICE guideline, Psoriasis: the management of psoriasis. (2012).

21 Balato, A., et al. Pathogenesis of Psoriasis: The Role of Pro-Inflammatory Cytokines Produced by Keratinocytes. (ed. Psoriais) 9-21 (2012).

Michelle De Deyn

Ciara Guerin

Kevin Moloney

Yasmine Roden

Neelam Nath

PSA I Love You: prostate cancer screening in Ireland

Eimear Keane
Fourth year Medicine, Trinity College Dublin

Abstract

Ireland has the second highest rate of prostate cancer (PCa) in Europe and this is largely due to the increased use of PSA testing in Ireland in recent years. Rapid Access Prostate Cancer Clinics (RAPC) were introduced in 2009, in order to increase the organisation and efficiency of PCa diagnosis in Ireland.

The use of PSA as a screening tool is controversial. At present, there is no evidence for the introduction of widespread, population-based screening for PCa. Two large randomised studies which evaluated the efficacy of PSA testing are examined in this review. These are the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) and The European Randomised Study for Prostate Cancer (ERSPC). These studies demonstrated conflicting results. The PLCO reported that there was no evidence that PSA screening reduced PCa-related mortality, while the ERSPC showed PSA screening was associated with a 21% decrease in PCa related mortality.

Following the publication of these trials, the European Association of Urology and the American Urology Association produced new PCa screening guidelines. These guidelines advised against widespread screening programmes but recommended PSA testing in men who met certain criteria (based on age, life-expectancy and other risk factors). In Ireland, there are currently very little national guidelines in relation to PCa screening. However, much opportunistic testing occurs and patients often request PSA tests in a GP setting. National guidelines for PSA screening, which are in line with the AUA (American Urology Association) and EAU (European Association of Urology) guidelines, need to be introduced in order to prevent overtreatment and over-diagnosis of asymptomatic prostate cancer.

Introduction

Prostate cancer (PCa) is the second most common cancer in males worldwide after lung cancer, with over 1.1 million cases diagnosed in 2012[1]. In Ireland today, PCa is the most prevalent non-skin cancer and the second most frequent cause of cancer mortality in men[2]. At present, it has an incidence rate of 156.4/100,000 in Ireland[2]. The majority of cases (97%) are diagnosed in patients older than 50 years[3].

The prostate is a small gland in the male reproductive system that helps to produce and store semen. It is divided into four anatomically distinct zones with the majority of cancers (70%) developing in the peripheral zone[4]. In most cases, PCa progress very slowly but there are rare aggressive forms. Most early stage cancers are asymptomatic but some can present with pain, urinary difficulties and sexual dysfunction[5]. The five-year survival for prostate cancer is very high (93.4%) and the majority of men die with the disease rather than from it[3].

According to the European Association of Urology (EAU) digital rectal exams[6], serum concentration of prostate specific antigen (PSA) and transrectal ultrasound (TRUS) are the main diagnostic tools for PCa[7]. Abnormal findings on a DRE or a raised serum PSA concentration may indicate PCa but a firm diagnosis depends on histological examination using TRUS. The exact cutoff for PSA serum concentration has yet to be defined but a threshold of >4ng/ml is commonly used in clinical practice[7]. The most prominent grading system used for prostate cancer is the Gleason grading system[8]. The Gleason grade is based

on the histological patterns of the tumour, using a 5-point scale. A Gleason score of 6 or less indicates a low-grade tumour, a Gleason score of 7 represents an intermediate grade tumour and Gleason scores 8-10 indicate high-grade tumours. The Gleason system is the most dominant prognostic factor in PCa today and is a powerful tool used to determine treatment choice. Prostate cancer treatment is largely dependent on the stage of the disease (see table 1). The EAU recommends active surveillance, radical prostatectomy (RP), radiation therapy (RT) and transperineal brachytherapy for clinically localized disease. Hormonal therapy (Lutenising hormone-releasing hormone (LHRH) agonists, gonadotrophin releasing hormone (GnRH) antagonists and androgen deprivation therapy (ADT)) is recommended for advanced disease. Patients who have a relapse following localized therapies should be treated with salvage RT, ADT, LHRH agonists or salvage RP. In patients with castration-resistant prostate cancer, ADT should be stopped and they may be started on docetaxel, abiraterone acetate or enzalutamide[9].

CLINICAL POINTS

In Ireland today, prostate cancer is the most prevalent non-skin cancer and the second most frequent cause of cancer mortality in men. At present, it has an incidence rate of 156.4/100,000 population

The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) and The European Randomised Study for Prostate Cancer (ERSPC) are two large randomised trials evaluating the efficacy of PSA screening

The PLCO reported that there was no evidence that PSA screening reduced PCa related mortality

The ERSPC showed PSA screening was associated with a 21% decrease in PCa related mortality

Following the publication of these trials, the European Association of Urology and the American Urology Association produced new PCa screening guidelines

PSA is a serine protease released solely by the prostate epithelial cells[10]. PSA serum levels rise in PCa but notably PSA also rises in a number of other pathologies such as benign prostatic hypertrophy, infection or chronic inflammation[11]. PSA is therefore prostate specific but not prostate cancer specific.

Prostate cancer incidence in Ireland

Ireland has the 4th highest rate of prostate cancer in Europe, 50% higher than the EU average[3]. However, Ireland's cancer mortality rates rank 12th in Europe, only 12% higher than the EU average[3]. Mortality: incidence ratios are second lowest in the EU, with only one death for every 10 men diagnosed[3]. The high rate of prostate cancer in Ireland has arisen due to the increased amount of PSA screening in recent years. The use of PSA screening increased by 19-fold between 1994 and 2005 in Ireland[13].

The rates of prostate cancer in Ireland have increased dramatically over the last 15 years. Currently, 3,267 new cases are diagnosed every year in Ireland, making it Ireland's most prevalent cancer[2]. The majority of deaths (70.3%) from prostate cancer occur in those aged above 75 years, at a median age of 80 (figure 1)[2]. According to data from the National Cancer Registry, Irish men have an

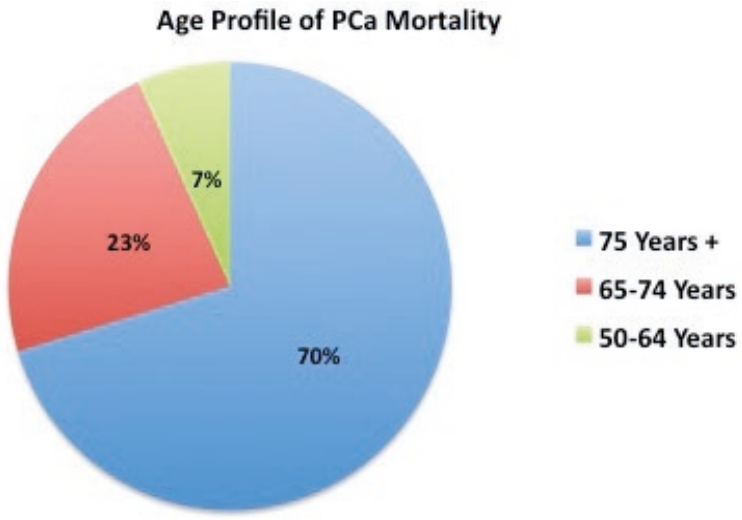


figure 1 The age profile of PCa-related deaths in Ireland[4]

table 1 EAU recommendation for treatment of prostate cancer[11]

Early stage disease	Advanced stage	Relapsed disease following localised treatment	Castration-resistant prostate cancer
Active surveillance	LHRH agonists • Goserelin • Leuprorelin • Buserelin • Triptorelin	Salvage radiation therapy	Stop ADT
Radical prostatectomy	GnRH • Degarelix	ADT • Bicalutamide • Flutamide • Cyproterone acetate	Docetaxel
Radiation therapy	ADT • Bicalutamide • Flutamide • Cyproterone acetate	LHRH agonists • Goserelin • Leuprorelin • Buserelin • Triptorelin	Abiraterone acetate
Transperineal brachytherapy		Salvage radical prostatectomy	Enzalutamide

11.9% cumulative lifetime risk of developing prostate cancer, but only a 1.1% risk of mortality from their disease[14]. The five-year survival rate for prostate cancer has risen from 68.8% in 1997 to 93.4% in 2009 (figure 2)[2].

Risk associated with PSA screening

Potential harms of the detection process

It is thought that approximately 42% of prostate cancers diagnosed by PSA screening would not have caused any clinical problems within the patient's lifetime meaning the patient would have died with the disease rather than from it[13]. This opens up the argument that perhaps widespread PSA screening could pose a significant health risk rather than act as a useful screening tool. Specifically, the PSA test itself causes mild complications (dizziness, fainting, bleeding and hematoma) occurring at a rate of 26.2 per 10,000[15]. Moreover, a significant number of false positives occur in PSA screening. When thresholds between 2.5 and 4.0 µg/L are employed, approximately 80% of positive results are found to be false positives[14]. Most false positives can be attributed to benign prostatic hyperplasia, ejaculation, prostatitis, perineal trauma, cystitis or recent use of instruments in the urinary tract[13]. Men who receive a false positive result may suffer from negative psychological effects such as anxiety about prostate cancer diagnosis. In addition, roughly one third of men who undergo a biopsy following a positive PSA result will suffer from pain, fever, haemorrhage, infection and transient urinary problems[14]. Undergoing a biopsy can be stressful for men and some may experience persistent anxiety even following a negative biopsy result[13].

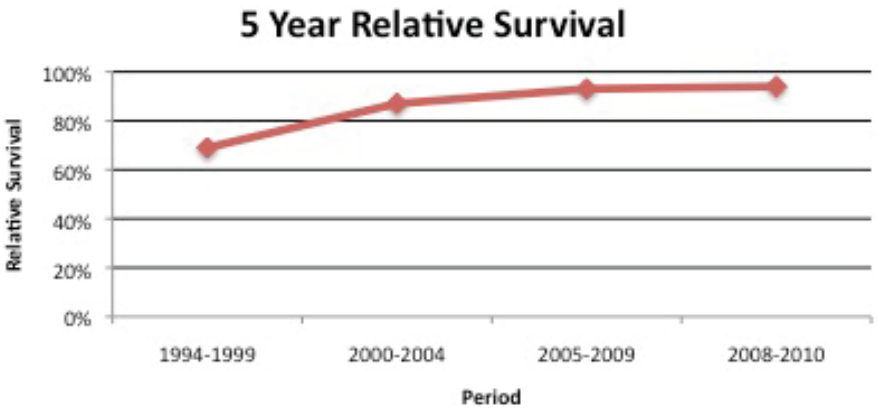


figure 1 the five-year survival rate since 1994[4]

Risks associated with earlier onset treatment

The main treatments for prostate cancer in Ireland are active surveillance, radical prostatectomy, external beam radiation therapy, brachytherapy, hormone therapy and chemotherapy[9]. Radiotherapy and surgery can result in some serious adverse effects; erectile dysfunction and urinary incontinence occurs in at least 20-30% of men treated with these therapies[14]. Androgen deprivation therapy, although not FDA approved, has been used as first-line therapy for early-stage PCa and is associated with hot flushes, erectile dysfunction, gynaecomastia, anaemia, osteoporosis, depression and fatigue[14]. According to the USPSTF, of the men that undergo PSA screening, a higher proportion will experience adverse events from the diagnostic tests or treatment, than will benefit from the screening.

International studies on PSA screening

Two large randomised trials, carried out in America and Europe, examining the efficacy of PSA screening, have been published. These are The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial[15] in the U.S. and The European Randomised Study of Screening for Prostate Cancer[16] in Europe. While both articles, were printed in the same issue of The New England Journal of Medicine in March 2009, both showed conflicting results!

The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

The PLCO was carried out between 1993 and 2001, in 10 different study centres across North America. Men (n = 76,693), aged between 55 and 74 years, were randomly assigned to either the standard care (control) group or the screening group (PSA & DRE annually for 4 years). At 7 years, more cases of prostate cancer were diagnosed in the screened group (2,820) than the control group (2,322), a relative increase of 22%[15]. At 10 years, there were 92 deaths in the screening group compared to 82 in the control group[15]. Therefore in comparison to the control group there was no reduction in PCa-related mortality in the screened group.

However, there are some important factors that may have played a part in lack of reduction in PCa mortality in the screening group. In the ERSPC a threshold of 3ng/ml was used. This increased the sensitivity of this test and allowed the diagnosis of more prostate cancers. By contrast, a PSA threshold of 4ng/ml was used in the PLCO trial. Secondly, 'PSA contamination' (a subject in the control group undergoing PSA screening) was carried out by 56% of control subjects by the end of the trial. This substantial number may have diluted down the results of the screening group. Another factor was that 44% of subjects in both groups had already undergone a PSA test at baseline. Fourthly, PCa therapy improved over the course of the trial, reducing the number of deaths in both groups. Lastly, the effect of PSA screening on PCa-related mortality may take many years to show a benefit so further evaluation on these trial subjects must be done at 15 years[15].

The European Randomised Study of Screening for Prostate Cancer

The ERSPC screening trial, randomly assigned 162,243 men aged between 55 and 69 years to either the control (receiving standard care) or screening group (receiving PSA screening every four years, on average). The trial was carried out in eight European countries and results evaluated at a median follow-up time of 9 years[16].Subsequent analysis of the data was at a median follow-up time of 11 years[17]. Prostate cancer incidence in the screened cohort was much greater than the control cohort (8.2% vs. 4.8%)[16]. The absolute risk difference was 0.71 deaths per 1000[16].Using this data, it was concluded that 1,410 males would require screening and 48 would need to be diagnosed in order to prevent one PCa death[16]. At 11 years, there was a relative reduction in PCa mortality of 21% in the screening group. In

order to prevent one PCa death, 1,055 men would require screening and 37 cancers would need to be diagnosed[17]. These improved figures in the subsequent analysis are in line with the view that the benefit of PSA screening increases with longer follow up time. The ERSPC concluded that PSA-based screening significantly decreases PCa-related mortality but is associated with a large amount of over-diagnosis[17].

International prostate cancer screening guidelines

The U.S. Preventative Services Task Force

Following the results of the PLCO and the ERSPC the U.S. Preventive Services Task Force (USPSTF) published a recommendation opposed to PSA screening, (table 5)[17]. The reason for this recommendation was due to the harms associated with PSA screening (overtreatment, bowel and erectile dysfunction and urinary incontinence) relative to its benefits (PCa- related deaths averted). Although the USPSTF discourages PSA screening it does acknowledge that some patients will request it. The USPSTF therefore recommends that any patient undergoing screening should be fully informed of the risks, as well as benefits, prior to testing.

The American Urological Association guidelines

The American Urological Association recently published new guidelines for the early diagnosis of PCa using PSA testing (table 2)[20]. These new guidelines are as follows:

1. PSA screening is not recommended in men <40 years
2. PSA screening is not recommended in men between 40-54 years with average risk
3. Shared decision making for PSA screening is recommended in men aged 55-69 years
4. The screening interval should be ≥2 years
5. PSA screening is not recommended in men >70 years or in men with a life expectancy of ≤10-15 years.

The European Association of Urology guidelines

The European Association of Urology guidelines differ from those of the AUA (table 5)[7]. The current EAU guidelines state that there is no evidence for the introduction of widespread population-based screening programmes for early diagnosis of PCa[21].

The patient and his physician should make a shared decision whether to undergo PSA testing for the early detection of PCa. A baseline PSA level should be obtained at 40-45 years of age and a subsequent screening interval should be determined from this level. Men aged 45-59 with an initial level of ≥1.0ng/ml should be tested every 2-4 years whereas men with a level of ≤1.0ng/ml need only to be screened every 8 years[7]. Men aged more than 75 years, with a level of ≤3ng/ml, do not require further PSA testing[21]. A significantly increased risk of PCa-related mortality and diagnosis of advanced disease is associated with patients that have a baseline serum PSA level ≥1ng/ml at 45 years or a baseline serum PSA level ≥2.0ng/ml at 60 years[7]. Using these levels, it is possible to target the high-risk group; this will avoid over diagnosis and reduce costs associated with frequent screening in the low risk group.

Guidelines in Ireland

There are currently very few guidelines in place in Ireland in relation to prostate cancer screening. Neither the HSE nor The Irish Cancer Society recommends widespread population based screening in Ireland. At present in Ireland, many men undergo optional PSA testing in a primary care setting. There is much variation in practice, most likely due to the absence of national guidelines. The HSE recommends that no patients undergo PSA screening without being fully informed on the implications of a positive result.

There is a need for more definite national prostate cancer screening guidelines that are in line with

those in other countries. The HSE needs to adopt a set of guidelines based on the EAU or AUA guidelines outlined above. National PSA screening is not recommended in any country but protocols must be put in place for those men who opt for PSA screening.

Drummond and colleagues showed that although PSA testing is used considerably throughout Ireland, it is not being used efficiently[13]. A significant percentage of PSA testing occurs in males aged <50 and >70 years and men with very low baseline levels (<1.0ng/ml), undergoing frequent repeat tests[19]. These findings suggest that many men in Ireland are undergoing nonessential PSA testing that is not cost effective and leads to over-diagnosis. Both the EAU and the AUA advise against testing men <40 years and those with a life expectancy of <10 years. Ireland must follow other European countries and form guidelines for PSA screening in order to prevent the unnecessary testing of men in these low risk groups.

