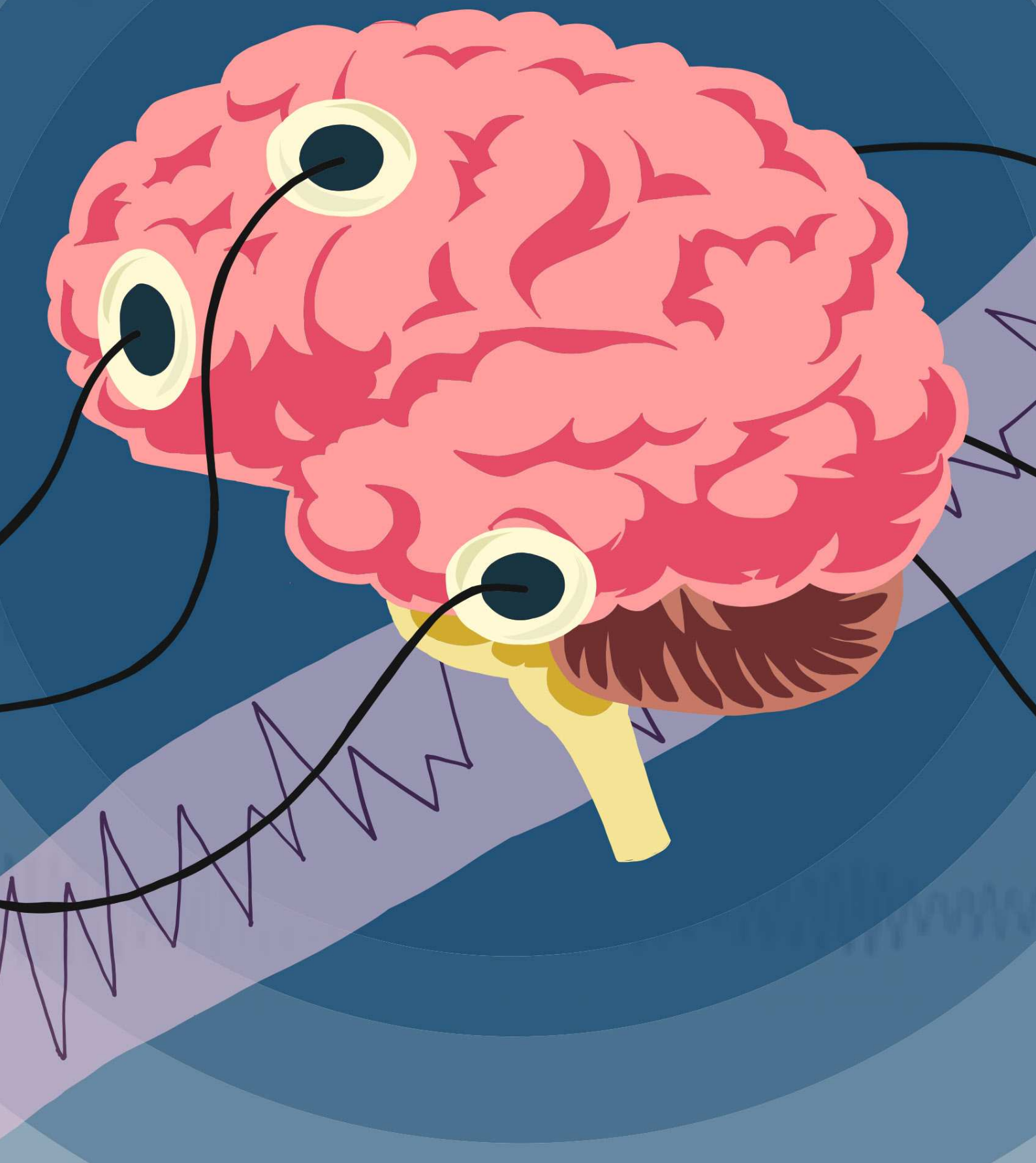
JSAMR
Journal of the National Student Association of Medical Research
Volume 3 Issue 1
30th April 2020



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2^{nd} year medical student, University of Oxford

Hadassah completed a degree in Neuroscience and is now in second year of graduate entry medicine. She's passionate about mental health and designed an app to help people who self harm (self-heal). All year round you can spot her swimming in rivers and lakes around Oxfordshire.

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Maria is an active member of various national and local committees, including the National DermSoc committee and is a British Journal of Dermatology Student editor. She has been involved with Birmingham Widening Access to Medical Sciences society and is a NICE Student Champion for evidence-based research ambassador.

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Gabriela di Scenza

4^{th} year medical student, St Georges University of London

Gabriela originally obtained a BA in English and History at the University of Southampton. Gabriela has been involved in many areas of student life at St George's including acting as an Educational Representative for her year group. She aspires to make opportunities in research more accessible to students and demonstrate its relevance to everyone.

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Amaal Masqsood-Shah

5^{th} year medical student, University of Leeds

Amaal has an interest in General Practice, ethics and research. She is currently undertaking an intercalated MA in Biomedical and Healthcare Ethics. Amaal is Vice President for the Student Clinical Ethics Forum at the Leeds School of Medicine, organising the annual ethics conference, monthly case-based discussions, workshops, student led presentations and the ethics debate team.

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3^{rd} year medical student, King's College London

Rhea works as a Money Mentor at King's, providing students with advice and support with regards to their finances. Before starting university, she worked as a healthcare assistant during her gap year. Rhea has a passion for teaching and tutors UCAT and BMAT. In her spare time she enjoys reading and public speaking.