Conclusion

Prostate cancer detection in Ireland has risen considerably in recent years due to increased use of PSA testing. There are still uncertainties as to whether the advantages of PCa screening using PSA outweigh the risks. Although there is no organisation in Ireland that recommends widespread population based screening, many men still undergo PSA testing in a primary care setting. Ireland urgently needs guidelines for PSA screening based on current evidence from the most recent literature. The guidelines should emphasise the importance of shared decision-making between patient and their physician prior to undergoing PSA screening. The guidelines should also outline the optimal PSA threshold for biopsy, the age groups and high-risk groups eligible for screening, the screening interval and the criteria for discontinuing screening. These guidelines are necessary to ensure that clinically significant prostate cancers are caught in an early treatable stage and also to minimise the amount of over-diagnosis and over treatment of asymptomatic tumours.

Acknowledgements

I would like to thank Dr. Antoinette Perry for her help with this literature review.

References

1 GLOBOCAN 2012 (IARC): Estimate on Cancer Incidence, Mortality and Prevalence Worldwide in 2012. Available at http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx [Accessed January 18th 2014].

2 National Cancer Registry in Ireland (NCRI) (2013) Cancer in Ireland 2013: Annual Report of the National Cancer Registry. Available at http://www.ncri.ie/publications/statistical-reports/cancer-ireland-2013-annual-report-national-cancer-registry [Accessed January 7th 2014].

3 National Cancer Registry in Ireland Prostate Cancer Fact Sheet (2013) Available at: http://www.ncri.ie/sites/ncri/files/factsheets/FACTSHEET_prostate.pdf [Accessed January 7th 2014].

4 McNeal, J.E. Normal and pathologic anatomy of prostate. Urology 17, 11-16 (1981).

5 Miller, D.C., Hafez, K.S., Stewart, A., Montie, J.E. & Wei, J.T. Prostate carcinoma presentation, diagnosis, and staging: an update form the National Cancer Data Base. Cancer 98, 1169-1178 (2003).

6 Fondrevelle, M.E., et al. The expression of Twist has an impact on survival in human bladder cancer and is influenced by the smoking status. Urologic oncology 27, 268-276 (2009).

7 Heidenreich, A., et al. Early detection of prostate cancer: European Association of Urology recommendation. European urology 64, 347-354 (2013).

8 Gleason, D.F. Classification of prostatic carcinomas. Cancer chemotherapy reports. Part 1 50, 125-128 (1966).

9 Mottet, N., et al. EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer. European urology 59, 572-583 (2011).

10 Lilja, H. A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. The Journal of clinical investigation 76, 1899-1903 (1985).

11 Pienta, K.J. Critical appraisal of prostate-specific antigen in prostate cancer screening: 20 years later. Urology 73, S11-20 (2009).

12 National Cancer Registry. Cancer Trends. Recent trends in prostate cancer. 2011 [cited; Available from: http://www.ncri.

ie/ pubs/pubfiles/prostate%20trends.pdf [Accessed January 7th 2013].

13 Hoffman, R.M., et al. Prostate cancer screening decisions: results from the National Survey of Medical Decisions (DECISIONS study). Archives of internal medicine 169, 1611-1618 (2009).

14 Moyer, V.A. & Force, U.S.P.S.T. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. Annals of internal medicine 157, 120-134 (2012).

15 Andriole, G.L., et al. Mortality results from a randomized prostate-cancer screening trial. The New England journal of medicine 360, 1310-1319 (2009).

16 Schroder, F.H., et al. Screening and prostate-cancer mortality in a randomized European study. The New England journal of medicine 360, 1320-1328 (2009).

17 Schroder, F.H., et al. Prostate-cancer mortality at 11 years of follow-up. The New England journal of medicine 366, 981-990 (2012).

18 Carter, H.B., et al. Early detection of prostate cancer: AUA Guideline. The Journal of urology 190, 419-426 (2013).

19 Drummond, F.J., Carsin, A.E., Sharp, L. & Comber, H. Trends in prostate specific antigen testing in Ireland: lessons from a country without guidelines. Irish journal of medical science 179, 43-49 (2010).

Eimear Keane

Anaesthetic Awareness: a past, present and future challenge

Daire Shanahan
Fifth year Medicine, Trinity College Dublin

Abstract

Anaesthetic awareness is a rare complication of general anaesthesia. Its occurrence is likely to be multifactorial, with young age, female sex, Chinese ethnicity, previous awareness, gestation, and pulmonary hypertension associated with a higher risk of awareness. Awareness is a distressing complication for patients, with 30% of patients who experience awareness developing psychiatric symptoms ranging from nightmares, flashbacks, and anxiety to post-traumatic stress. Therefore, attempts to monitor awareness are desirable. The aim of this short article is to look at past achievements and future challenges in detecting, monitoring, managing and avoiding awareness.

Introduction

On a cold and frosty Friday morning in 1846, twenty-one-year-old Gilbert Abbot sat nervously in the main operating theatre of the Massachusetts General Hospital. He felt a cold bead of sweat run down his temple. The room felt as if it was getting smaller, hotter- suffocating in tension. The audience sat on edge in the surgical amphitheater, eyes fixed on the clock above the operating table. The dentist, Mr. Morton was late. Time was ticking by; each stroke of the second hand seemed louder than the last.

Morton burst through the theatre doors, a glass dome apparatus in his hand. Inhaling deeply from Morton's ether vaporiser, Abbot 'sank into a state of insensibility'. Mr. John Collins Warren, the attending surgeon then proceeded to dissect a tumor from Abbots jaw and the patient "did not experience any pain at the time, although aware that the operation was proceeding." The first public demonstration of ether anaesthesia - a success![1]

The above account is taken from an article published by Warren in 1846[1]. Not only was it one of the first demonstrations of general anaesthesia, but it was also one of the first reported cases of anaesthetic awareness. The challenge of awareness during anaesthesia was further complicated in 1942 with the introduction of curare to achieve complete paralysis which allowed patients to be fully aware but unable to alert operating staff[2, 3]. One hundred and-sixty-seven years on from Morton's achievement, awareness continues to be an issue which presents many challenges for anaesthetists.

CLINICAL POINTS

Anaesthetic awareness is a rare complication of general anaesthesia occurring in 1-2/1,000 population

It is associated with postoperative psychological sequelae for the patient and medico-legal consequences for the anaesthetist

It has a multifactorial aetiology: patient, operation and anaesthetic factors

Monitoring intra-operative awareness is both challenging and often inaccurate

Intra-operative awareness management protocols are required

Causes of anaesthetic awareness

Awareness is defined as postoperative recollection, with or without prompting, of events following general anaesthesia[4, 5]. It can be either explicit, where the patient can consciously recall events such as hearing conversations and sounds, or implicit, where the patient has unclear memories and is unable to recall exact details[6]. The incidence of awareness is in the range of 1-2 per 1,000 general anaesthetics administered[7-10], while a higher occurrence has been reported in a paediatric population[11, 12, 13]. However, the actual incidence is difficult to measure because

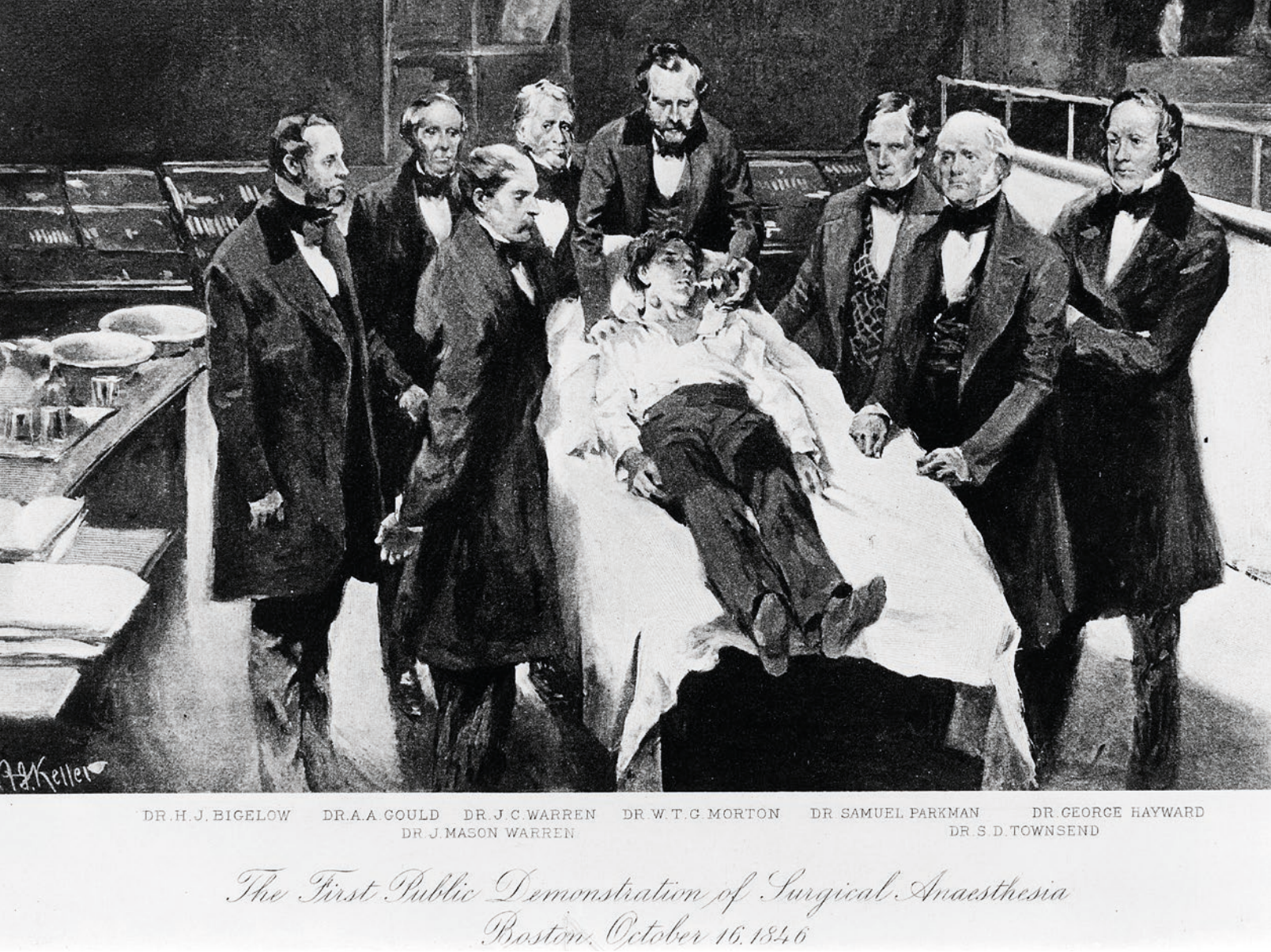


image 1 The first public demonstration of surgical anaesthesia, Boston, 16th October 1846: Dr W. T. G. Morton is administering the anaesthetic, ether. c. 1897
Credit for this image is to the Wellcome Library, London©

there may be under-reporting by medial staff and over-reporting of dreams and false memories by patients which are classified as awareness[14].

It is important to identify which groups of patients are especially at risk of experiencing awareness. Risk groups can be divided into patient, operation and anaesthetic factors[15]. Patient factors include young age[16], female sex[17], Chinese ethnicity[18], ASA III or above (defined as patients with severe systemic disease)[19], previous awareness[20], and pulmonary hypertension[21], are all associated with a higher risk of awareness. Patients undergoing cardiac, obstetric and trauma surgery are at an increased risk, as intentional reduction of anaesthesia is used to maintain a higher blood pressure[22]. Under-dosing and use of muscle relaxants are regarded as the primary cause of awareness during general anaesthesia[23].

Awareness is a distressing complication for patients. One third of patients who report this experience have developed psychiatric symptoms ranging from nightmares, flashbacks, and anxiety to post-traumatic stress disorder[24, 25, 26]. Although awareness is rare, it does have serious consequences for patients. Attempts to monitor awareness are

image 2 This is a copy of the inhaler Morton used in his public demonstration, which took place in Boston, Massachusetts, United States. The air is drawn into the glass jar where ether-soaked sponges emit vapour which is inhaled by the patient through the glass mouthpiece at the top. The patient's expired air is diverted by a valve in the mouthpiece. This type of inhaler was widely used and adapted by a large number of dentists. The original is on show at Massachusetts General Hospital in Boston
Credit for this image is to the Science Museum, London, and Wellcome Images©

therefore highly desirable.

Detecting awareness

Awareness may be detected from clinical signs generated through the sympathetic nervous system. These signs include tachycardia, hypertension, tear formation, sweating, pupil dilatation, pupil reactivity to light, and movement. However; these signs can be masked by some diseases such as autonomic neuropathy (diabetes, renal failure), heart block, and hypothyroidism, while some concurrent medications have a similar effect, such as beta antagonists, anti-muscarinic (atropine, glycopyrrolate) and opioids[27].

Monitoring awareness

One way to prevent awareness is to monitor the depth of anaesthesia. This can be achieved by assessing the end tidal volume of volatile anaesthetic agent concentration, which in turn is measured by the minimum alveolar concentration (MAC). MAC is defined as the concentration of the vapour in the lungs that is needed to prevent a motor response in 50% of subjects in response to surgical stimulus[28]. It is useful because it allows real time monitoring of the depth of anaesthesia. However, MAC does present a number of problems: firstly, it is a median value and so 50% of patients may still demonstrate a

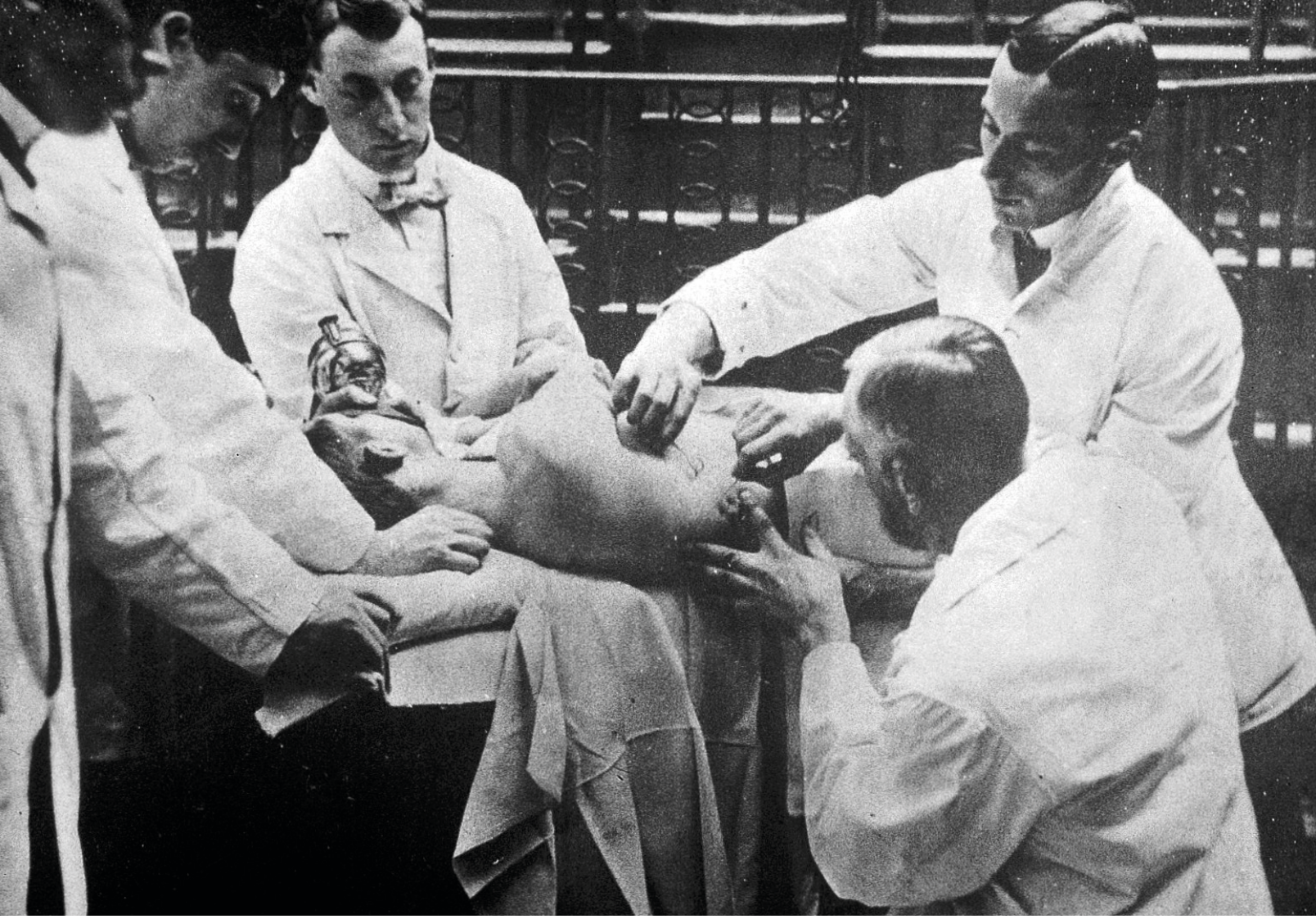


image 3 Surgeon Sir Rickman Godlee operating at University College Hospital. The anaesthetist is using a Clover's portable inhaler or a modification
Credit for this image is to the Wellcome Library, London©

motor response to surgical stimulation. Secondly, MAC is affected by a number of physiological and pathological factors such as age[29], pregnancy, metabolic diseases, and chronic use of drugs such as opioid analgesics[30]. Thirdly, MAC is only applicable to inhaled agents and as of yet there is no similar method to measure intravenous anaesthetic agents.