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Arunima Batra

3^{rd} year medical student, King's College London

Arunima has a range of roles in student societies including Vice President of King's Medtech, leading the organisation of major events on innovative topics such as artificial intelligence in radiology. She is passionate about teaching and in the future hopes to get involved in strategies to improve medical education and enhance clinical learning environments.

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Thomas Franchi

4^{th} year medical student, The University of Sheffield

Thomas has strong interests in both research and surgery, and aims to combine these in his future practice as an academically active surgeon. He is currently undertaking an intercalated MSc Human Anatomy with Education and hopes to apply for the Academic Foundation Programme before pursuing a PhD. He is currently President of the Sheffield Academic Medicine Society, and having experienced the difficulties students face when trying to publish their work, Thomas is a great supporter of student-only journals.

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Reviewers

Journal of the National Student Association of Medical Research
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Issue Summary: Volume 3, Issue 1

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Not peer-reviewed

Available online: 30th April 2020

A welcome from our Deputy Editor-in-Chief, A, Banerjee:

We are delighted to present our third issue of JSAMR. It has been an incredible journey so far and we are amazed at how quickly interest from authors and readers have grown! After an incredible amount of submissions, we have put together this latest issue! Our front cover was designed by Oishee Ghosh, Year 12 student. Oishee is a passionate student, who wishes to pursue a career in medicine. Her design for the front cover is inspired by one of the articles in this journal on 'Brain Oscillations. The image artistically captures the use of electroencephalography in measuring brain activity – a diagnostic tool that has revolutionised neurological sciences and medicine.

In this issue's Editorial, our Editor-in-Chief Anna Harvey discusses the ongoing covid-19 crisis and the need for everyone, particularly those who work in healthcare, to be alert to misinformation and how to tackle it.

We then move to present a variety of research articles. The first by K. Patel is an interesting piece exploring 'Brain Oscillations'. We then change direction with a clinical research paper by P. Hurley looking into whether angiotensin converting enzyme inhibitors are suitable for paediatric patients. Following this, we move to immunology and molecular biology with Kanbar et al exploring the use of murine fluorescent timer technology to study cell signalling.

Next, we present a fascinating review article exploring research into 'intermediate syndrome' – a perhaps less well known subject, but one with important consequences medical professionals should know! We then have two interesting audits. Whilst Price explores 'venous cannulation', Ali asks a simple but interesting question applicable to modern medicine, 'Who is your consultant?' Having moved from the simple times where the family or village doctor would care for all, medicine has expanded to such a vast multidisciplinary effort, many patients and teams often have multiple consultants leading their care! Therefore, this question is very relevant to medicine today.

In our education section, our Education Editors R. Lopes and C. Charalambides write a very useful guide to critical appraisal. This is a key skill that all those in medicine and science will require in their career, especially as greater academic involvement is encouraged for students and medical professionals alike. This is followed by a letter to the editor by Okoli and Akinwuntan on the very relatable subject of 'test anxiety'. Leading from this, A. Banerjee provides some insights into emotional intelligence through an opinion piece, which may help our readers to address any anxieties.

We then have an interesting reflection by J. Brown titled 'An Irish Packet Arriving' – must read to find out more! Finally, we finish with our poster presentations. We commence with Franchi et al.'s work on Selenium, followed by Bhalla et al's audit looking at adherance to prescribing pathway for overactive bladder treatment in general practice. Lastly, the team of JSAMR present our conference poster sharing our journey of creating, developing and running a student-run peer-reviewed journal.

We are very grateful to all our readers, editors, authors and peer-reviewers who have joined us in our journey and continued to help JSAMR grow! We hope you continue to be a part of the team and if you are new to the journal we hope our work encourages you to learn something new and take part as well!

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Author statements

Conflicts of interest statement

Anamika Banerjee is the current Deputy Editor-in-Chief of the Journal of the National Student Association of Medical Research. She has previously held role as Education Editor 2018-19.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html. All authors have read and approved the final version, and accept responsibility for information published in this article.

Ethics statement

Authors declare that no ethical approval was required for this article.

Editorial and peer review statement

This article was submitted as an editorial and it did not pass through the peer review process.

Open access and distribution statement

Journal of the National Student Association of Medical Research
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Posters

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Covid-19 and the Importance of a Robust Science Communication Workforce

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Not peer-reviewed

Available online: 30th April 2020

Keywords: science communication

It is an odd time to be writing a note to the readers of a journal run in its entirety by medical students. Most of our worlds have ground to a halt, or, for finalists, accelerated as the NHS battens down its hatches to weather a storm unlike any other. There is much still to learn about the increasingly mysterious disease cause by SARS-CoV-2. What's clear is that the healthcare world those who are currently studying medicine will graduate into is likely to be very different to the one that existed before.

To me, the covid-19 crisis has in many ways highlighted the great value of science communication as a field; be that new papers published in traditional or open access journals or preprint servers; science journalism in specialist publications or national newspapers; and even the new media of Tweetorials, Facebook group posts and public Zoom webinars. In a world where, at the click of a mouse or the tap of a finger, a piece of information can reach millions or billions of people, it is all the more important that the scientific process, and the way we communicate the outcomes of scientific enquiry, is highly scrutinised. It is pertinent, then, that in this issue of JSAMR, our Education team provides a comprehensive guide to critical appraisal of scientific papers.