Specialised monitoring equipment has been developed to alert the clinician to awareness. Electro-encephalography (EEG) allows for detection of brain responsiveness by measuring spontaneous electrical activity. A number of processing devices are available on the market that both analyse EEG activity, and present this data numerically. The most widely used EEG machines is the Bi-spectral (BIS). BIS will display a number on a scale of 0 to 100, where 40-60 indicates an appropriate level for surgical anaesthesia[31, 32].

Many studies have reported a reduction in the incidence of awareness from use of BIS[33, 34], while others have demonstrated no effect[35, 36, 37]. BIS does have limitations- senile dementia, nitrous oxide and ketamine have all been reported to affect the interpretation of BIS[38, 39]. Despite these issues, the National Institute of Health and Clinical Excellence (NICE) recommend the use of BIS (and other EEG machines) for the reduction of the risk of awareness[40].

Other methods include, “evoked potential monitors”, which are similar to EEG except that they

image 4 *Amputation* by Thomas Rowlandson, coloured aquatint, 1793. Five surgeons participating in the amputation of a man's leg while another oversees them
Credit is to the Wellcome Library, London©

detect electrical responses at localised parts of the brain. The use of auditory evoked potential in particular has been reported to detect unintentional awareness[41, 42]. The isolated forearm technique, forehead galvanometry and lower oesophageal motility assessment are of historical interest; but due to their unreliability and they do not have a place in current practice[27].

Managing awareness

Pre-operatively, a detailed history and examination of the patient may identify potential risk factors. Intra-operatively, the use of benziodiapine during induction, appropriate titration of anaesthetic agents and the avoidance of neuromuscular blockade where possible, all reduce the incidence awareness[27].

If awareness is detected intra-operatively, stop any painful stimuli, verbally re-assure, rapidly deepen anaesthesia and consider amnestic drugs such as benzodiazepines. Post operatively, interview the patient, reassure and explain what has happened in a honest, open and sympathetic manner, and arrange appropriate follow up[43].

Future challenges

The ability to detect and monitor awareness during general anaesthesia is essential for its prevention. However, in order to do this, it is necessary to un-

derstand what it means to be "aware". This is not a simple question. Philosophers have long debated the meaning of awareness long before anaesthetists - Descartes famously proposed that "I think therefore I am"[44], while Locke describes awareness as "that conscious thinking thing, (whatever substance, made up of whether spiritual, or material, simple, or compounded, it matters not) which is sensible, or conscious of pleasure and pain, capable of happiness or misery, and so is concerned for itself, as far as that consciousness extends"[45]. In philosophy, awareness could be considered to be the ability to appreciate "self" when experiencing sensations. From the prospective of neuroscience, the theory is not much clearer. Difficult questions raised in this field include: how does neural activity form thoughts and process the concept of awareness? How can one monitor "self" and "self in situation"? What should monitors actually be checking? How can a monitor which is itself "unaware" measure "awareness"?[46] In short, awareness is a difficult concept to understand, on which neither philosophers nor scientists have reached a consensus.

The National Audit Project (NAP5) of the Royal College of Anaesthetists (UK) in collaboration with the Association of Anaesthetists of Great Britain and Ireland, found that only 1.5% of anaesthetists used an EEG machine despite over two-thirds of centres possessing monitoring equipment, and only 4.5% of centres had intra-operative awareness management protocols in place[14]. Such results suggest that the future challenge is to move away from developing monitoring equipment and focus on the development of polices to prevent and manage accidental awareness[14, 47-49].

First encountered in 1846, awareness is a rare occurrence, but its consequences can be devastating. The greatest, most impelling challenge for the future of anaesthetics is to translate the abstract concept of consciousness into a concrete, scientific model. Once this is achieved, it will be desirable to develop effective neuromonitoring techniques, which can be used to detect awareness in real-time, with all current anaesthetic drugs, and in all disease states. It will also be important to implement evidence based management protocols for those suffering from awareness.

References

1 Warren, JC. Inhalation of ethereal vapour for the prevention of pain in surgical operations. Boston Med Surg J. 35, 363-65 (1846).

2 Mainzer, J. Awareness, muscle relaxants and balanced anaesthesia. Can J Anesth.26.5, 386-393 (1979).

3 Lennmarken, C. Sandin, R. Neuromonitoring for awareness during surgery. Lancet. 363, 1747-1748 (2004).

4 Aceto, P. Relationship between awareness and middle latency auditory evoked responses during surgical anaesthesia. Br J Anaesth. 90,630-635 (2003).

5 Myles, P. et. al. Bispectral index monitoring to prevent awareness during anaesthesia: the B-Aware randomised controlled trial. Lancet. 363, 1757-1763 (2004).

6 Ewart, L. Are you aware of awareness? J Periop Pract. 21,93-6 (2001).

7 Sandin, RH. Enlund, G. Samuelsson, P. Lennmarken, C. Awareness during anaesthesia: a prospective case study. Lancet. 355, 707–11 (2000).

8 Wennervirta, J. Ranta, SO. Hynynen, M. Awareness and recall in outpatient anesthesia. Anesth Analg 95, 72-77 (2002).

9 Sebel, P. et al. The incidence of awareness during anesthesia: a multicenter United States study. Anesth Analg. 99, 833-839 (2004).

10 Avidan, M. et al. Anesthesia awareness and the bispectral index. NEJM. 358, 1097–108(208).

11 Davidson, AJ et al. Awareness during anesthesia in children: a prospective cohort study. Anesth Analg.100, 653–661 (2005).

12 Lopez ,H. et al. Intra-operative awareness in children: the value of an interview adapted to their cognitive abilities. Anaesth. 62, 778–89 (2007).

13 Andrade, J. Deeprose, C. Barker, I. Awareness and memory function during paediatric anaesthesia. Br J Anaesth. 100,389-396 (2008).

14 Pandit, J. Cook, T. Jonker, W. O'Sullivan, E. A national survey of anaesthetists (NAP5 Baseline) to estimate an annual incidence of accidental awareness during general anaesthesia in the UK. Anaesth. 68, 343-353 (2013).

15 MacGregor, K. A waking nightmare: How can we avoid accidental awareness during general anaesthesia? J Periop Pract. 23,185-90 (2013).

16 Davidson, AJ. et al. Awareness in children: a secondary analysis of five cohort studies. Anaesth. 66, 446-54 (2011).

17 Errando, C. et al. Awareness with recall during general anaesthesia: a prospective observational evaluation of 4001 patients. Br J Anaesth. 101,178-185 (2008).

18 Xu, L. Wu, A.S. Yue, Y. The incidence of intraoperative awareness during general anesthesia in China: a multi-center observational study. Anaesthesiol Scand. 53, 873-882 (2009).

19 Sebel, P. et al. The incidence of awareness during anesthesia: a multicenter United States study. Anesth Analg. 99, 833-

839 (2004).
20 Osborne, G. Runciman, W. & Helps, S. Crisis management during anaesthesia: awareness and anaesthesia. Qual Saf Health Care .14, e16 (2005).
21 Radovanovic, D. & Radovanovic, Z. Awareness during general anaesthesia - implications of explicit intraoperative recall.Eur Rev Med Pharmacol Sci. 15, 1085-9 (2011).
22 Niranjan, N. Wilson, I.Tutorial of the week: Awareness under anaesthesia 2008. Available from: www.aagbi.org/sites/default/files/108-Awareness-under-anaesthesia.pdf [Accessed Novmeber 2014].
23 Ghoneim, M.M. Incidence of and risk factors for awareness during anaesthesia. Best Pract Res Clin Anaesthesiol. 21, 327-343 (2007).
24 Moerman, N. Bonke, B.Oosting, J. Awareness and recall during general anaesthesia: Facts and feelings. Anesthesiol 79, 454-464 (1993).
25 Osterman, J. et al. Awareness under anesthesia and the development of post-traumatic stress disorder. Gen Hosp Psych 23, 198–204 (2001).
26 Lennmarken, C. & Sydsjo, G. Psychological consequences of awareness and their treatment. Best Pract Res Clin Anaesthesiol. 21, 357-367(2007).
27 Hardman, J. & Aitkenhead, A. Awareness during anaesthesia. Continuing Education in Anaesth, Crit Care Pain. 5,183-186 (2005).
28 Eger, E. Saidman, L. Brandstater, B. Minimum alveolar anesthetic concentration: a standard of anesthetic potency. Anesthesiol. 26.6,756-763 (1995).
29 Stevens, W. et al. Minimum alveolar concentrations (MAC) of isoflurane with and without nitrous oxide in patients of various ages. Anesthesiol. 42,197-200(1975).
30 de Wolff, M. Leather, H. Wouters, P. Effects of tramadol on minimum alveolar concentration (MAC) of isoflurane in rats. Brit J Anaesth. 83, 780-783(1999).
31 Sigl, J.C. & Chamoun, N.G. An introduction to bispectral analysis for the electroencephalogram. J Clin Monit. 10, 392-404 (1994).
32 Song, D. Joshi, GP. White, PF. Titration of volatile anesthetic using bispectral index facilitates recovery after ambulatory anesthesia. Anesthesiol. 87, 842-848 (1997).
33 Dressler, O. Schneider, G. Stockmanns, G. Kochs, E. Awareness and the EEG power spectrum: analysis of frequencies. Brit J Anaesth 93, 806–809 (2004).
34 Chiu, C.L. Ong, G. Majid, A. A. Impact of bispectral index monitoring on propofol administration in patients undergoing cardiopulmonary bypass. Anaestha Intensive Care. 35, 342–347 (2007).
35 Schneider, G. et al. Detection of awareness in surgical patients with EEG-based indices bispectral index and patient state index. Brit J Anaesth. 91: 329-335 (2003).
36 Bruhn, J. et al. Bispectral index and A-line AAI index as guidance for desflurane-remifentanil anaesthesia compared with a standard practice group: a multicentre study. Brit J Anaesth. 94, 63–69 (2005).
37 Zohar, E. et al. Bispectral index monitoring does not improve early recovery of geriatric outpatients undergoing brief surgical procedures. Can J Anesth. 53, 20–25 (2006).
38 Morioka, N. et al. Ketamine causes a paradoxical increase in the bispectral index. Anesthesiol. 87,A502 (1997).
39 Puri, G.D. Paradoxical changes in bispectral index during nitrous oxide administration. British J Anaesth. 86,141-142 (2001).
40 National Institute for Health and Care Excellence. Depth of anaesthesia monitors (E-Entropy, BIS and Narcotrend) 2012; DG6. http://www.nice.org.uk/nicemedia/live/13965/61547/61547.pdf [Accessed November 2014].
41 Plourde, G. Auditory evoked potentials. Best Pract Res Clini Anaesthesiol. 20,129–139 (2006).
42 Aceto, P. et al. Relationship between awareness and middle latency auditory evoked responses during surgical anaesthesia. Brit J Anaesth. 90, 630-635 (2003).
43 Osborne, G. Bacon, A. Runciman, W. Helps, S.Crisis management during anaesthesia: awareness and anaesthesia. Qual Saf Health Care. 14,16-21 (2005).
44 Lafleur, L. Discourse on Method and Meditations. New York :The Liberal Arts Press (1960).
45 Nidditch, P. Locke, John. (1690–4). An Essay Concerning Human Understanding. Oxford: Oxford University Press (1979).
46 Pandit J. National Audit Project 5, Accidental awareness during general anaesthesia partnership of RCOA/AAGBI. Presented at The Royal College of Anaesthetists Annual Congress, London 2012. http://webcast.rcoa.ac.uk/Mediasite/Play/5d3f2ae3788544b292aac63e3d297a761d?cata [Accessed Novemeber 2014].
47 Avidan, M, Mashour, G.II. The incidence of intraoperative awareness in the UK: under the rate or under the radar? Brit J Anaesth. 110, 494-497 (2013).
48 Pryor, K. O., & Root, J. C. III. Intraoperative awareness: a pound of prevention, an ounce of cure? Brit J Anaesth. 111, 529-531 (2013).
49 Panditt, J.J. et al. 5th National Audit Project (NAP5) on accidental awareness during general anaesthesia: summary of main findings and risk factors. Br J Anaesth.113, 549-59 (2014).

Daire Shanahan

Autoantibodies to the NMDA Receptor in Schizophrenia: a literature review

Liam Kennedy

M.Sc. Neuroscience

Abstract

Schizophrenia is a chronic mental illness characterised by positive and negative symptoms, and cognitive dysfunction. The aetiology of the condition is poorly understood; among the various proposed hypotheses are theories involving glutamate transmission and autoimmunity. The N-methyl-D-aspartate receptor (NMDAR) is an ionotropic glutamate receptor expressed widely in the CNS, it is involved in long-term potentiation, memory and learning, and regulation of glutamatergic transmission via GABA-ergic interneurons. Pharmacological blockade of NMDARs induces positive and negative symptoms of schizophrenia in healthy subjects, and exacerbates symptoms in patients. NMDA hypo-function has been demonstrated to cause localised glutamate excito-toxicity in schizophrenia. Anti-NMDAR auto-encephalitis is a recently described neuro-immune condition, which often presents with acute psychosis. Due to the behavioural symptoms caused by the condition, 65% of those who develop anti-NMDAR auto-encephalitis are initially seen by psychiatric services. Studies of anti-NMDAR antibody prevalence in schizophrenic patients and the general population have found conflicting results. Analysis is complicated by variance between studies in terms of antibody specificity and assays used. Further work in this expanding field will help delineate the exact relevance of antibody seropositivity to the development of schizophrenia or psychosis. Anti-NMDAR auto-encephalitis represents an important aetiological factor of psychosis to be considered in the clinical assessment of acute psychosis.

Introduction

Schizophrenia is a severe, chronic, and debilitating mental illness characterised primarily by negative symptoms such as hallucination, delusion, and thought disorder[1]. Despite major research efforts spanning decades, no clear picture of a precise aetiology underpinning the disorder has emerged[1]. However, hypotheses implicating dopamine, glutamate, serotonin, neurodevelopment, and neuro-immune interactions have been proposed[2]. Factors that appear to be associated with the development of schizophrenia include genetic variance, maternal or perinatal infection, urban upbringing, lower socioeconomic status, and regular cannabis use[1]. A diagnosis of schizophrenia carries with it a significantly lowered life expectancy with major causes of death including suicide, cardiovascular disease, respiratory disease, and infection[1].

For decades, the immune system has been investigated as a potential causal factor in the aetiology of schizophrenia[2]. A correlation between immunity and schizophrenia has long been established. Specifically, those with a diagnosis of schizophrenia have a significantly higher rate of autoimmune diseases[3]. The recent descriptions of a spontaneous organic form of psychosis seen in anti-NMDAR auto-encephalitis have sparked fresh interest in the field, birthing the concept of an antibody-mediated treatable form of "schizophrenia"[4-7].

This report aims to review:

- The NMDA hypofunction hypothesis of schizophrenia
- Recent descriptions of the psychiatric and behavioural symptoms commonly seen in anti-NMDAR auto-encephalitis

- The prevalence of IgG anti-NR1 NMDAR autoantibodies in the general population, and those diagnosed with schizophrenia and first episode psychosis (FEP)

NMDA hypofunction in schizophrenia

For decades, altered dopamine transmission has been considered the key neurochemical driving force in schizophrenia. Although dopaminergic blockade has been the primary mode of pharmacological treatment, antipsychotic therapies remain limited and imperfect. More recently, interest has grown in the role of glutamate transmission as an aetiological defect and therapeutic target in schizophrenia with some suggesting it may even be primary to dopaminergic dysfunction[8].In contrast to the dopamine hypothesis, the positive and negative symptoms of schizophrenia, as well as cognitive deficits, are plausible for a glutamatergic theory[9].

NMDARs

The NMDAR is an ionotropic glutamate receptor, widely expressed both pre- and post-synaptically on the cell surface of brain neurons. It has a role in regulating glutamatergic transmission and is involved in synaptic plasticity, memory, and learning[10]. The NMDAR is composed of one or more NR1 subunits in addition to a combination of NR2 and/or NR3 subunits. Different formations of these subunits give rise to several structurally and functionally distinct NMDARs[11].The natural ligand for the receptor is glutamate, but it also requires co-stimulation from glycine. At resting membrane potential, the ion channel is blocked by a magnesium ion. Depolarisation unblocks the channel and allows influx of calcium and sodium ions, and efflux of potassium (figure 1). Calcium ion influx plays an important role in long-term potentiation and, thus, in learning and memory[10]

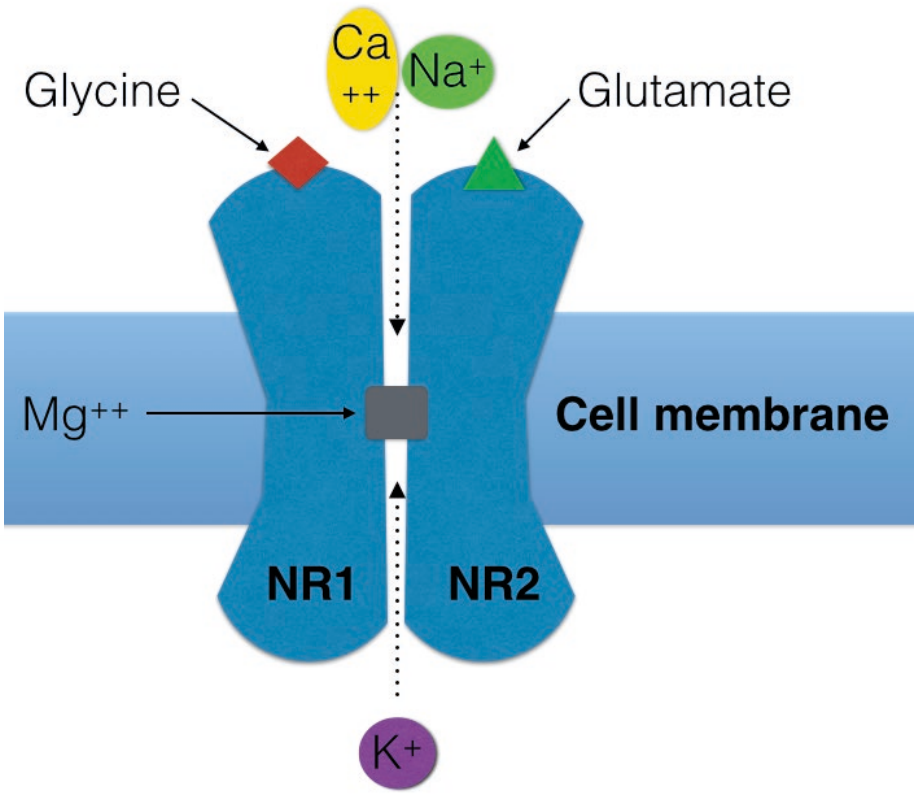


figure 1 Structure of NMDAR

Glutamate in schizophrenia

The core observations underlying the glutamatergic model of schizophrenia have resulted from pharmacological studies. Blockade of NMDARs using dissociative anaesthetics (e.g. phencyclidine or ketamine) cause schizophrenia-like behavioural and cognitive symptoms in healthy subjects, and the exacerbation of psychotic symptoms in subjects with schizophrenia[12, 13]. Rodent models demonstrate schizophrenia-like behaviours, such as catatonia and stereotyped movements in response to administration of NMDA antagonists[14].

Initial hypotheses involving glutamate transmission speculated that overall glutamate deficiency might play a role in the development of schizophrenia. It is now thought that hypofunction of

the NMDAR on GABAergic inhibitory interneurons causes an overall excitotoxic effect with glutamate levels increased rather than deficient[15]. Administration of NMDA antagonists have been shown to cause extracellular glutamate levels to be increased specifically in the anterior cingulate cortex, part of the limbic system[16].

Post-mortem studies examining the levels of expression of the NMDAR have found conflicting results with various studies showing increased, normal, or decreased levels of NMDAR mRNA or protein expression[17, 18, 19]. Without definitive evidence that receptor expression is significantly altered in schizophrenia, researchers have looked to modulatory and downstream factors that may affect receptor function. A number of genes which have been linked to schizophrenia are important in glutamatergic signalling via the NMDAR[20].

CLINICAL POINTS

Anti-NMDA autoencephalitis is a recently described neuro-immune condition which often presents with acute psychosis

While first described exclusively in those with ovarian teratoma, the condition has since been seen in males, and in females without malignancy

65% of those who develop anti-NMDA autoencephalitis are initially seen by psychiatry services

NMDAR autoencephalitis therefore represents an important organic aetiology to be considered in first episode psychosis

Interest lies in the prevalence of anti-NMDAR antibodies in those already diagnosed with schizophrenia, as seropositivity may represent an organic cause for previously diagnosed psychiatric disease

NMDAR autoencephalitis and psychosis

Anti-NMDA receptor autoencephalitis is a recently described autoimmune condition. Initially described in 2005 as a paraneoplastic phenomenon in young women with ovarian teratomas[4], anti-NMDAR autoencephalitis has more recently been shown to occur in males and females with no underlying malignancy[21]. Clinical expression consists of a prodromal phase of nausea, headache, fever, vomiting, and diarrhoea which develop within days into a variable presentation of psychiatric symptoms such as delusions, mania, anxiety, catatonia and insomnia[6]. Motor dysfunction such as choreiform movements and ataxia may also manifest. Approximately 65% of adults first present with psychiatric symptoms and the majority are initially assessed by psychiatric services making anti-NMDA autoencephalitis a valid differential diagnosis to be considered in presentations of acute psychosis[20].

According to Dalmau and associates, IgG antibodies targeting the extracellular domain of the NR1 subunit of the NMDAR are pathognomic of the condition[5]. This pathology is consistent with the clinical course of the autoencephalitis, with many symptoms similar to those observed in pharmacological NMDA blockade and NMDAR knockout in animal studies[22, 23]. In vitro and in vivo studies have demonstrated that anti-NMDAR autoantibodies act pathogenically by causing a reduction in the number of expressed neuronal surface NMDARs leading to a selective functional attenuation of the excitatory post-synaptic potential (EPSP)[24]. The synaptic and clinical effects of this process have been demonstrated to be titre-dependent: cerebral spinal fluid (CSF) and serum samples taken at symptom presentation or symptom worsening displayed higher anti-NR1 antibody titres than samples taken during symptomatic improvement. High-titre samples had a greater observed effect on reducing in vitro post-synaptic receptor cluster concentrations than relatively low-titre samples[24]. The pathological reduction in post-synaptic receptor density has been shown to reverse with removal of antibody, providing a basic mechanistic explanation for the symptom relief observed once patients receive therapeutic intervention[5].

Prevalence of anti-NMDAR autoantibodies

Several studies have investigated the prevalence of anti-NMDA autoantibodies in populations with schizophrenia, but found conflicting results. Initial studies discovered minor or zero prevalence of antibody in case populations, with many of these studies having small sample size and failed to compare with control populations (figure 2)[25, 26, 27].

The first substantial comparison of seroprevalence rates between cases and matched controls came in the form of a prospective study by Steiner and colleagues in 2013 (n=459). The authors found non-specific antibodies against NMDA in 9.9% of acutely ill patients with an initial diagnosis of schizophrenia, contrasting strongly with a seropositivity rate of only 0.4% (1/230) of controls[28]. Studying a significantly larger cohort population (n=2817) and controls, Hammer and co-workers found anti-NMDAR autoantibodies in 10.5% of all subjects, with no significant difference in seropositivity between cases and controls[29].

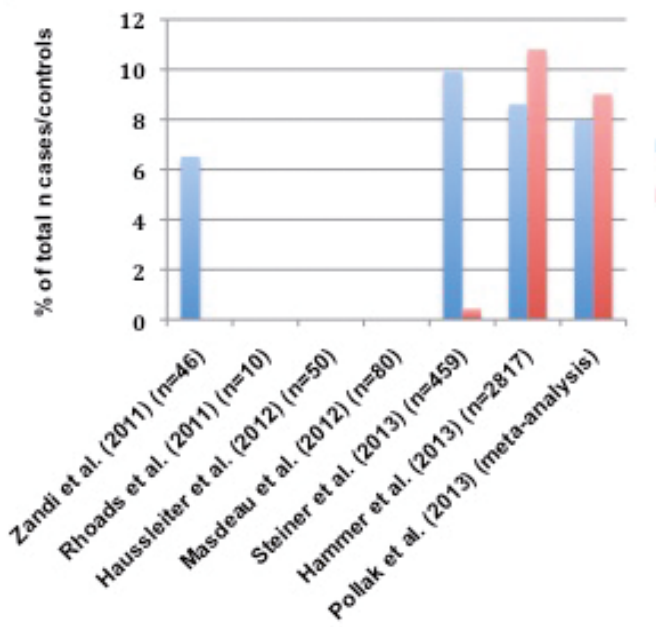


figure 2 Prevalence rates of anti-NMDA autoantibodies as repored in recent literature

Pollak and colleagues carried out a systematic review and meta-analysis, including data from seven studies comprising 1,441 patients and 1,598 healthy controls[30]. Meta-analysis gave figures of 7.98% prevalence of anti-NMDAR autoantibodies in patients and 9.01% prevalence in controls. Studies were noted to be inconsistent in assaying for specific classes of immunoglobulin and receptor subunit epitope. Notably, while 7.98% of patients tested positive for anti-NMDAR antibody, only 1.46% of patients were found to carry IgG antibodies specifically, with the remainder testing positive for IgA and IgM anti-NMDAR antibodies. If IgA and IgM antibodies were excluded from analysis, antibody prevalence was significantly greater in cases than in controls.

Blood-brain barrier integrity

The cited study by Hammer and colleagues stands in stark contrast to others included in the systematic review of Pollak and co-authors in that it found anti-NMDAR autoantibodies to be prevalent in a significant proportion of the general population. One possible reason for this discrepancy is the slightly higher average age of the control population studied as autoantibody prevalence is correlated with age. Interpreting this finding, the authors propose that in light of equal seroprevalence rates across cases and controls, impaired blood-barrier function may confer susceptibility to autoantibody-mediated impairment of function in seropositive populations. Supporting this hypothesis is their finding of a significant correlation, within seropositive individuals between proxy indicators of temporary blood-brain barrier damage (past neurological trauma/birth complication) and schizophrenia. Future approaches to this hypothesis might investigate a correlation between anti-NMDA autoantibody seropositivity, psychosis and blood-brain barrier damage in relation to more sensitive indicators of blood-brain barrier function, e.g. serum/CSF markers or imaging studies.

Implications for practice

The relatively recent discovery of anti-NMDA autoencephalitis and subsequent research on its underlying pathology, course, and treatment raise important considerations among physicians and psychiatrists. Given the large proportion of those eventually diagnosed with anti-NMDA autoencephalitis who initially present to psychiatric services, psychiatrists must be fully aware of the presentation and clinical course of the condition and keep it in mind as an important organic cause of psychosis. Maneta and associates propose a list of features which should raise the index of suspicion of anti-NMDA autoencephalitis in anyone presenting with first episode psychosis: these include a flu-like prodrome, rapid onset of symptoms, seizures, and catatonia, among other features[31].

Another important consideration is screening in the existing population of diagnosed schizophrenics. A finding of seropositivity for anti-NMDAR autoantibodies in a treatment-resistant schizophrenic may provide a novel approach for therapeutic intervention in the form of immune therapies.

Conclusion

The discovery of antibodies targeting the NMDAR as a cause of organic psychosis has interesting ramifications for future management and treatment in mental healthcare. It underlines the strong link between the medical and psychiatric fields and strongly reinforces the NMDA hypofunction model of schizophrenia as a biological basis for mental illness. The clinical course of anti-NMDA autoencephalitis is in agreement with pharmacological and genetic knockout studies of NMDA hypofunction and serves as another direct link between NMDA hypofunction and psychotic symptoms. Future clarification of the currently inconclusive evidence regarding the prevalence of anti-NMDA autoantibodies in schizophrenic and general populations will help further delineate the relevance of antibody seropositivity to the development of psychotic disease.

References

1 Wright, P., Stern, J. & Phelan, M. Core Psychiatry. (Elsevier Health Sciences UK, 2012).

2 Davison, K. Autoimmunity in psychiatry. The British journal of psychiatry : the journal of mental science 200, 353-355, doi:10.1192/bjp.bp.111.104471 (2012).

3 Chen, S.-J. et al. Prevalence of autoimmune diseases in in-patients with schizophrenia: nationwide population-based study. The British journal of psychiatry : the journal of mental science 200, 374-380, doi:10.1192/bjp.bp.111.092098 (2012).

4 Vitaliani, R. et al. Paraneoplastic encephalitis, psychiatric symptoms, and hypoventilation in ovarian teratoma. Annals of neurology 58, 594-604, doi:10.1002/ana.20614 (2005).

5 Dalmau, J. et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet neurology 7, 1091-1098, doi:10.1016/S1474-4422(08)70224-2 (2008).

6 Dalmau, J., Lancaster, E., Martinez-Hernandez, E., Rosenfeld, M. R. & Balice-Gordon, R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. The Lancet Neurology 10, 63-74, doi:10.1016/s1474-4422(10)70253-2 (2011).

7 Lennox, B., Coles, A. & Vincent, A. Antibody-mediated encephalitis: a treatable cause of schizophrenia. The British journal of psychiatry : the journal of mental science 200, 92-94, doi:10.1192/bjp.bp.111.095042 (2012).

8 Moghaddam, B. & Javitt, D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 37, 4-15, doi:10.1038/npp.2011.181 (2012).

9 Lin, C. H., Lane, H. Y. & Tsai, G. E. Glutamate signaling in the pathophysiology and therapy of schizophrenia. Pharmacology, biochemistry, and behavior 100, 665-677, doi:10.1016/j.pbb.2011.03.023 (2012).

10 Gilmour, G. et al. NMDA receptors, cognition and schizophrenia--testing the validity of the NMDA receptor hypofunction hypothesis. Neuropharmacology 62, 1401-1412, doi:10.1016/j.neuropharm.2011.03.015 (2012).

11 Kristiansen, L. V., Huerta, I., Beneyto, M. & Meador-Woodruff, J. H. NMDA receptors and schizophrenia. Current opinion in pharmacology 7, 48-55, doi:10.1016/j.coph.2006.08.013 (2007).

12 Lahti, A., Koffel, B., LaPorte, D. & Tamminga, C. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 13, 9-19, doi:10.1016/0893-133X(94)00131-I (1995).

13 Malhotra, A. et al. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsychopharmacology : official

publication of the American College of Neuropsychopharmacology 17, 141-150, doi:10.1016/S0893-133X(97)00036-5 (1997).

14 Chartoff, E., Heusner, C. & Palmiter, R. Dopamine is not required for the hyperlocomotor response to NMDA receptor antagonists. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 30, 1324-1333, doi:10.1038/sj.npp.1300678 (2005).

15 Marek, G. et al. Glutamatergic (N-methyl-D-aspartate receptor) hypofrontality in schizophrenia: too little juice or a miswired brain? Molecular pharmacology 77, 317-326, doi:10.1124/mol.109.059865 (2010).

16 Rowland, L. et al. Effects of ketamine on anterior cingulate glutamate metabolism in healthy humans: a 4-T proton MRS study. The American journal of psychiatry 162, 394-396, doi:10.1176/appi.ajp.162.2.394 (2005).

17 Beneyto, M. & Meador-Woodruff, J. Lamina-specific abnormalities of NMDA receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33, 2175-2186, doi:10.1038/sj.npp.1301604 (2008).

18 Schmitt, A. et al. Gene expression of NMDA receptor subunits in the cerebellum of elderly patients with schizophrenia. European archives of psychiatry and clinical neuroscience 260, 101-111, doi:10.1007/s00406-009-0017-1 (2010).

19 Akbarian, S. et al. Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. The Journal of neuroscience : the official journal of the Society for Neuroscience 16, 19-30 (1996).

20 Timms, A. E. et al. Support for the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia from exome sequencing in multiplex families. JAMA psychiatry 70, 582-590, doi:10.1001/jamapsychiatry.2013.1195 (2013).

21 Titulaer, M. et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. Lancet neurology 12, 157-165, doi:10.1016/S1474-4422(12)70310-1 (2013).

22 Gunduz-Bruce, H. The acute effects of NMDA antagonism: from the rodent to the human brain. Brain research reviews 60, 279-286, doi:10.1016/j.brainresrev.2008.07.006 (2009).

23 Forrest, D. et al. Targeted disruption of NMDA receptor 1 gene abolishes NMDA response and results in neonatal death. Neuron 13, 325-338, doi:10.1016/0896-6273(94)90350-6 (1994).

24 Hughes, E. G. et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. The Journal of neuroscience : the official journal of the Society for Neuroscience 30, 5866-5875, doi:10.1523/JNEUROSCI.0167-10.2010 (2010).

25 Rhoads, J., Guirgis, H., McKnight, C. & Duchemin, A.-M. Lack of anti-NMDA receptor autoantibodies in the serum of subjects with schizophrenia. Schizophrenia research 129, 213-214, doi:10.1016/j.schres.2010.12.018 (2011).

26 Haussleiter, I. et al. Investigation of antibodies against synaptic proteins in a cross-sectional cohort of psychotic patients. Schizophrenia research 140, 258-259, doi:10.1016/j.schres.2012.05.006 (2012).

27 Masdeu, J. et al. Serum IgG antibodies against the NR1 subunit of the NMDA receptor not detected in schizophrenia. The American journal of psychiatry 169, 1120-1121, doi:10.1176/appi.ajp.2012.12050646 (2012).

28 Steiner, J. et al. Increased prevalence of diverse N-methyl-D-aspartate glutamate receptor antibodies in patients with an initial diagnosis of schizophrenia: specific relevance of IgG NR1a antibodies for distinction from N-methyl-D-aspartate glutamate receptor encephalitis. JAMA psychiatry 70, 271-278, doi:10.1001/2013.jamapsychiatry.86 (2013).

29 Hammer, C. et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. Molecular psychiatry, doi:10.1038/mp.2013.110 (2013).