In my field of interest, medical journalism, scrutiny is important too. The way medical information is presented and communicated to the public may in some ways have more impact than the content of a paper in a niche journal. Whilst the Lancet may have published (and subsequently retracted) Andrew Wakefield's infamous paper linking the MMR vaccination and autism, would we be faced with a spate of outbreaks of mumps in adolescents who never received an MMR vaccine as a child had publications like The Daily Mail had not picked up the paper, and presented its findings as truth to their readers?

The ability and opportunity to practice the various skills involved in different areas of science communication is no doubt invaluable to medical students, who, as doctors, will be required not only to publish papers, but to counsel patients (and perhaps even family and friends) who may receive much of their medical information from lay publications, be that in print or online. Combating misinformation is something anyone can, and should, be doing from behind their laptop or phone screen.

Author statements

Conflicts of interest statement

Anna Harvey is the Editor-in-Chief of JSAMR. She works in a full time paid capacity for the British Medical Journal as Editorial Scholar. She is a member of the editorial advisory panel of the GKT Gazette. She is a Medics.Academy Fellow and sits on the National Student Widening Participation Working Group and is President of the British Undergraduate Society of Obstetrics and Gynaecology.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html. All authors have read and approved the final version, and accept responsibility for information published in this article.

Ethics statement

Authors declare that no ethical approval was required for this article.

Editorial and peer review statement

This article was submitted as an editorial and it did not pass through the peer review process.

Open access and distribution statement

JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Brain Oscillations in Stroke Rehabilitation: What can they tell us about Impairment, Recovery and Response to Training?

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Received: 27th January 2020
Revised: 24th February 2020
Accepted: 8th March 2020

Keywords: stroke rehabilitation EEG

Abstract

Introduction: Stroke is the third leading cause of disability in the world with 80 percent of stroke survivors suffering from some degree of motor impairment. Yet the degree of motor impairment and subsequent recovery varies largely between individuals. Whilst neurorehabilitation programmes are available, not all individuals benefit from them equally. Hence this literature review aims to summarise the current literature around the use of EEG in predicting the degree of motor impairment, recovery and response to training in stroke patients.
Method: A non-systematic literature search of PubMed was conducted to identify articles reporting changes in brain oscillations in stroke patients.
Results: Here we discuss how changes in different parameters of brain oscillations, indices of different types of waves as well as EEG patterns are associated with different degrees of motor functions both in acute and chronic stages of stroke. The review demonstrates how recovery of motor function depends on re-establishment of symmetry between the two hemispheres and increased shift of activity from the unaffected to affected hemisphere and back to normal levels.
Conclusions: Recognition of such EEG patterns has furthered our understanding of causal relationship between pathophysiological processes and motor function, opening further opportunities to identify biomarkers which will allow us to predict the response of individual to training and tailor the therapeutic intervention in a personalised way to maximise motor recovery after stroke.

1 Introduction

Stroke is the second leading cause of death in the world (WHO, 2016). Despite increasing availability and effectiveness of acute treatment, 80 percent of stroke survivors are still left with some degree of motor impairment and disability (Langhorne, Coupar, & Pollock,). According to the World Health Organisation (WHO), this makes stroke the third leading cause of disability worldwide (WHO, 2016). Neurorehabilitation programs aim to reduce the motor impairment and improve long-term functional outcome mainly through training (Kitago & Marshall,). However, motor recovery appears to be a multifactorial process as reflected by the significant differences in motor improvement seen in patients with similar clinical profiles (Kitago & Marshall,).

Whilst re-absorption of cerebral oedema and growth of collateral blood vessels leads to some recovery of motor function in short-term, complete recovery of motor function is highly variable between individuals due to the range of physiological repair processes which underlie restoration of function (Rossini, Calautti, Pauri, & Baron,). These recovery processes encompasses a range of changes in neuronal circuit

such as: recruitment of functionally homologous pathways, activation of previously inhibited neurones, increased cortical representation, axonal sprouting around the injured tissues and the formation of new synapse and reorganisation of neuronal networks (Kitago & Marshall,). These vast number of complex changes in neuronal circuits leading to recovery are collectively referred to as the process of 'brain plasticity' (Rossini et al.,).

One of the most important mechanism is the increase in strength of synapses and hence an improvement in synaptic efficacy in the remaining neurones by a process called long-term potentiation (Rossini et al.,). Most of the patients regain function partially or completely through these mechanisms in the first 3 months after stroke (Di Pino et al.,) because this time marks the "sensitive period" for neuronal plasticity (Kitago & Marshall,). This is the period of hyperexcitability of cortical neurones characterised by increased in glutamate-mediated excitation and decreased GABA mediated inhibition (due to downregulation of GABA-A receptors) creating the optimal environment for these neuronal reorganisation (Di Pino et al.,). It is therefore believed that it is essential to take advantage of this natural "sensitive period" in order to maximise the activity-dependent plasticity and functional recovery.

EEG waves record the sum of post-synaptic potentials of a number of neurones located at the surface of the cortex (J. E. Hall,). The waves have a range of frequency (0.5-500 Hz) (Rabiller, He, Nishijima, Wong, & Liu,) and intensities (0-200 mv) (J. E. Hall,). The intensity or amplitude of the wave depends on the number of neurones firing synchronously. EEG oscillations have been divided into 5 main frequency groups which are observed in different parts of cortex during different activities: (1-4Hz), (4-8 Hz), (8-12 Hz), (12-30Hz) and (30-80 Hz) (Rabiller et al.,).

This review discusses some of the recent advances on the use of EEG to predict impairment, recovery and response of individuals to training. EEG allows us to monitor changes in the activity of brain overtime in a non-invasive way and allows us to follow these recovery processes more closely during rehabilitation.

2 Aims

The aim of this review was to synthesize the current literature regarding changes in brain oscillations observed following stroke as well as changes seen during recovery from motor impairment in stroke patients.

A non-systematic literature search of PubMed was conducted to identify articles reporting changes in brain oscillations in stroke patients.

3 Results

3.1 Interhemispheric Changes in Brain Oscillations

Following stroke, there is interhemispheric imbalance of activity. There is a reduction in the function of the affected hemisphere due to loss of neurones. There is also shift of activity from the primary motor cortex of affected hemisphere to contralesional primary and ipsilesional as well as contralesional secondary motor cortices (Rossini et al.,). These changes are thought to be acute recovery mechanisms to compensate for the loss of function (Rossini et al.,). Interhemispheric inhibition (IHI) is the inhibition of the activity of one hemisphere by the contralateral hemisphere (Di Pino et al.,). IHI measurements are taken through protocols involving TMS (Transmagnetic Stimulation) (Di Pino et al.,).

Additionally, there is greater interhemispheric inhibition (IHI) from unaffected hemisphere to affected hemisphere (Yang, Sau, Lai, Cichon, & Li,). Normally, when healthy individuals try to move a hand, there is a reduction in the interhemispheric inhibition from ipsilateral to contralateral hemisphere during preparation, allowing activation of contralateral hemisphere neurones. However, in stroke patients, this decrease in interhemispheric inhibition from unaffected (ipsilateral) to affected (contralateral) hemisphere does not occur for movement of affected hand which makes initiation of movement in stroke patients difficult (Murase, Duque, Mazzocchio, & Cohen,). This explains the negative correlation between the degree of IHI from unaffected to affected hemisphere and motor function (Murase et al.,).

Three models of recovery have been developed around this idea of interhemispheric inhibition: Vicariation model, Interhemispheric Competition Model and Bimodal balance-recovery model. Vicariation model suggests that recovery occurs through increased activity of the neurones in unaffected hemisphere (Di Pino et al.,). Interhemispheric Competition Model suggests that decreased activity of affected hemisphere leads to decreased inhibition of unaffected hemisphere which then causes increased inhibition of affected hemisphere and hence poor motor function. Since the evidence for these models are contradictory (Di Pino et al.,), bimodal balance model was proposed.

Bimodal balance-recovery model suggests that the dominance of the previous two models depends on the structural reserve which is defined as the amount of functionally remaining neurones. If there is increased damage, it could mean greater dependence on unaffected hemisphere for function as even removal of inhibition on affected hemisphere will not yield enough recovery. If there is increased functional reserve, interhemispheric model dominates as removal of inhibition on these neurones could be enough to regain functionality (Di Pino et al.,). This model is supported by by a meta-analysis (McDonnell & Stinear,) which suggests that in severe stroke, increased excitation of affected hemisphere using TMS is of little benefit as vicariation model dominates and reliance on unaffected hemisphere is greater.

However not many studies can be found which have used

EEG to test these models. Hence, using EEG to determine the level of interhemispheric imbalance could allow stratifying patients depending on the level of injury, and hence deciding which therapeutic intervention will provide maximum recovery in the individual.

Individuals with good recovery after rehabilitation show a shift of dominance of activity from unaffected to affected hemisphere (Hummel & Gerloff,) (Tangwiriyasakul, Verhagen, Rutten, & van Putten,). This interhemispheric symmetry predicts recovery. This observation is exemplified by studies comparing beta-waves in the two hemispheres. A study which compared beta-ERD of the two hemispheres using laterality index (Shiner, Tang, Johnson, & McNulty,), showed that higher laterality index, which signifies increased contribution of affected hemisphere, were associated with better motor function. Studies which have compared activity of both hemisphere using indices such as BSI (Brain Symmetry Index) have shown that higher BSI scores, which means greater asymmetry of waves between hemispheres, is associated with worse motor function (Agius Anastasi, Falzon, Camilleri, Vella, & Muscat,) (Van Putten & Tavy,). Yet another study also suggested that recovery involves restoration of symmetry between hemispheres (Rossiter, Boudrias, & Ward,).

3.2 Intrahemispheric Changes in Brain Oscillations

Following acute stroke, there is an increase in the amplitude of lower frequency waves (delta waves) (Iyer,2017) and a decrease in the amplitude of higher frequency waves (alpha and beta waves)(Iyer,2017) in ipsilesional sensorimotor cortex and contralesional parietal cortex (Wu et al.,). These increase and decrease in delta and beta waves respectively have been positively correlated with motor impairment (Wu et al.,). Furthermore higher delta wave amplitude in ipsilesional hemisphere was associated with lower transferrin levels (Assenza et al.,). Since transferrin has been cited in the literature as a scavenger to counteract mediators of oxidative stress following reperfusion after stroke, lower level explains the greater degree of infarct and impairment seen in these individuals (Assenza et al.,). Hence, the identification of these waves not only allows us to quantify the degree of impairment before rehabilitation but also allows us to understand the processes which lead to impairment.