30 Pollak, T. A., McCormack, R., Peakman, M., Nicholson, T. R. & David, A. S. Prevalence of anti-N-methyl-d-aspartate (NMDA) antibodies in patients with schizophrenia and related psychoses: a systematic review and meta-analysis. Psychological medicine, 1-13, doi:10.1017/S003329171300295X (2013).

31 Maneta, E. & Garcia, G. Psychiatric Manifestations of Anti-NMDA Receptor Encephalitis: Neurobiological Underpinnings and Differential Diagnostic Implications. Psychosomatics 55, 37-44, doi:10.1016/j.psym.2013.06.002 (2014).

Liam Kennedy

Targeted Drug Therapies for BRAF Mutated Tumours

Michael McCrohan
Fourth year Medicine, Trinity College Dublin

Abstract

Mutations in the Ras/Raf/MEK/ERK pathway are frequently present in human cancer. Following extracellular signaling, the G protein Ras becomes activated which further leads to activation of a member of the Raf kinase family. Subsequent activation of other cascade members, such as MEK (MAP/ERK kinase) and ERK (extracellular signal-regulated kinase) eventually results in the activation of transcription factors, which regulate key cellular processes such as growth, differentiation, and apoptosis. The v-raf murine sarcoma viral oncogenes homolog B1 (BRAF) is frequently mutated in a range of human cancers including melanoma, papillary thyroid carcinoma, and colorectal carcinoma. Consequently, BRAF has been identified as a therapeutic drug target with the development of BRAF inhibitors in recent years. Researchers are focusing on understanding the resistance mechanisms present in BRAF-mutated cancers and have identified combination therapy as a potential treatment option that may overcome resistance to BRAF inhibitors. In this review of the literature, the development of BRAF inhibitors and their clinical use is investigated. In addition, resistance to BRAF inhibitors and combination therapy are discussed.

Introduction

The Ras/Raf/MEK/ERK pathway (figure 1) is involved in multiple cellular processes including cell differentiation, proliferation, cell cycle-arrest, and apoptosis, which are initiated following extracellular signaling[1]. The v-raf murine sarcoma viral homolog B1 (BRAF) belongs to the Raf (Rapidly accelerated fibrosarcoma) family of serine/threonine kinases and is the most dominant activator of the MAPK/ERK kinase (MEK) pathway[2, 3]. BRAF mutations are found in approximately 8% of human cancer cases with a significant number harboring the BRAF-V600E mutation. Thus, BRAF mutations and, in particular, the BRAF-V600E mutation have become a focal point of research into targeted therapies to treat specific cancer types. In this article, BRAF inhibitors will be reviewed, focusing mainly on the findings of preclinical and clinical trials. Resistance to BRAF inhibitors and the potential and efficacy of combination therapy in combating this resistance will also be discussed.

CLINICAL POINTS

BRAF mutations are found in approximately 8% of human cancer cases

Vemurafenib and dabrafenib are BRAF inhibitors used for the treatment of BRAF-V600E mutated unresectable or metastatic melanoma

BRAF inhibitor resistance can be divided into multiple subtypes based on the mechanism of the resistance

MAP/ERK kinase (MEK) is one of the most successful drug targets utilised in combination therapy targeting BRAF mutations to date

Trametinib/dabrafenib combination therapy is used for the treatment of unresectable or metastatic melanoma harbouring a BRAF V600E or V600K mutation

Future research should concentrate on innovative combination therapies to target resistance mechanisms more aggressively

BRAF inhibitors

Vemurafenib (Zelboraf) was developed to treat mutant-BRAF melanomas as well as other solid tumours with BRAF mutations5. Trunzer and colleagues demonstrated that vemurafenib initially works by inhibiting the MAPK signalling pathway, resulting in a substantial decrease in ERK phosphorylation after 15 days of therapy being given to the patient. This leads to downstream suppression of cyclin D1, expression of the cell-cycle inhibitor p27, and eventually cell-cycle arrest[6]. Vemurafenib is effective in xenograft models of melanoma harbouring a

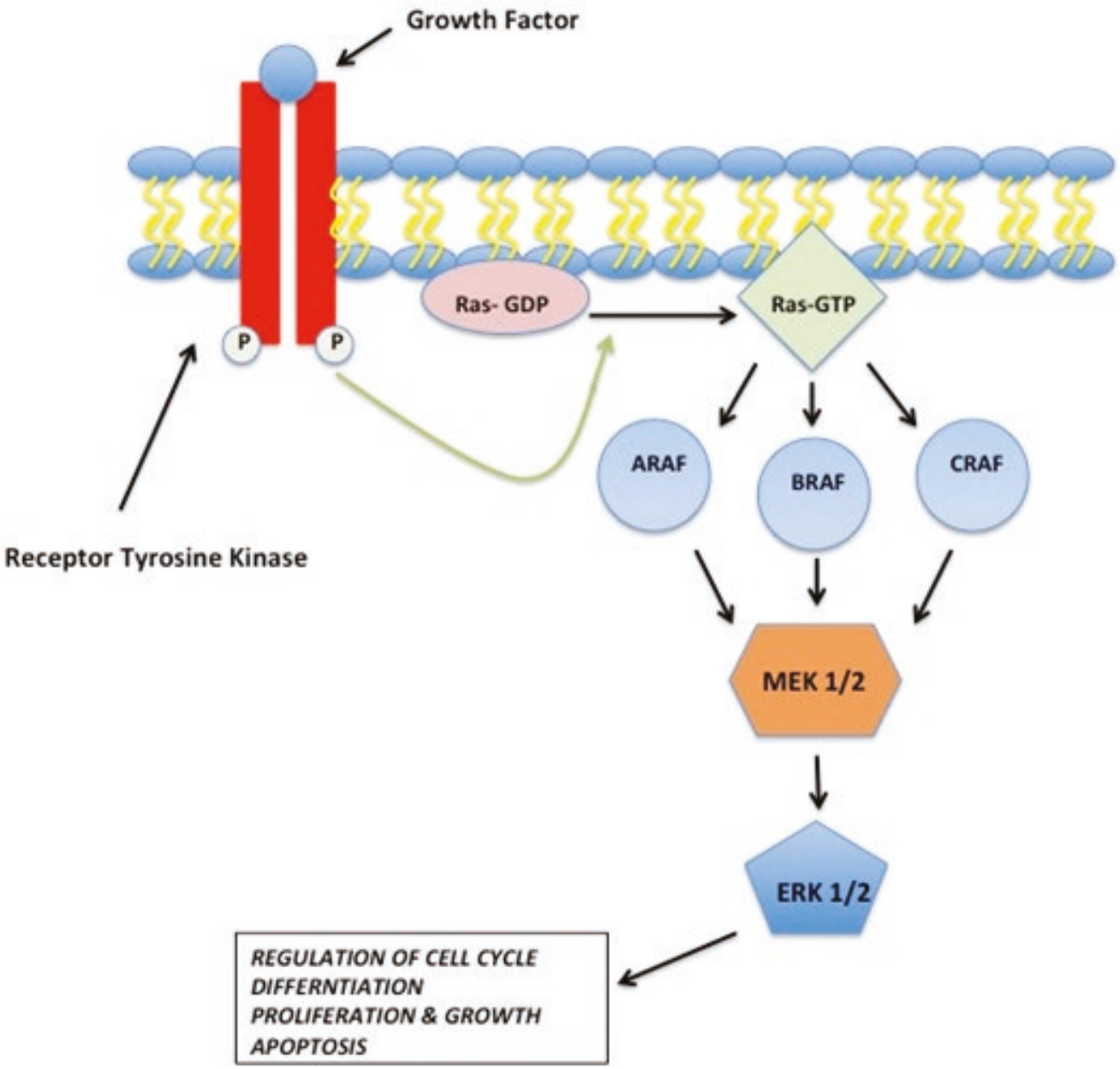


figure 1 The Ras/Raf/MEK/ERK pathway: Tyrosine kinase receptors are stimulated by growth factors resulting in the activation of the G protein Ras. Ras activates members of the RAF family (ARAF, BRAF, CRAF), which subsequently activate MEK 1/2. Following the activation of ERK 1/2 by MEK 1/2, transcription factors are activated leading to an array of key cellular processes[4]

BRAF-V600E mutation. It inhibits proliferation in BRAF-V600E melanoma cells and other melanoma cells with BRAF mutations at residue 600 such as V600R, V600D and V600K[7]. A randomised phase 3 clinical trial compared vemurafenib to dacarbazine: the only current chemotherapeutic drug with U.S Food and Drug Administration (FDA) approval for treating metastatic melanoma. This trial demonstrated that single-agent vemurafenib resulted in an improvement in response rate, progression-free survival (PFS), and overall survival in comparison to dacarbazine in patients with metastatic melanoma harbouring a BRAF-V600E mutation[8]. Subsequently in 2011, the FDA approved the oral BRAF inhibitor, vemurafenib, for the treatment of BRAF-V600E mutated unresectable or metastatic melanoma.

Dabrafenib is an ATP-competitive RAF kinase inhib-

itor which inhibits the BRAF-V600E mutant kinase. A phase 3 trial showed that patients responded positively to dabrafenib with better PFS in comparison to dacarbazine. The trial reported a median PFS for patients treated with dabrafenib for 5.1 months, which was higher than the 2.7 months observed for the dacarbazine patient group[9]. Dabrafenib has also shown efficacy in inhibiting the MAPK pathway in melanoma cell lines with BRAF-V600D/R mutations. Significant inhibition of cell proliferation was observed in melanoma cell lines harbouring a BRAF-V600D or BRAF-V600R mutation. In addition, melanoma cells with BRAF-V600D/R mutations exhibited a stronger and more rapid inhibition of phosphorylated ERK when compared to wildtype BRAF control cells[10]. In May 2013, dabrafenib was approved by the FDA for the treatment of patients with unresectable or metastatic melanoma encompassing a BRAF-V600E mutation[11].

LGX818 is a potent RAF inhibitor with apoptotic and anti-proliferative properties in cells expressing a BRAF-V600E mutation. In the BRAF-V600E human melanoma cell line, LGX818 causes a decrease in the phosphorylation of ERK ultimately resulting in downstream inhibition of proliferation. Studies in human melanoma xenograft models harbouring a BRAF-V600E mutation reported that low oral doses of LGX818 led to a substantial and sustained decrease in the phosphorylation of MEK. In addition, several mutant-BRAF human tumour xenograft models grown in rats and mice showed that low doses of LGX818 can cause tumour regression[12]. At present, LGX818 is undergoing multiple clinical trials.

Sorafenib is a multi-kinase inhibitor with FDA approval for the treatment of hepatocellular carcinoma and renal cell carcinoma. As of November 2013, sorafenib also gained approval for the treatment of "locally recurrent or metastatic, progressive, differentiated thyroid carcinoma (DTC) refractory to radioactive iodine treatment"[13]. In preclinical studies, Sorafenib was active against a number of kinases including BRAF, CRAF, VEGFR-2, PDGFR-h, CKIT, and FLT314. Therefore, it inhibits cancer growth via two mechanisms: inhibition of tumour angiogenesis and, secondly, inhibition of cancer cell proliferation. It has exhibited anti-tumour effects, accounted for by its inhibition of the Ras/Raf/MEK/ERK pathway, in preclinical models of pancreatic, breast, colon, ovarian and lung carcinoma[15].

Regorafenib is a type-2 kinase inhibitor with FDA approval for the treatment of advanced gastrointestinal stromal tumours (GIST) that cannot be treated by means of surgical removal and that are unresponsive to other FDA-approved drugs. It is also an approved treatment for patients with metastatic colorectal cancer (mCRC) who have undergone specific therapies such as anti-VEGF therapy. Regorafenib is an oral multi-kinase inhibitor with potent anti-tumour activity. It has been shown to inhibit both wildtype and BRAF-V600E; However, other studies suggest no correlation between the mutation status of BRAF and regorafenib's effectiveness in inhibiting in vitro tumour cell proliferation or in vivo tumour growth[16]. These findings suggest that BRAF is not the primary target kinase of importance in terms of regorafenib efficacy.

RAF265 and XL281 are BRAF inhibitors currently under evaluation in clinical trials. RAF265 is an oral inhibitor of wildtype BRAF, BRAF-V600E, and CRAF. In addition, it also has inhibiting effects on c-Kit, VEGFR2 and PDGFRβ[5]. RAF265 is currently being evaluated in a phase 2 trial (NCT00304525) on patients with locally advanced or metastatic melanoma. XL281 is an orally bioavailable RAF inhibitor that has displayed a greater than 250-fold selectivity for RAF compared to other kinases[5]. A phase 1/2 trial (NCT01086267) involving XL281 treatment has recently been completed, with results pending. This study investigated XL281 alone or combined with the EGFR inhibitor, cetuximab, in patients with ad-

vanced or metastatic colorectal cancer harbouring mutations in KRAS or BRAF[17].

Mechanisms of resistance to BRAF inhibitors

There are multiple mechanisms in which resistance can be developed against BRAF inhibitors. In particular, evidence of resistance is seen in melanoma and the drugs used to treat it: vemurafenib and dabrafenib. For example, a substantial amount of variation exists in the amount of tumour reduction observed in patients responding to these treatments. In clinical trials with dabrafenib and vemurafenib, approximately 80-90% of patients who respond to treatment initially exhibit progression of their cancer within a year, demonstrating that BRAF inhibitor resistance is established quite rapidly[8, 9].

Typically, these mechanisms of resistance to BRAF inhibitors can lead to the MAPK pathway becoming reactivated or alternatively result in activation of other pathways supporting proliferation and survival. Thus, BRAF inhibitor resistance is probably a multifactorial process[18].

BRAF inhibitor resistance can be divided into multiple subtypes based on the mechanism of the resistance. These subtypes include changes in BRAF itself, resulting in reactivation of the MAPK pathway, as well as alterations occurring in constituents other than BRAF in this pathway, such as MEK1 or NRAS. The PI3K-Akt pathway has also been implicated in BRAF resistance with particular emphasis on loss of PTEN in this process. In addition, alterations in the components regulating the cell cycle have been linked to BRAF resistance[18].

A RAF kinase switch has been implicated in BRAF resistance in melanoma patients. This involves cancer cells relying less on BRAF for signalling and instead utilising the other RAF isoforms, ARAF and CRAF. Inhibition of BRAF forces melanoma cells to rely on a different signalling pathway. This kinase switch enables the tumour to continue utilising the MAPK pathway to support its growth and proliferation[19]. Montagut and associates also observed this switch from BRAF to CRAF dependency in cancer cells and proposed that it accounted for resistance to BRAF inhibitors. The potential of drugs combating CRAF was also highlighted in this study. Geldanamycin, a Heat Shock Protein 90 (HSP90) inhibitor, was observed to promote the degradation of CRAF and revealed the potential of targeting CRAF to reduce resistance to BRAF inhibition[20]. Ganetespib is also a HSP90 inhibitor and its activity has been studied in BRAF-V600E mutated melanoma lines. It has shown promising results in tackling intrinsic and acquired resistance to the BRAF inhibitor vemurafenib[21].

Activation of the PI3K/AKT/mTOR pathway has been linked to malignancy and there is evidence for its role in melanoma, which typically occurs alongside activation of MAPK signalling[22]. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a tumour suppressor gene that decreases Akt activity and its mutant form is associated with BRAF mutated melanoma[23]. The PI3K-AKT pathway and PTEN may be implicated in the mechanisms of BRAF resistance. In particular, crosstalk between the PI3K/AKT/mTOR and Ras/Raf/MEK/ERK pathway following treatment with a BRAF inhibitor is important in cell survival and resistance. There is mounting evidence to suggest an important role for PTEN in this mechanism of resistance with studies showing that PTEN-null melanoma lines display increased resistance to the inhibitor PLX4720[24, 25].

Combination therapy and its potential to overcome resistance

BRAF resistance can occur through multiple mechanisms. Therefore, molecular inhibitors within various pathways are being studied to decipher the best combination of drugs to counteract resistance. The PI3K/AKT/mTOR pathway is being evaluated as a

possible pathway to co-target with BRAF inhibitors. A study conducted on BRAF-V600E mutated melanoma cells demonstrated that combining inhibitors of both the MAPK and AKT pathways decreases the growth rate of a number of cell lines resistant to vemurafenib. The study suggested that adding an AKT or mTOR inhibitor to vemurafenib therapy might enhance overall inhibition[26].

Other combination strategies being investigated include the use of HSP90 inhibitors and selective inhibitors of ERK 1/2, located downstream of BRAF. HSP90 is a molecular chaperone that is important for the stability and function of multiple signaling proteins. Moreover, it stabilizes many proteins, for example BRAF, that are involved in tumour growth[27]. The importance of the HSP90 inhibitor Ganetespib in combating BRAF resistance was discussed above. XL888 is an additional HSP90 inhibitor that has demonstrated positive results in overcoming BRAF resistance. A recent study reported that in vitro and in vivo, XL888 was successful in inhibiting and inducing tumour regression and apoptosis in melanoma cell lines resistant to vemurafenib[28].

MAP/ERK kinase (MEK) is possibly the most successful drug target utilised in combination therapy targeting BRAF mutations to date. MEK 1/2 catalyses the phosphorylation of threonine and tyrosine residues located on ERK 1/2, allowing ERK 1/2 to translocate to the nucleus and activate key transcription factors essential for cell growth and proliferation[29]. BRAF mutations rely on the MEK/ERK pathway more than ras mutations and this reliance appears to increase the sensitivity of BRAF for MEK inhibition[30].

Selumetinib is a potent MEK 1/2 inhibitor that is showing promising results in clinical trials[31]. Patel and colleagues conducted a phase 1 trial on melanoma patients assessing the efficacy of selumetinib in combination with other drugs. Results demonstrated improved response rates and a prolonged time to progression in patients with BRAF mutated tumours when compared to patients with wildtype BRAF tumours[32]. Another recent phase 2 trial found that selumetinib effectively induced tumour regression in melanoma patients harbouring a BRAF mutation[33].

In terms of MEK inhibition integration into combination therapies to combat BRAF resistance, trametinib (Mekinist) has been the most promising MEK 1/2 inhibitor developed. In January 2014, the FDA approved the combination treatment consisting of trametinib and the BRAF inhibitor, dabrafenib for the "treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation as detected by an FDA-approved test"[34]. This combination treatment was approved following the results of an open-label trial (NCT01072175) investigating trametinib/dabrafenib combination therapy in patients with BRAF-mutated melanoma[34]. Patients receiving the combination therapy of trametinib and dabrafenib had complete or partial response rates of 76% and response duration of 10.5 months. This was compared to patients receiving dabrafenib monotherapy who had complete or partial response rates of 54% and response duration of 5.6 months. Overall response rates were similar for patients with V600E and V600K mutations[35, 36]. Unfortunately, resistance is still a problem even with trametinib/dabrafenib combination therapy. Further research is necessary to investigate the resistance patterns causing the decreased efficacy of this combination of drugs in an effort to develop more successful drug combination strategies[36].

Conclusion

There has been much success in the development of BRAF targeted drug therapies in recent years. However, this recent success underscores the importance of drug resistance in combating BRAF-mutated cancers. It is clear that combination therapy is a promising method to effectively treat cancers harbouring BRAF mutations. BRAF inhibitor monother-

apy has improved survival rates for cancer patients but, as discussed, is not able to overcome resistance. The fact that resistance to BRAF inhibitors, like vemurafenib or dabrafenib, can occur through multiple mechanisms highlights the need for treatment options that target multiple components of different pathways involved in tumour progression. The FDA approval of dabrafenib and trametinib combination therapy for the treatment of metastatic and unresectable melanoma harbouring BRAF-V600E/K mutations is very promising. However, resistance is inevitable even with the use of this effective combination of drugs and future research should concentrate on innovative combination therapies to target resistance mechanisms more aggressively.

In summary, BRAF is a promising drug target that has been at the centre of recent clinical treatment successes. Since the discovery of BRAF as an oncogene in 2002, our knowledge of its role in cancer, its resistance mechanisms and its potential as a drug target have improved greatly. In the future, we hope to see combination therapy at the forefront of BRAF-mutated tumour treatment with the hope of improved survival rates amongst this group of cancer patients.

References

1 Peyssonnaux, C. and A. Eychene, The Raf/MEK/ERK pathway: new concepts of activation. Biol Cell. 93(1-2): p. 53-62 (2001).

2 Robinson, M.J. and M.H. Cobb, Mitogen-activated protein kinase pathways. Curr Opin Cell Biol. 9(2): p. 180-6 (1997).

3 Improta, G., et al., Biological insights into BRAF mutations in melanoma patient: Not mere therapeutic targets. Oncoimmunology 2(8): p. e25594 (2013).

4 Pratilas, C.A. and D.B. Solit, Targeting the mitogen-activated protein kinase pathway: physiological feedback and drug response. Clin Cancer Res 16(13): p. 3329-34 (2010).

5 Zambon, A., et al., Small molecule inhibitors of BRAF in clinical trials. Bioorg Med Chem Lett 22(2): p. 789-92 (2012).

6 Trunzer, K., et al., Pharmacodynamic effects and mechanisms of resistance to vemurafenib in patients with metastatic melanoma. J Clin Oncol 31(14): p. 1767-74 (2013).

7 Yang, H., et al., RG7204 (PLX4032), a selective BRAFV600E inhibitor, displays potent antitumor activity in preclinical melanoma models. Cancer Res 70(13): p. 5518-27 (2010).

8 Chapman, P.B., et al., Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 364(26): p. 2507-16 (2011).

9 Hauschild, A., et al., Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 380(9839): p. 358-65 (2012).

10 Gentilcore, G., et al., Effect of dabrafenib on melanoma cell lines harbouring the BRAF(V600D/R) mutations. BMC Cancer 13: p. 17 (2013).

11 Goldenberg, M.M., Pharmaceutical approval update. P T 38(11): p. 705-7 (2013).

12 Stuart DD, L.N., Poon DJ, Aaralen K, Kaufman S, Merritt H et al, Preclinical profile of LGX818: A potent and selective RAF kinase inhibitor. Cancer Res 72(8 Suppl)(8) (2012).

13 FDA., U.S. Sorafenib (NEXAVAR). (2013); Available at: http://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm376547.htm. (Accessed: 25th January 2014)

14 Wilhelm, S.M., et al., BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 64(19): p. 7099-109 (2004).

15 Clark, J.W., et al., Safety and pharmacokinetics of the dual action Raf kinase and vascular endothelial growth factor receptor inhibitor, BAY 43-9006, in patients with advanced, refractory solid tumors. Clin Cancer Res 11(15): p. 5472-80 (2005).

16 Wilhelm, S., et al., Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. International journal of cancer. Journal international du cancer 129(1): p. 245-255 (2011).

17 Clinicaltrials.gov., Safety and Efficacy Study of BMS-908662 Alone or in Combination With Cetuximab in Subjects With K-RAS or B-RAF Mutation Positive Advanced or Metastatic Colorectal Cancer. (2011); Available at: http://clinicaltrials.gov/ct2/show/NCT01086267?term=xl281&rank=2. (Accessed: 28th January 2014).

18 Bucheit, A. and M. Davies, Emerging insights into resistance to BRAF inhibitors in melanoma. Biochemical pharmacology 87(3): p. 381-389 (2014).

19 Villanueva, J., et al., Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell 18(6): p. 683-95 (2010).

20 Montagut, C., et al., Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. Cancer Res 68(12): p. 4853-61 (2008)

21 Acquaviva, J., et al., Overcoming Acquired BRAF Inhibitor Resistance in Melanoma via Targeted Inhibition of Hsp90

with Ganetespib. Mol Cancer Ther, (2014).

22 Davies, M.A., The role of the PI3K-AKT pathway in melanoma. Cancer J 18(2): p. 142-7 (2012)

23 Wu, H., V. Goel, and F.G. Haluska, PTEN signaling pathways in melanoma. Oncogene 22(20): p. 3113-22 (2003).

24 Deng, W., et al., Role and therapeutic potential of PI3K-mTOR signaling in de novo resistance to BRAF inhibition. Pigment Cell Melanoma Res 25(2): p. 248-58 (2012)

25 Paraiso, K.H., et al., PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. Cancer Res 71(7): p. 2750-60 (2011)

26 Atefi, M., et al., Reversing melanoma cross-resistance to BRAF and MEK inhibitors by co-targeting the AKT/mTOR pathway. PLoS One 6(12): p. e28973 (2011)

27 Neckers, L. and S.P. Ivy, Heat shock protein 90. Curr Opin Oncol 15(6): p. 419-24 (2003).

28 Paraiso, K.H., et al., The HSP90 inhibitor XL888 overcomes BRAF inhibitor resistance mediated through diverse mechanisms. Clin Cancer Res 18(9): p. 2502-14 (2012)

29 Roskoski, R., Jr., ERK1/2 MAP kinases: structure, function, and regulation. Pharmacol Res 66(2): p. 105-43 (2012).

30 Solit, D.B., et al., BRAF mutation predicts sensitivity to MEK inhibition. Nature 439(7074): p. 358-62 (2006).

31 Adjei, A.A., et al., Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J Clin Oncol 26(13): p. 2139-46 (2008).

32 Patel, S.P., et al., Clinical responses to selumetinib (AZD6244; ARRY-142886)-based combination therapy stratified by gene mutations in patients with metastatic melanoma. Cancer 119(4): p. 799-805 (2013).

33 Catalanotti, F., et al., Phase II trial of MEK inhibitor selumetinib (AZD6244, ARRY-142886) in patients with BRAFV600E/K-mutated melanoma. Clin Cancer Res 19(8): p. 2257-64 (2013).

34 FDA., U.S. Trametinib and Dabrafenib. (2014); Available at: http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm381451.htm (Accessed: 2nd February 2014).

35 GlaxoSmithKline. Study ID 113220 results summary. (2013); Available at: http://www.gsk-clinicalstudyregister.com/study/113220-rs. (Accessed: 2nd February 2014).

36 Flaherty, K.T., et al., Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med 367(18): p. 1694-703 (2012).

37 Davies, H., et al., Mutations of the BRAF gene in human cancer. Nature 417(6892): p. 949-54 (2002).

Michael McCrohan

The Holy Grail of Tissue Engineering? Islet cell microencapsulation in type 1 diabetes mellitus

Brian Woods

Fourth year Medicine, Trinity College Dublin

Abstract

The immunoisolation of transplanted islet cells represents a promising future therapy for the treatment of type 1 diabetes mellitus (T1DM). Microencapsulation is one avenue being explored to restore insulin independence while simultaneously protecting islet cells from destruction by the immune system. A variety of techniques have been developed to encapsulate the islet cells, with alginate being the most commonly employed biomaterial. The great challenge in microencapsulation is to ensure that the capsule is permselective, allowing for the free diffusion of oxygen, nutrients and waste products while providing an effective barrier to cytokines and immune identification. Other alternatives including nanoencapsulation and conformal coating are emerging. In vivo work is now beginning to be translated into clinical trials.

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by pancreatic β-cell destruction and an absolute deficiency of insulin. Affecting over 500,000 children under 15 worldwide, the International Diabetes Federation estimates that an additional 79,000 children developed type 1 diabetes in 2013[1, 2].

Over the last decades, clinical islet transplants have emerged as a possible therapy to replace those originally destroyed. In the early 1990's, 7 patients were transplanted with pancreatic islet cells and remained insulin independent for an average of 12 months[3]. The Edmonton protocol, a method of islet cell transplantation that uses immunosuppressive medications, developed as a result of this success and remains the current standard[4]. Islets are saved from destruction by the immune system through the usage of a concoction of various immunosuppressive agents. However, there are a whole host of adverse effects of immunosuppressive drugs including, but not limited to, an increased susceptibility to illness and cancers.

The possibility of transplanting an endogenous insulin source into a diabetic patient to control their blood sugar is one of the holy grails of tissue engineering. One of the more commonly used procedures for immunoprotection of transplanted tissues is the microencapsulation of islets in an alginate-based capsule[5]. This procedure was originally described by Lim and Sun but in recent years important advances have been made to bring this technology from beyond the realms of science fiction[6]. With the commencement of human trials, temporary but significant survival of human microencapsulated tissue has been observed[7, 8]. One substantial hurdle that has to be overcome is the issue of the variability of graft survival[9]. A wide variety of approaches have been used to improve capsule properties.

Why encapsulate?

The concept of islet microencapsulation is to create a permselective membrane around a cluster of islet cells using an immunoprotective biomaterial. Ideally the encapsulation should eliminate, or at least limit, any immunological response to the graft.

Allowing for the clinical use of xenogeneically-derived islets or engineered insulin-secreting stem cells, encapsulation may offer a solution to the shortage of donors for clinical islet transplantation.

Encapsulation devices to date generally range from microscale to macroscale devices. Macrocapsules can be up to 3cm by 8 cm and hold up to 250 µL of tissue[10]. In contrast, microcapsules are droplets ranging from 100 nm to 1mm in size[11,12]. To optimize oxygen and nutrient delivery and waste removal, ideally capsules would be placed intravascularly. However, this strategy has been, in the main, abandoned due to the increased risk of thrombosis and haemhorrhage[13]. The membrane that encapsulates the islets should be porous enough to allow diffusion of glucose, insulin, oxygen and other nutrients while remaining selective enough to omit immune cells, toxic cytokines, complement proteins and cytokines[14].

In microencapsulation, individual or small clusters of islets are incorporated into a spherical hydrogel polymer formed through various techniques. This offers improved diffusion capacity due to a better surface/volume ratio[15]. Another major advantage of microencapsulation is that individual islets are protected from any immune attack. This means that, as long as the failure rate is kept low when forming the microcapsules, only the affected islets will be destroyed. However, if there is a small failure rate in a macroencapsulated device, the whole population of encapsulated islets is at risk of destruction[16]. One downfall of microencapsulation to date has been their sometimes very large size in comparison to the islets or groups of islets contained within. This may result in capsules made up predominantly of hydrogel with only a comparatively, very small amount of islets.

CLINICAL POINTS

Type 1 diabetes mellitus is an autoimmune disease characterized by pancreatic β-cell destruction and an absolute deficiency of insulin

It affects over 500,000 children under 15 worldwide

Over the last decades, clinical islet transplants have emerged as a possible therapy to replace those originally destroyed

With microencapsulation, individual or small clusters of islets are incorporated into a spherical hydrogel polymer that offers improved diffusion capacity and immuno-protection

In in vivo animal and human models, transplanted encapsulated islet cells can survive when placed in the peritoneal cavity. These transplantations could reduce the requirement for exogenous insulin injections

Issues that need resolving include optimization of insulin release and further data on the length of functional survival of microcapsules once implanted. Further in vivo animal models and extensive clinical trials are required to optimize and evaluate this therapy

In vivo studies

In a recent pilot study, Jacobs-Tulleneers-Thevissen and colleagues showed that transplanted human islets contained within alginate microcapsules could reverse diabetes in mice when placed in the peritoneal cavity[41]. These encapsulated cells performed significantly better than non-encapsulated cells implanted under the fibrous capsule of the kidney[41]. The microcapsules were retrieved after 5 weeks and found to have retained functional, healthy, insulin secretory responses to glucose and glucagon stimulation. This was in contrast to the non-encapsulated cells which were found to be completely non-functional and only retained 10% of their initial β-cell population[14,41].

Barium-alginate microbeads have been the most extensively studied non-coated alginate beads for microencapsulation. One group, using an in vivo T1DM model, managed to normalize the blood glucose of a non-obese-diabetic (NOD) mouse over the course of one year using allogenic islets embedded in barium-alginate microcapsules[17]. The protection of these uncoated beads was effective in T1DM and it was believed that the encapsulated islets may survive for longer than a year[17]. The group also suggested that regeneration of islets occurs within the capsules as the average life span for a β-cell is 3 months[18].

An encapsulated islet allotransplantation was first performed as a clinical trial in a 38 year old T1DM male. Human islets isolated from a cadaveric pancreas were encapsulated in alginate microcapsules. They were then injected into an intra-peritoneal location at a dose of 10,000 islet equivalent per kilogram (IEq/kg) body mass with a booster of 5,000 IEq/kg six months later. The patient was able to survive purely from the endogenous insulin secreted from the implanted islets for a period of nine months proving that encapsulated islets are capable of achieving adequate glycemic control in a T1DM subject. However, one aspect of this particular case worth noting is the fact that this man was already on an immunosuppressive regimen prior to receiving the transplanted islet[20]. Thus, it was not possible to state whether or not the alginate microcapsules offered any immune protection to the encapsulated islets. A follow up study done in four more T1DM patients transplanted with microencapsulated islets displayed a statistically significant decrease in insulin requirements for several months. However, at follow-up seven years later all patients were entirely dependent on exogenous injections of insulin again[19]. Similar results were noted in a separate trial where two patients were implanted with islets encapsulated within alginate microcapsules. Both patients had significant reductions in their insulin dosage requirements but complete insulin independence was never achieved[20].

One potentially promising product currently in clinical trials is the Diabecell by Living Cell Technologies. This is an alginate-based microcapsule for the implantation of pig islets[21, 22]. Viable encapsulated porcine islets were reported after a 9.5 year laproscopic biopsy in one patient. Another trial of the same product in Moscow in 2008 found that 2 out of 3 patients receiving 10,000 islet equivalent per kilogram (IEq/kg) body mass were insulin independent 6 months post implantation[23]. Another trial also had its subject have his islets inspected under laparoscopy. Although this patient still had a detectable C-peptide level, his encapsulated islets were not producing enough insulin to attain control of his diabetes. They were found to be surrounded by a fibrous tissue and some of the islets necrotic[11].

Challenges in islet microencapsulation

Studies have frequently reported that the kinetics of insulin are delayed when islets are microencapsulated. This appears to be related to microcapsule volume[16]. Perfusion work performed in vitro has shown that insulin is released 10-15 minutes earlier from cells that are not encapsulated as compared to microencapsulated islets. There is a parallel delay in the return toward basal rates when concentrations of glucose decrease from high to low values.

The kinetics of insulin release may also be affected by its anatomical placement during surgery. Often an intra-peritoneal location is chosen. If the encapsulated islets fail to adhere to the recipient's tissues then, when the recipient assumes an upright position, the capsules may fall into the pelvis. The oxygen carrying capacity of the peritoneal fluid is quite low and thus, a large amount of the encapsulated islets die off when packed into the pelvis of primates[16]. However, if the microcapsules are adequately adhesive, they will resist falling into the pelvis[16].