Additionally, the relative power of these high and low frequency waves have been used to calculate Quantitative EEG Indices. These indices have also been shown to be significant predictors of outcomes after stroke. The most significant index reported by many studies is the ratio of amplitude of Delta:Alpha waves, known as Delta:Alpha Ratio (DAR) , where high DAR (S. P. Finnigan, Walsh, Rose, Chalk, 2007; Leon-Carrion, Martin-Rodriguez, Damas-Lopez, Barroso y Martin, Dominguez-Morales, 2009; Trujillo et al., 2017) has been associated with lower recovery following training.

Use of other Indices such as Power Ratio Index (PRI), ratio of amplitude of Delta+Theta:Alpha+Beta waves, have shown contradictory conclusions. Some studies report negative correlation between PRI and recovery (Trujillo et al.,) whilst others find this correlation to be non-significant (S. P. Finnigan, Walsh, Rose, & Chalk,). These inconsistencies between studies despite similar number of participants, 10 in (Trujillo et al.,) and 13 in (S. P. Finnigan et al.,), could be due to fact that the outcome measure of recovery were different in both studies. The study which demonstrated negative correlation used FMA (Fugl-Meyer Assessment) (Trujillo et al.,), which is more specific to stroke recovery, whereas the other study which did not find statistically significant different used the generic NIHSS (National Institute of Health Stroke Scale) score (S. P. Finnigan et al.,). However, considering the sample size of both studies were quite small, the use of these indices is yet debatable and studies with larger sample size are needed.

3.3 Event-Related Potentials in Beta Waves

Event-related potentials (ERP) are changes in the amplitude of cortical EEG waves during different stages of movement (Pfurtscheller & Lopes,):

- Firstly, Event-Related Desynchronization (ERD) occurs which is characterised by a decrease in amplitude of beta waves during preparation and planning of movement. ERD occurs over the contralateral hemisphere due to an increase in desynchronised firing of neurones (Pfurtscheller & Neuper,), hence suggestive of an activated cortex (Neuper, Wörtz, & Pfurtscheller,);
- Secondly, ERD spreads to the ipsilateral hemisphere and therefore is present in both hemispheres during execution of movement (Neuper et al.,);
- Thirdly, Event-Related Synchronisation (ERS) occurs which is characterised by an increase in the amplitude of beta waves (Neuper et al.,). This increase in amplitude indicates reestablishment of synchronous neuronal firing, hence suggestive of deactivated cortex (Neuper et al.,).

Event Related Desynchronization (ERD) in Beta Waves is a very useful parameter to determine motor function because ERD is smaller in the affected hemisphere of stroke patients compared to control (Rossiter et al.,). This means that following stroke, there is decreased attenuation or modulation of beta wave activity (Rossiter et al.,). Furthermore, smaller ERD was associated with greater motor impairment within the stroke group (Rossiter et al.,).

Since oscillations arise due to feedback circuits between excitatory and inhibitory neurones (Rabiller et al.,), decreased ability to modulate these oscillations can be attributed to imbalances in these two processes. This idea is supported by the evidence that stroke patients with reduced ERD have decreased GABA-mediated inhibition (Muthukumaraswamy et al.,). Studies have not only shown reduced GABA levels in primary motor cortex of stroke patients compared to control (Blicher et al.,), but also shown that these decreased GABA levels are associated with decreased ERD. In particular, ERD seems to be mediated by GABA-A receptors since ERD is increased on administration of GABA-A receptor agonist, diazepam, to healthy individuals (S. D. Hall et al.,).

Even though low GABA levels is thought to decrease ERD detrimentally in acute phase, follow-up studies suggest that this low GABA levels may be beneficial in terms of rehabilitation. A study by Blicher et al. (2015) showed that changes in GABA levels over rehabilitation was negatively correlated with motor improvement, where greater improvement was seen in individuals with smallest increase in GABA levels after training (Blicher et al.,). Since long-term potentiation occurs in the presence of increased glutamergic excitation and removal of GABAergic inhibition (Rossini et al.,), the reduced GABA mediated inhibition in stroke could explain the increased plasticity (Rossini et al.,) seen in stroke patients in acute phase. This explains why individuals with reduced GABA levels over the course of rehabilitation had better recovery (Blicher et al.,). Additionally, lower GABA levels have been shown to increase use-dependent plasticity which leads to greater representation of the trained limb in the motor cortex (Paik & Yang,).

Event-Related Synchronisation (ERS) in Beta Waves (Adam, Isabella, & Chan,) is another parameter recorded in the first second following termination of movement (Ramos-Murguialday & Birbaumer,). Studies have reported that lower -ERS is observed in unaffected hemisphere on tactile stimulation in stroke patients following acute stroke (Laaksonen et al.,). However, subsequent measurements at 1 and 3 months showed that -ERS increased overtime with recovery (Laaksonen et al.,). Based on evidence from previous studies, it has been proposed that this lower beta-ERS in the acute phase suggests increased cortical excitability of motor cortex which gradually decreases overtime with recovery (Laaksonen et al.,). These findings of tactile stimulation are of interest in terms of rehabilitation because studies have shown positive correlation between -ERS and motor function, where -ERS have been shown to be influenced by integrity of sensory proprioceptive afferents (Laaksonen et al.,), (Shiner et al.,). This highlights the role of afferent signals from hands in determining the functionality of motor cortex.

Putting all these observations together, it can be interpreted that during motor impairment following stroke, there is reduced sensory afferent signals to the brain which causes disinhibition of the motor cortex. This results in increased cortical excitability that can be observed as reduced beta-ERS. Increased excitability in acute phase leads to poor control of movement which leads to impaired motor function (Laaksonen et al.,). Furthermore, increase in -beta-ERS is correlated with improved motor function highlighting that role that sensory afferent signals plays in changing the cortical excitability is important for motor recovery following stroke (Laaksonen et al.,).