Sufficient supply of oxygen and nutrients is crucial to the longevity of islets. For effective oxygen and nutrient transfer, islets cannot be located more than 200 µm from capillaries[24]. There is no convection movement within the microcapsule and thus a nutrient gradient is induced from the capsule surface in to the islet centre[25, 26]. One obvious solution to combat this apparent nutrient-gradient would be to reduce the capsule size. However, there are significant disadvantages to reducing capsule thickness as the number of capsules that contain islets that are partially protruding will proportionally increase[27,

[28]. Thus, the number of microcapsules affected by an inflammatory response would also increase.

Many studies initially assumed a loss of 2-10% due mainly to the reasons mentioned. What could not be explained, however, was the failure of the remaining 90-98% of the capsules[29, 30]. It was unclear what was causing failure since it was generally assumed that the loss of 2-10% of capsules cannot explain the failure of the cells in the remaining 99-90% of the capsules[29, 30].

However, new insight has been brought to the situation over the last number of years. In a series of experiments it became clear that it was the islet cells (as opposed to any failing of the biomaterial) that was at fault for the failure. Cytokines such as MCP-1, MIP, nitric oxide, and IL-6 are secreted by islet cells when stressed[31]. These cytokines recruit and activate immune cells[31, 32, 33]. A follow-up experiment showed that activated macrophages surrounding the 2-10% of overgrown capsules secreted IL-1β and TNF-α when co-cultured with microencapsulated islets but not with capsules that are empty[32, 33]. IL-1β and TNF-α are known to place islets under stress. A progressive loss of function of the encapsulated tissue was observed[33, 34]. Essentially, this series of experiments showed that cytokines derived from the graft diffuse out of the capsules, activating macrophages which go on to secrete cytokines in a feed forward cycle.

What is it that sets off this destructive cycle? Transplantation of microencapsulated cells into the peritoneal cavity requires just minor surgery. However, this procedure is still associated with an element of tissue damage. Chemotactic proteins such as fibrinogen, thrombin, histamine and fibronectin are released[35]. An influx of large numbers of inflammatory cells (granulocytes, basophiles, mast cells, and macrophages) is induced into the peritoneal site in the early days after the implantation procedure[36].

In the first months after transplantation, the release of bioactive factors from the encapsulated tissue is responsible for the loss of 60% of the engrafted tissue[29]. The diffusion of graft-derived and inflammatory cell-derived cytokines is a major threat for the longevity of the encapsulated grafts[37]. One potential way of overcoming the issue of islet-derived cytokines is to reduce the permeability of the capsule. However, how is it possible to protect against harmful cytokines with a similar molecular weight to insulin or other vital nutrients (5-15kDa)? Up until recent years it was thought that this diffusion of cytokines into capsules was an impossible barrier to adequate immunoisolation.

Interestingly, several studies have shown this to not be the case. The ultimate effect of cytokines depends on a combined presence of various cytokines and on the concentrations of these respective cytokines[32, 33, 38]. In vitro, it has been shown that by decreasing the permeability via chemical modification, it is possible to prevent large, multimeric cytokines such as TNF-α diffusing into the capsule. Another group showed that damage induced by cytokines is minor in 'medium size' (400-500 µm) microcapsules and increases in smaller microcapsules[39]. With respect to immunoprotective capacity, this observation tends to suggest that microcapsules may display superior performance than conformal coatings (whereby a thin protective chemical covering or film is used)[39].

An interesting revelation in recent years has been the fact that, in the case of xenografting encapsulated islets in humans, cytokines may not interfere with islet functioning to the same extent as allografting. This is due to the fact that xenogenic islets (i.e. islets derived from a different species) do not readily bind to and take up human cytokines[40]. The implication of these findings is that xenogenic islets will be less affected if encapsulated in a cytokine-permeable biomaterial5.

Microscale cellular encapsulation methods allow for the precise control of cell size and shape. However, the scale-up to mass production using cost-effective and labour-efficient methods and automation represents another great challenge for the future.

Discussion

The idea that implanted islet cells can be protected from immune system identification and destruction through the use of a permselective biomaterial barrier remains an attractive therapeutic approach in spite of the challenges being faced. Advances in the clinical translation of laboratory research have somewhat slowed down since the initial great pioneering developments in islet transplantation. However, an appreciation for the subtleties and nuances of the diffusional characteristics of the encapsulating material combined with an improved understanding of the workings of the cytokine and complement systems has led to a renewed interest and enthusiasm in the field.

It has been shown in vivo animal and human models that transplanted encapsulated islet cells can survive when placed in the peritoneal cavity. In a growing number of instances these transplantations have reduced the requirement for exogenous insulin injections. However, one issue that needs resolving is how long can encapsulated islet cells survive and function when transplanted? There is a great degree of discordance in the literature on the lifetime of microencapsulated islets. In its current form, islet encapsulation appears to be at most a medium-term solution for patients as grafts appear to be functionally useful from several months to just over a year. Most likely, if this becomes a therapeutic option, patients may need to replenish lost islets with annual 'top-ups'.

Another issue affecting islet microencapsulation is whether or not the kinetics of insulin release will be adequate. The choice of anatomical location may need to be optimized in future to ensure that there is an appropriate vasculature for insulin levels to rise and fall quickly either side of meals while, at the same time, allowing for minimally-invasive delivery. The biomaterial encapsulating the cells also requires further optimization to ensure that oxygen, nutrients and waste products can freely diffuse in and out of the capsule, avoiding any 'nutrient-gradients'.

New techniques for islet encapsulation are beginning to show promising results. Further in vivo animal models and extensive clinical trials are required to optimize and evaluate this therapy. Islet transplantation is potentially the most important advance in the treatment of T1DM since Fredrick Banting and Charles Best discovered insulin in 1921. With the possibility of significantly improving the quality of life of patients and, essentially, even curing the disease itself, it is hard not to be at least cautiously optimistic about what the future has in store for islet cell microencapsulation.

References

1 Patterson C., Guariguata L., Dahlquist G., Soltész G., Ogle G., Silink M. Diabetes in the young - a global view and worldwide estimates of numbers of children with type 1 diabetes. Diabetes Res Clin Pract. 103: 161-75 (2014).

2 International Diabetes Federation. IDF Diabetes Atlas, 6th edn. Brussels, Belgium: International Diabetes Federation (2013).

3 Shapiro A.M., Lakey J.R., Ryan E.A., et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 343, 230-238 (2000).

4 Shapiro A.M., Ricordi C., Hering B.J., Auchincloss H., Lindblad R., Robertson P., Secchi A. et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med. 355, 1318-1330 (2006).

5 De Vos, P, Marijke M. Faas, Strand B., and Calafiore R. Alginate-based microcapsules for immunoisolation of pancreatic islets. Biomaterials 27(32) 5603-5617 (2006).

6 Lim F., Sun A.M. Microencapsulated islets as bioartificial endocrine pancreas. Science 210, 908-910 (1980).

7 Hasse, C., G. Klöck, A. Schlosser, U. Zimmermann, and M. Rothmund. Parathyroid allotransplantation without immunosuppression. The Lancet 350,1296-1297 (1997).

8 Soon-Shiong, Patrick, Edward Feldman, Richard Nelson, Jan Komtebedde, Olav Smidsrod, Gudmund Skjak-Braek, Terje Espevik, Roswitha Heintz, and Martin Lee. Successful reversal of spontaneous diabetes in dogs by intraperitoneal microencapsulated islets. Transplantation. 54,(5) 769-774 (1992).

9 O'Shea, Geraldine M., Mattheus FA Goosen, and Anthony M. Sun. Prolonged survival of transplanted islets of Langerhans encapsulated in a biocompatible membrane. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 804, 133-136 (1984).

10 Tarantal AF, Lee CCI, Itkin-Ansari P. Real-time Bioluminescence Imaging of Macroencapsulated Fibroblasts Reveals Allograft Protection in Rhesus Monkeys (Macaca mulatta). Transplantation;88(1):38-41 (2009).

11 Tuch, Bernard E., Gregory W. Keogh, Lindy J. Williams, Wei Wu, Jayne L. Foster, Vijayganapathy Vaithilingam, and Robert Philips. "Safety and viability of microencapsulated human islets transplanted into diabetic humans." Diabetes care 32, no. 10; 1887-1889 (2009).

12 Elliott, Robert B., Livia Escobar, Paul LJ Tan, Maria Muzina, Sahar Zwain, and Christina Buchanan. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 years after xenotransplantation. Xenotransplantation 14(2) 157-161 (2007).

13 Vijayaganapathy V., Bernard E. Tuch. "Islet transplantation and encapsulation: an update on recent developments." The review of diabetic studies: RDS 8(1) 51 (2011).

14 Weir, G. C. Islet encapsulation: advances and obstacles. Diabetologia 56, no. 7: 1458-1461. (2013)

15 Clayton, H. A., N. J. London, P. S. Colloby, P. R. Bell, and R. F. James. A study of the effect of capsule composition on the viability of cultured alginate/poly-l-lysine--encapsulated rat islets. Diabetes research (Edinburgh, Scotland) 14,3:27-132 (1990).

16 Scharp D.W., Marchetti P. Encapsulated islets for diabetes therapy: History, current progress, and critical issues requiring solution. Adv Drug Deliv Rev.67, 35-73 (2014).

17 Duvivier-Kali, Valérie F., Abdulkadir Omer, Richard J. Parent, John J. O'Neil, and Gordon C. Weir. Complete protection of islets against allorejection and autoimmunity by a simple barium-alginate membrane. Diabetes 50, (8) 1698-1705 (2001).

18 Finegood, Diane T., Luisa Scaglia, and Susan Bonner-Weir. Dynamics of -cell mass in the growing rat pancreas: estimation with a simple mathematical model. Diabetes 44, 3:249-256 (1995).

19 Valdés-González, Rafael A., Luis M. Dorantes, G. Nayely Garibay, Eduardo Bracho-Blanchet, Armando J. Mendez, Roberto Dávila-Pérez, Robert B. Elliott, Luis Terán, and David JG White. Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. European Journal of Endocrinology 153, 3:419-427 (2005).

20 Valdes Gonzalez, R., A. L. Rodriguez Ventura, D. J. G. White, E. Bracho Blanchet, A. Castillo, B. Ramírez González, M. G. López Santos, B. H. León Mancilla, and L. M. Dorantes. Long term follow up of patients with type 1 diabetes transplanted with neonatal pig islets. Clinical & Experimental Immunology 162, 3:537-542 (2010).

21 Elliott, R. B., L. Escobar, R. Calafiore, G. Basta, O. Garkavenko, A. Vasconcellos, and C. Bambra. Transplantation of micro-and macroencapsulated piglet islets into mice and monkeys. Transplantation Proceedings, 37, (1):466-469 (2005).

22 Elliott, R. B., L. Escobar, P. L. J. Tan, O. Garkavenko, R. Calafiore, P. Basta, A. V. Vasconcellos, D. F. Emerich, C. Thanos, and C. Bambra. Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates. Transplantation Proceedings, 37, 8:3505-3508 (2005).

23 Skinner, Stephen JM, Paul LJ Tan, Olga Garkavenko, Marija Muzina, Livia Escobar, and Robert B. Elliott. "Cell Replacement Therapy: The Rationale for Encapsulated Porcine Islet Transplantation." in: D. Wagner (Ed.), Type 1 Diabetes Complications, In Tech Europe, Croatia (2011).

24 Dionne, Keith E., Clark K. Colton, and M. Lyarmush. Effect of hypoxia on insulin secretion by isolated rat and canine islets of Langerhans. Diabetes 42,1: 12-21 (1993).

25 Schrezenmeir, J., L. Gerö, C. Laue, J. Kirchgessner, A. Müller, A. Hüls, R. Passmann, H. J. Hahn, L. Kunz, and W. Mueller-Klieser. "The role of oxygen supply in islet transplantation. In Transplantation proceedings, 24, 2925. (1992).

26 Schrezenmeir, Jürgen, Judith Kirchgessner, Lazlo Gerö, Leoni A. Kunz, Jürgen Beyer, and Wolfgang Mueller-Klieser. Effect of microencapsulation on oxygen distribution in islets organs. Transplantation 57, 1308-1314 (1994).

27 De Vos, Paul, Bart De Haan, Jenny Pater, and Reinout Van Schilfgaarde. Association between capsule diameter, adequacy of encapsulation, and survival of microencapsulated rat islet allografts. Transplantation 62, 893-899 (1996).

28 De Vos, Paul, Bart De Haan, Gerrit HJ Wolters, and Reinout Van Schilfgaarde. Factors Influencing the Adequacy of Microencapsulation of Rat Pancreatic Islets. Transplantation 62, 888-893 (1996).

29 De Vos, Paul, J. F. Van Straaten, Arie G. Nieuwenhuizen, Martijn de Groot, Rutger J. Ploeg, Bart J. De Haan, and Reinout Van Schilfgaarde. Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? Diabetes 48, 1388 (1999).

30 King, Aileen, Arne Andersson, Berit L. Strand, Joey Lau, Gudmund Skjåk-Bræk, and Stellan Sandler. "The role of capsule composition and biologic responses in the function of transplanted microencapsulated islets of langerhans1." Transplantation 76, 275-279 (2003).

31 Cardozo AK, Proost P, Gysemans C, Chen MC. IL-1beta and IFN-gamma induce the expression of diverse chemokines and il-15 in human and rat pancreatic islet cells, and in islets from pre-diabetic NOD mice. Diabetologia 46:255-66 (2003).

32 De Vos, P., I. Smedema, H. Van Goor, H. Moes, J. Van

Zanten, S. Netters, L. F. M. de Leij, A. De Haan, and B. J. De Haan. "Association between macrophage activation and function of micro-encapsulated rat islets." Diabetologia 46, 666-673 (2003).

33 De Vos, Paul, Bart J. de Haan, Aalzen de Haan, Jacoba van Zanten, and Marijke M. Faas. Factors influencing functional survival of microencapsulated islet grafts. Cell transplantation 13, 515-524 (2004).

34 De Vos, P., B. J. De Haan, D. Vegter, J-L. Hillebrands, J. H. Strubbe, J. E. Bruggink, and R. Van Schilfgaarde. Insulin levels after portal and systemic insulin infusion differ in a dose-dependent fashion. Hormone and Metabolic Research. 30, 721-725 (1998).

35 Ři hová, Blanka. Immunocompatibility and biocompatibility of cell delivery systems. Advanced drug delivery reviews. 42, 65-80 (2000).

36 De Vos, Paul, Chris G. van Hoogmoed, Bart J. de Haan, and Henk J. Busscher. Tissue responses against immunoisolating alginate PLL capsules in the immediate posttransplant period. Journal of biomedical materials research. 62, 430-437 (2002).

37 Bolzán, Alejandro D., and Martha S. Bianchi. Genotoxicity of streptozotocin. Mutation Research/Reviews in Mutation Research. 512, 121-134 (2002).

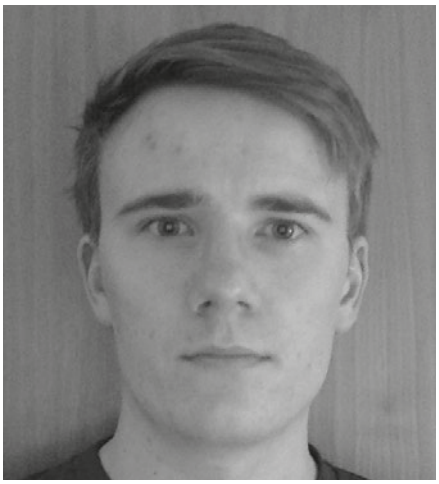
38 King, Aileen, Arne Andersson, and Stellan Sandler. Cytokine-Induced Functional Suppression of Microencapsulated Rat Pancreatic Islets in Vitro. Transplantation. 70, 380-383 (2000).

39 Basta, G., P. Sarchielli, G. Luca, L. Racanicchi, C. Nastruzzi, L. Guido, F. Mancuso et al. Optimized parameters for microencapsulation of pancreatic islet cells: an in vitro study clueing on islet graft immunoprotection in type 1 diabetes mellitus. Transplant immunology. 13, 289-296 (2004).

40 Piro, Salvatore, Roberto Lupi, Francesco Dotta, Giovanni Patan, Maria A. Rabuazzo, Lorella Marselli, Carmela Santangelo et al. Bovine Islets Are Less Susceptible Than Human Islets to Damage by Human Cytokines. Transplantation 71, 21-26 (2001).

41 Jacobs-Tulleneers-Thevissen, D., M. Chintinne, Z. Ling, Pieter Gillard, Luc Schoonjans, G. Delvaux, B. L. Strand, F. Gorus, B. Keymeulen, and D. Pipeleers. Sustained function of alginate-encapsulated human islet cell implants in the peritoneal cavity of mice leading to a pilot study in a type 1 diabetic patient. Diabetologia 56, 1605-1614 (2013).

42 De Vos, P, van Hoogmoed C G , van Zanten J, Netter S., Strubbe J H, Busscher H J. Long-term biocompatibility, chemistry, and function of microencapsulated pancreatic islets. Biomaterials 24, 305-312 (2003).



Brian Woods

Discretionary Medical Cards: ethical considerations

Neasa Fitzpatrick, Isabelle Hunt, Éabha Manley, Michael McCrohan, Amy Nash, Michael Ryan, Andrew Stokes and Conor Toale
Fourth year Medicine, Trinity College Dublin

Abstract

The General Medical Service (GMS) scheme governs access to medical cards in Ireland. A medical card entitles the holder to free health services, including free GP care, inpatient and outpatient hospital services, and prescription medications. Eligibility for medical card cover is based on income, while those whose income is above the maximum threshold for eligibility may be granted a Discretionary card. This is on the basis that to fail to offer these cards would result in 'exceptional personal and financial burdens arising from medical or social circumstances' being placed on the applicant. In practice, this scheme covers many people with chronic, life-limiting illnesses. A recent governmental review of the Discretionary medical card scheme led to the cancellation and subsequent re-approval of 15,000 cards, and was met with much controversy both in the Dáil and in the media. Ultimately the ensuing debate centred on the issue of resource allocation, and arguments were made for and against the current means-based system of card allocation versus a disease-based model where factors other than income are taken into account in order to determine an applicant's eligibility for cover. This article examines some of the arguments both in favour and against these approaches, and questions whether the proposed changes to the scheme, as recommended by the Report of the Expert Panel on Medical Need for Medical Card Eligibility, meet the HSE's own stated policy targets of equity, fairness, proportionality, openness and accountability, solidarity, and sustainability.

Introduction

In Ireland, eligibility for free health care is based on the grounds of residency and means. The General Medical Service (GMS) scheme governs access to medical cards which entitle the holder to free health services, including free GP care, inpatient and outpatient hospital services, and prescription medications (although prescription charges do apply). The legislative basis for entitlement to medical cards is governed by Section 45 of the Health Act 1970[1]. This legislation has been subject to a number of revisions since its conception, yet still explicitly states that income and expenditure must be taken into account when assessing eligibility for free health services: 'Adult persons, who in the opinion of the Health Service Executive, are unable without undue hardship to arrange general practitioner medical and surgical services for themselves and their dependants' should be granted a medical card. Those who do not qualify for a medical card may be entitled to a GP visit card under Section 58 of the Health Act 1970 (as amended by the Health Amendment Act 2005), which similarly describes eligibility on the grounds that it would be "unduly burdensome" for the patient to provide GP services for themselves and their dependents[1]. People aged over 70, are assessed on gross income at higher thresholds and expenditure is not taken into account. However, if individuals demonstrate significant outgoings such as health expenses, this added expenditure may be taken into account in determining eligibility. As of July 2014, there were 1,804,376 medical card and 142,668 GP card users in Ireland. This equates to 39.3% and 3.11% of the population, respectively. These figures reflect a 57% increase in the number of medical card holders from the period 2004 to August 2014[4].

Discretionary medical card eligibility

The services which medical card holders are entitled to free of charge include GP visits, prescribed medicines, inpatient and outpatient services in public hospitals, medical services for mothers and infants and some dental, aural and ophthalmic visits. GP visit cards allow for GP services only[2]. Discretionary medical cards may be granted in exceptional circumstances when a person's weekly income (gross less tax, USI and PRSI) is in excess of HSE's stated guidelines. Currently the threshold is set at €184 per week for a single person under aged 66 living alone. This threshold changes in accordance with the number of dependents in the recipient's household, or if the application is made by a married/co-habiting couple or by civil partners[2]. Discretionary medical cards entitle the holder to access the same services as general medical cards do.

The HSE takes a number of factors into account when deciding eligibility for these discretionary medical cards. Again, the concepts of 'undue hardship' and being 'unduly burdensome' are expressed, with the act stating that 'the HSE must have regard to a person's overall financial situation and not just their income' when assessing a person's eligibility for discretionary medical card provision[3]. When the cost of providing medical care compromises one's ability to meet these essential costs, a discretionary medical card may be granted[3]. These discretionary medical cards have been the source of recent controversy, although in practice, the vast majority of medical cards are granted to applicants whose income is below the guideline. As of July 2014, there were 65,993 discretionary medical cards and 28,423 discretionary GP visit cards in circulation[4], amounting to a significant proportion of the health budget. The sitting government recently moved to review the eligibility of discretionary medical card recipients, resulting in the cancellation of a number of cards during the period 1 July 2011 to 31 May 2014.

CLINICAL POINTS

Current policy surrounding discretionary medical card allocation can appear to be subjective and discriminatory to the outside observer

While a disease-based approach has its merits, the government is right to be concerned about compiling a list of the "hierarchy of diseases" that would be required under such a scheme

Disease-based approaches have been implemented in other jurisdictions such as the State of Oregon, USA

Quality Adjusted Life Years (QALYs) may represent an objective method by which a disease-based allocation model could be constructed

Disease-based cover already exists in Ireland in the form of the long-term illness scheme. We believe this scheme is arbitrary and restrictive, and that there is scope for expansion of this policy to cover other debilitating illnesses

Ultimately it is clear that objective, transparent methods are required to assess discretionary medical card eligibility. While we welcome the debate on the merits of a means-based versus disease-based approach, we question whether new proposals put forward by the Expert Panel on Medical Need for Medical Card Eligibility meet the HSE's own stated aims of equity, fairness, proportionality, openness and accountability, solidarity, and sustainability

Recent eligibility review and resulting controversy

The allocation of discretionary medical cards has not been without its controversies. Approximately 17,000 discretionary cards were withdrawn between March 2011 and October 2011 in a recent governmental review of the scheme (2,300 were due to holders passing away)[5]. This fallout highlighted a great deal of weakness and discrepancy in the medical card system and, particularly in this case, the allocation of discretionary medical cards. In June of 2014, the government then announced they would be re-issuing the 15,000 discretionary medical cards withdrawn under the eligibility review "within a matter of weeks"[5]. The eligibility review of discretionary medical cards was also suspended and an expert panel was put in place to "advise on the broader issue" of

medical card allocation. This panel published their report in September of 2014[4], in which, on the topic of discretionary medical cards, they cited the legacy issues in revoking medical cards granted to individuals with a disease that was unlikely to change. They conceded that much of these legacy issues came from the lack of a uniform approach to the granting of the discretionary medical cards in the various HSE districts prior to the centralisation, but admonished the HSE for not handling the matter more delicately. Furthermore, this controversy and the findings surrounding it may highlight the hazards of a non-objective/discretionary method of awarding medical cards, with many calling for a complete overhaul of the system.

Ethical considerations

Thus, the recent review and surrounding controversy focused on the core issue of eligibility: should medical card distribution be based solely on financial need, or should the severity and impact (either functional or financial) of a person's illness be taken into account? If the latter approach is favoured, to what extent should the nature of a person's illness be considered, and how exactly would the impact of each disease be measured? Decisions regarding the allocation of finite resources must be made, hopefully in a way that is fair, equitable and ethical. To this aim, the HSE has identified 6 principles on which all healthcare policy decisions should be made: equity, fairness, proportionality, openness and accountability, solidarity, and sustainability. Many people, including the current Fine Gael led coalition, believe that means-tested medical card distribution is the most appropriate way to achieve these aims[6]. This is based on the universal observation that the burden of illness is greatest in those of lower socio-economic status[7]. However, in the aftermath of public and media outrage regarding the cancelling of discretionary medical cards to children and adults with serious life-limiting illnesses, questions pertaining to the ethics of a solely income-based model of provision are raised.

This now begs the question; how would a system in which free healthcare is granted based on disease severity compare to the existing means-tested model, particularly in relation to achieving the HSE's ethical aims? Regarding equity and fairness, we believe there are doubts that the newly reviewed system is the best way forward. Specifically promises to de-centralise the decision process and to allow for flexibility with medical card provision could lead to new moral challenges. Take for example the "post-code lottery" phenomenon observed in the UK, where regional healthcare trusts make decisions on whether certain treatments are covered by the NHS[8]. This has resulted in notable regional inequalities in healthcare provision. It is entirely plausible that a similar phenomenon could emerge in Ireland over the coming years, where a patient's chance of securing medical cover differs according to geography. In order to overcome this inequity in the UK, new NICE guidelines have been drawn up in an attempt to base funding decisions on more objective analysis, by using Quality-adjusted life years (QALYs)[9]. With this in mind we wonder whether or not a similar system be employed in Ireland?

To ascertain the equity and fairness of disease-based provision, a system would have to be devised whereby the functional, financial, and/or psychological aspects of disease burden could be accurately measured, which is itself fraught with ethical challenges[10,11]. Options exploring a more nuanced approach to healthcare rationing have suggested the use of QALYs as a means by which the functional and not just financial aspects of disease can be considered when allocating treatment resources[12]. This would require the HSE or a relevant body to compose a list of diseases/treatments to be covered by the medical card, a policy initiative to which the government is somewhat justifiably opposed[13]. A similar approach to healthcare provision was adopted by the State of

Oregon in the United States, which consulted with the public at large in order to compile a list of services which would be covered by Medicaid for those under a specific income level[14]. A provisional list based on a cost-benefit algorithm came under considerable scrutiny for covering such procedures as toothcaps but not appendicitis[15]. This example demonstrated the difficulties underpinning the proposed illness-based model of health-care provision: how much moral weight should be given to competing influences such as cost-effectiveness, quality of life, financial hardship and pain/suffering? However, the inclusion of the public at large in the decision making process would seem to fulfil the HSE's goals regarding openness and accountability in making decisions regarding healthcare provision[20].

Issues around sustainability are invariably raised by a system of health provision based on medical need. What is the correct "hierarchy of diseases"? Who is to decide what diseases or treatments are worth covering? Minister for Health, Leo Varadkar, has raised his concern over such proposals, suggesting that resources would not stretch as far as the medical need of Ireland's populace would warrant - "If you look at the international classification of disease, things like overweight and obesity are considered to be illnesses, too, so you would be potentially extending medical cards to almost the entire population, which would not be realistic"[18]. In the aforementioned Oregon Example, healthcare benefits were expanded under the disease-based approach[20]. With 44% of the Irish population currently in possession of a medical card or GP visit card, would it be equitable to propose that the ever-decreasing majority pay for the increased medical card cover? Of course, it would be possible as in Oregon to decide a set line based on cost-effectiveness under which treatments would not be covered, although this raises its own ethical dilemmas[20]. Furthermore, if the aim of medical card provision is to support those who are unable to afford their own medical care; is it fair, equitable or sustainable to provide free cover to those who have the means to support themselves? This approach would surely go against the HSEs stated aim of "solidarity" in deciding policy decisions.

There are many valid arguments however, for providing medical cards to those with chronic or life-limiting illnesses irrespective of a patient's financial means. Individuals with diagnoses of chronic medical conditions such as motor neuron disease or chronic cardiac failure who do not possess a medical card incur major expenses for a wide number of reasons. They have increased medication costs, attend the GP more regularly and may have to be admitted to hospital frequently. Furthermore, without a medical card they may have difficulty accessing community services like public health nursing and primary care counselling, or acquiring appliances and tools from physiotherapy and occupational therapy in the community. There are also a whole host of incidental costs such as travel expenses, supplements and foodstuffs that add to a patient's financial burden[16].

Should medical card provision take these factors into account? Should consideration be given to the loss of life-years, reduction in quality of life, and psychological hardship incurred by those with chronic diseases? Moreover, can a financial cost be assigned to these sufferings? The existing legislation, whereby a discretionary card may be granted if it can be proven that the absence of provision of a medical card is unduly burdensome to the patient or will cause 'undue hardship', goes some way towards addressing this. However, there appears to be little clarity as to what constitutes 'hardship', particularly as the concept of 'exceptional personal and financial burdens arising from medical or social circumstances' is rather subjective. There is also a lack of instruction as to how much weight should be given to these contributing social and financial factors when it comes to deciding the eligibility of patients

applying for medical cards. Thus, in spite of this legislation, many patients who suffer from ailments considered by many to cause undue hardship or to impose a financial burden will not qualify[7].

The long-term illness scheme is an existing disease-based model which provides free medical care to patients with the following conditions: mental handicap, mental illness (for people under 16 only), diabetes insipidus, diabetes mellitus, haemophilia, cerebral palsy, phenylketonuria, epilepsy, cystic fibrosis, multiple sclerosis, spina bifida, muscular dystrophies, hydrocephalus, parkinsonism, acute leukaemia and conditions arising from the use of thalidomide[1]. Implicitly this suggests that these diseases are so debilitating that it would be immoral to refuse a medical card to those diagnosed. With the huge changes that have been observed in both the diagnosis and treatment of several conditions over the last 40 years, perhaps the Health Act of 1970 is in need of some revision. For example, while diabetes mellitus is now a controllable chronic illness with reduced impact on a patient's quality of life, it is notable that it is explicitly covered by the scheme while a wide range of debilitating illnesses such as stroke, spinal injuries, and other severe congenital and acquired conditions are not. An expansion of the long-term illness scheme to cover a wider range of diseases and disabilities could go some way to alleviating the uncertainty and subjectivity currently overshadowing medical card provision. Again however, issues of sustainability and eligibility would inevitably feature in any debate on this issue, with an enormous range of diagnoses and an infinite number of combined diagnoses vying for coverage. The concept of a "hierarchy of diseases" would once again be a contentious issue. Furthermore, such a scheme could discriminate against patients whose symptoms don't neatly fit into restrictive diagnostic criteria, an issue which has been raised by the Irish cancer society[10].

Conclusion

The objective of this review was to highlight and explore the ethical and legal controversies surrounding policy and legislation that currently dictates discretionary medical card eligibility and distribution. The benefits and limitations of means-tested provision versus a disease-based model have been debated extensively throughout this period of HSE reorganisation. A means-tested approach is arguably much easier to categorise than disease-based models and this of course appeals to politicians and policy makers. The need to decide what illness is debilitating, chronic and costly enough to warrant a medical card is problematic particularly with the added challenge of fulfilling the 6 principles that health policy should uphold, and thus is a less appealing approach to policy makers. However, is it ethical to deprive people with severe chronic disease free access to medical care only because of the difficulty of defining parameters? Regardless of income, patients with chronic disease incur major expenses to pay for medication, access to therapeutic services and community support as well as frequent visits to general practitioners. Discretionary medical cards have until now provided free medical care to those with chronic illness at the discretion of the HSE, in a system that appears arbitrary and discriminatory to the outside observer. Therefore it is clear that objective methods and criteria are needed to avoid many of the issues that have arisen throughout this 3 year period of medical card revoking and reinstating.

References

1 Health Act of 1970 (1970). Available at http://www.irishstatutebook.ie/1970/en/act/pub/0001/.

2 Citizens Information Board. Medical cards. Relate; Volume 41(3). (2014) Available at:
Available from http://www.citizensinformationboard.ie/publications/relate/relate_2014_03.pdf.

3 Health Service Executive. Medical Card/GP Visit Card National Assessment Guidelines. (2014) Available at: http://www.hse.ie/eng/services/list/1/schemes/mc/forms/assessmentguidelines.pdf.

4 Health Service Executive. Report of the Expert Panel on Medical Need for Medical Card Eligibility (2014) Available at: http://www.hse.ie/eng/services/publications/corporate/expertpanelmedicalneed.pdf.

5 Department of Health. Government approves method for the return of medical cards lost in discretionary review (2014) Available at: http://health.gov.ie/blog/press-release/government-approves-method-for-the-return-of-medical-cards-lost-in-discretionary-review/.

6 Health Service Executive. Healthy Ireland: A Framework for Improved Health and Wellbeing 2013-2025 (2013) Available at: http://www.hse.ie/eng/services/publications/corporate/hieng.pdf.

7 Marmot MG, Kogevinas M, Elston MA. Social/economic status and disease. Annual review of public health; 8(1), 111-135 (1987).

8 Bungay H. Cancer and health policy: the postcode lottery of care. Social Policy & Administration; 39(1), 35-48 (2005).

9 Delamothe T. NHS at 60: Universality, equity, and quality of care. British Medical Journal; 336(7656), 1278 (2008).

10 Williams A. QALYs and ethics: a health economist's perspective. Social Science & Medicine 43(12), 1795-1804 (1996).

11 La Puma J, Lawlor EF. Quality-adjusted life-years: ethical implications for physicians and policy makers. Journal of the American Medical Association; 263(21), 2917-2921 (1990).

12 Schwappach DL. Resource allocation, social values and the QALY: a review of the debate and empirical evidence. Health Expectations; 5(3), 210-222 (2002).

13 Cullen P. Varadkar promises to reform discretionary medical card. The Irish Times (2014)
Available at: http://www.irishtimes.com/news/health/varadkar-promises-to-reform-discretionary-medical-cards-1.1951657.

14 Ganiats TG, Kaplan RM. Priority setting—the Oregon example. Fixing health budgets: experience from Europe and North America. Sussex, England: Wiley and Sons, 21-47 (1996).

15 Bodenheimer T. The Oregon health plan: lessons for the nation. N Engl J Med 337(10), 720-3 (1997).

16 Reilly P. Submission for Medical Card Eligibility: Special Interest Group in Primary Care. Irish Association of Social Workers (2014) Available at: https://www.iasw.ie/attachments/36810ecc-901d-466b-ab40-9c58aa093b60.PDF.

Neasa Fitzpatrick

Isabelle Hunt

Eabha Manley

Michael McCrohan

Amy Nash

Michael Ryan

Andrew Stokes

Conor Toale