3.4 Limitations of EEG

EEG is a great tool in understanding the pathophysiology of stroke as it is widely available, relatively inexpensive and non-invasive (S. Finnigan & van Putten,). However, as the potentials generated by individual neurones are very small and the current has to pass through layers of the scalp, it is more difficult to detect low energy waves. Furthermore, human factors which are difficult to control such as blinking, movement and muscle activity such as respiration can create artefacts even in otherwise still individuals (Rabiller et al.,). In addition, the wakefulness of the patient at the time of measurement could also affect the recordings as the delta wave measurements may be affected by sleep/drowsiness of the patient which may be due to medications (S. Finnigan & van Putten,). Another limitation of EEG is the inability to detect the activity of deeper structures, which may be of great value in future research. Use of such technique is limited in human studies due to the invasive nature of the technique (Rabiller et al.,).

3.5 Clinical Implications and Future Development

EEG has a huge potential for developing predictive models of arm recovery which can then not only be used to determine the prognosis for an individual in acute phase, but also used to track the progress of recovery for the individual and tailor rehabilitation to maximise their recovery. Whilst some predictive models have been developed, these do not explain recovery in all patient groups (Kwah & Herbert,). As suggested by the bimodal balance recovery model, the recovery depends on the structural reserve. Hence being able to stratify patient groups depending on pattern and degree of injury and then developing recovery models for individual groups could allow us to track recovery more realistically on individual basis, particularly for individuals with more severe impairment.

4 Conclusions

Distinct features and patterns of EEG waves have been seen in stroke patients with good and poor recovery in both acute and chronic stages. Recovery of function has been most in individuals with greatest restoration of symmetry and baseline activity. EEG can provide an avenue for development of biomarkers to determine efficacy of therapeutic interventions on individual basis and provide a personalised rehabilitation program.

Even though many studies have demonstrated the use of EEG to predict impairment and recovery after stroke, the evidence of using these oscillations to predict response after training is weak. Few studies have been reported where EEG markers of patients have been recorded before and after rehabilitation program. Hence this is an area which requires further research and would allow us to administer therapeutic interventions more effectively based on individual predicted outcomes, which could be our step towards bringing personalised medicine to the field of stroke rehabilitation.

Author statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Are Angiotensin Converting Enzyme Inhibitors an Appropriate Therapy in Paediatric Patients with Single Ventricle Physiology?

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Received: 25th January 2020
Revised: 28th February 2020
Accepted: 8th March 2020

Keywords: ACEIs | paediatric | single ventricle physiology

Abstract

Introduction: The term single ventricle defect (SVD) is used to define a set of congenital cardiac malformations characterised by the failure of correct development of a lower chamber of the heart. Despite the prognosis for these patients being significantly improved by the Fontan procedure, these patients still commonly develop heart failure. Despite being recommended in adult heart failure, the use of angiotensin converting enzyme inhibitors (ACEIs) in paediatric single ventricle physiology is 6 not evidence-based.
Current Best Evidence: The only randomised controlled trial (RCT) on this topic found that patients prescribed ACEIs do not exhibit the expected benefits, such as improved weight-to-age z-score and Bayley score of infant development, and are therefore not recommended.
Self-Reported ACEI use in Single Ventricle Paediatric Patients: Two recent largescale surveys displayed that a significant proportion of the health care community were either not aware of the evidence or had chosen purposefully not to alter their practice Future research: The next major study to look into this issue will be phase II of the PANORAMA-HF study which will compare entresto (sacubitril/valsartan) with enalapril over a 52 week period.
Discussion: The current best evidence is not without flaws and as such it's validity has been called into question by physicians.
Conclusion: Further dissemination of the current research and addition of superior trials is required before ACEI efficacy in this patient group can be truly ascertained.

1 Introduction

The term single ventricle defect (SVD) is used to define a set of congenital cardiac malformations characterised by the failure of correct development of a lower chamber of the heart. While only accounting for slightly greater than 1 % of all congenital cardiovascular defects(Steinberger, Ferencz, & Loffredo,), SVDs are disproportionately associated with morbidity and mortality (Hsu et al.,). There are numerous types of SVD, each with a distinct anatomical anomaly. The types of SVD include:

- Tricuspid atresia;
- Double outlet right ventricle;
- Hypoplastic left heart syndrome;
- Pulmonary atresia with intact ventricular septum;
- Mitral valve atresia;
- Single left ventricle;
- Ebstein's anomaly;
- Double inlet left ventricle;
- Atrioventricular canal defect.

Survival rates for individuals born with SVDs has improved dramatically over the past 41 five decades (Kempny,

Dimopoulos, & Gatzoulis,). This shift in survival can be attributed initially to the work of Francois Fontan in 1971 and his development of the surgical procedure to produce a 'Fontan circulation'(Fontan & Baudet,). In simplest terms, the Fontan circulation (shown in figure 1) allows for the redirection of systemic venous blood to the pulmonary arteries either directly or via the right atrium, meaning the deoxygenated blood does not pass through a subpulmonary ventricle (Kempny et al.,). Unfortunately, due to the nature of the Fontan circulation, patients exhibit a reduced cardiac output and progressive dysfunction of the systemic ventricle (Lambert et al.,). This eventually leads to heart failure. As well as this, patients having undergone the Fontan procedure are at an increased risk of arrhythmia, thromboembolism, protein losing enteropathy, plastic bronchitis and death (Iyengar et al.,).

Due to these long-term complications, medical management is required to maximise life expectancy of these patients. Whether or not this management should include the use of Angiotensin Converting Enzyme inhibitors (ACEIs) has long been a matter of contention (Wilson, Iyengar, & d'Udekem,). ACEIs function by inhibiting angiotensin converting enzyme from cleaving angiotensin I to angiotensin II (CV Pharmacology | Angiotensin Converting Enzyme (ACE) Inhibitors, n.d.). Angiotensin II has multiple roles within the body, including, but not limited to, systemic vasoconstriction and sympathetic nervous stimulation (Fyhrquist, Metsärinne, & Tikkanen,). Alongside other medications, ACEIs are currently recommended by NICE for the long term treatment of adult heart failure with reduced ejection fraction (Heart failure—Chronic—NICE CKS, n.d.). ACEIs have also been reported to reduce cardiac remodelling (Ferrario,) ,the term given to progressive pathological change at a cellular and interstitial level within the myocardium resulting in altered geometry and function (Azevedo, Polegato, Minicucci, Paiva, & Zornoff,). This process can take place in untreated chronic heart failure, thus resulting in a gradual worsening of cardiac dysfunction. It therefore stands to reason that ACEIs would be a beneficial pharmacological therapy in single ventricle physiology (both physiological and Fontan) in which heart failure is a common outcome. However, the only large scale RCT to look into this clinical scenario showed that ACEIs offer no observable benefit, and as such should not be recommended (Hsu et al.,). Despite this evidence, surveys of healthcare professionals in Europe and the US have shown the persistent use of ACEIs in single ventricle physiology in significant numbers. This essay will address the current evidence for the use of ACEIs in single ventricle physiology and issues in clinical practice regarding their use.

1.1 Self-reported ACEI use in single ventricle paediatric patients

In order to investigate the current trends in pharmacotherapeutic management of paediatric heart failure, Díez et al. (2019) conducted a survey of physicians in Europe providing paediatric cardiology services between January to May 2015. This survey took into account both congenital heart defects and dilated cardiomyopathies as causes of paediatric heart failure. Of a potential 200 participants, 100 doctors representing 27 countries took part. As a whole the cohort were well experienced, with 96 % having had 5 years or more experience in this field. Díez et al. (2019) reported the most surprising result being that 87 % of those surveyed would recommend the use of ACEIs in paediatric patients with single ventricle physiology. This is surprising as the current best evidence provided by Hsu et al. (2010) reported no benefit in the use of ACEIs in single ventricle paediatric patients. This implies that within the 87 % some may be unaware of the evidence (published in 2010) and as such rely in part on the common understanding of ACEIs use in adult heart failure, and some may simply disagree with the results of the findings. A similar 2017 survey performed by Zak et al. (2017) with the US Paediatric Heart Network focused on ACEI use in paediatric single ventricle patients solely. This trial was targeted at health professionals involved in paediatric cardiology care (69 % being doctors). Respondents were similarly well experienced to their European counterparts with 65 % having greater than 10 years' experience. This survey showed that 22 % of the 629 respondents were not aware of the findings of Hsu et al. (2010). Of the 78 % that were aware of the study, 28 % had purposefully chosen not to alter their practice. Therefore, when combined with the 22 % who were unaware of the evidence, potentially up to 43.8 % of total respondents had not altered their practice, despite having evidence to the contrary. The reasons provided for non-conformance included concerns about the small number of patients involved, differing interpretation of the results, as well as disagreement with trial design and end-point choice. Despite the large percentage of healthcare professionals who had not changed their practice, Zak et al. (2017) did note significant decrease ($p<0.001$) in reported use of ACEIs as preventative medication amongst participants familiar with Hsu et al. (2010).

1.2 Current best evidence

Hsu et al. conducted the largest study to date to investigate the use of ACEIs in single ventricle patients (Hsu et al., 2010). This randomised double-blind study was performed with the US Paediatric Heart Network and divided 230 infants into a placebo arm and atreatment arm. The treatment consisted of enalapril initially prescribed at 0.1mg/kg/day and titrated up to 0.4mg/kg/day over two weeks. For inclusion, patients were required to be 45 days of age and >1 week if born at 35 weeks gestation, they were followed to 14 months of age, this allowed for assessment of patients at least 6 months following from superior cavopulmonary connection (SCPC) surgery (the first of two operations in the Fontan procedure). At the end of the study, patients were assessed using:

- Weight-for-age z-score (main outcome measurement);
- Serum brain natriuretic peptide level;
- Height-for-age z-score;
- Ross heart failure class;
- Bayley score of infant development;
- Ventricular ejection fraction.

Baseline characteristics were comparable between the two arms of the study except for the gestational age which

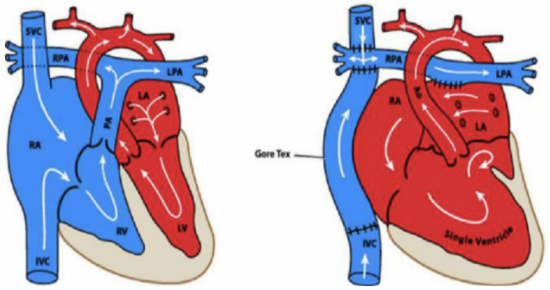

Figure 1: The diagram on the left shows normal circulation through the heart, while the diagram on the right shows a 'Fontan circulation' (James et al., 2016).

was significantly lower in the placebo group (median age of 38 weeks in comparison to 39 weeks P=0.01). 185 infants completed the study (20 % drop out rate), there was no significant difference in withdrawal/death rates between study arms. Primary analysis was performed according to the intention-to-treat principle. A secondary non-intention-to-treat analysis was also performed due to a high rate of discontinuation of the study drug (53/185 participants-29 %). This involved comparison of patients who had undergone at least 10 consecutive months of placebo/no ACEI therapy with those who had undergone at least 10 consecutive months of study drug and/or open-label enalapril. In both the intention-to-treat and non-intention-to-treat analysis there was no significant difference between the enalapril and placebo group in either primary or secondary outcomes.

As expected, the ventricular mass-to-volume ratio and z-score were significantly higher than reference normal values at both pre- and post-SCPC checks. Interestingly, despite the mean ventricular mass-to-volume ratio and z-score being significantly lower in the enalapril arm than the placebo arm before the SCPC surgery (P=0.02), at the post-SCPC check this significant difference was no longer present (P=0.34). There was no significant difference between the trial arms in terms of serious adverse events. As well as these analyses, the authors also conducted sub-group analysis to assess whether any cohort of patients within the study had demonstrated a significant improvement in an outcome measurement whilst receiving enalapril. They were unable to identify any subgroup which showed this.

Despite being an excellent therapeutic option in adult heart failure and demonstrating preservation of ventricular function in children with volume overload conditions (Calabrò, Pisacane, Pacileo, & Russo,)(Mori, Nakazawa, Tomimatsu, & Momma,), ACEIs failed to show any significant benefit over placebo in this study. The authors suggest the growth suppression noted in single ventricle physiology may be due to factors that are non-modifiable by ACEIs, such as gastrointestinal reflux. Interestingly, Shaw et al. (1988), Scammell et al. (1987), and Frenneaux et al. (1989) all report improvements in growth rate in paediatric heart failure following treatment with ACEIs. This lends support to the suggestion by Hsu et al. (2010) that retardation of growth rate in paediatric patients with single ventricle physiology may be due to a non-cardiac cause.

1.3 Future research

The next large scale study to address the issue of ACEI use in single ventricle paediatric patients will be phase 2 of the PANORAMA-HF study (Shaddy et al.,). This global multi-centre study will be divided into two parts. Phase 1 will assess the pharmacology of entresto (sacubitril/valsartan), a new category of drug termed an angiotensin receptor NEP inhibitor (ARNI), in paediatric patients with reduced systemic left-ventricular function. Entresto has already been shown to be superior to enalapril in adult heart failure in terms of relative risk of cardiovascular death, relative risk of all-cause mortality, risk of heart failure related hospitalisation and clinical progression of heart failure in the PARADIGM-HF study (McMurray et al.,). Phase 2 will compare the efficacy of entresto with enalapril over a period of 52 weeks in 360 patients. Outcome measurements will include adverse events such as death, progression onto mechanical life support and listing for urgent cardiac transplant. As well as this, the study will look at clinical markers of health in heart failure such as measures of functional capacity (i.e. New York Heart Association classification and Ross scores)and patient reported experience.

2 Discussion

In a 2007 online poll conducted by the British Medical Journal, evidence-based medicine (EBM) ranked seventh of the 15 most important milestones to shape modern medicine (Thoma & Eaves,). EBM has been defined as 'the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients' by Sackett et al. (1996). In the proceeding 23 years since this definition, EBM has continued to gather momentum as a component of responsible clinical practice. However, EBM is far from ubiquitous and as noted by Greenhalgh et al. (2014), a criticism of its use is that it may supersede clinical reasoning earned through years of experience. As identified by Zak et al. (2017), a notable number of health care providers (22 % of respondents) were aware of the findings of Hsu et al. (2010) but had chosen not to alter their practice. This raises a central issue in EBM as to the correct course of

action if one disagrees with the methodology or conclusion of the best evidence available. This is relevant as Hsu et al. (2010) is not without drawbacks. As well as a significant dropout rate, which the authors did attempt to account for with rigorous statistical analysis, there were multiple issues with the study methodology. These were addressed in part by Singh (2011) who highlighted that ventricular function was normal in patients at baseline and that ventricular dysfunction is rarely noted in the first year of life. However, Singh (2011) also raises the point of challenges faced by clinical researchers in organising large scale paediatric heart failure studies. As a means to circumvent this difficulty, surrogate endpoints are suggested as an alternative. These outcome measures function as substitute indicators of disease progression and as therapeutic responders in place of morbid end-events (Cohn, 2004).

3 Conclusions

In summary, the current evidence for use of ACEIs in single ventricle paediatric patients requires greater dissemination and the addition of higher quality studies. This will enable physicians to be confident in making evidence-based decisions on pharmacotherapeutic management, which will in turn provide better outcomes for these vulnerable patients.

Author statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at `http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html`. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Murine Fluorescent Timer Technology can be used to study the Impact of PD1 Ligation on Intracellular T Cell Receptor Signalling

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[*]Source of Support: Sir Arthur Thomson Trust Student Vacationship, Wellcome Trust

Received: 27th January 2020
Revised: 24th February 2020
Accepted: 8th March 2020

Keywords: murine | flourescent | T Cell

Abstract

Introduction: PD1 is an inhibitory protein which interacts with its ligands to restrict T cell function. This interaction may prevent immune clearance of cancers and is a target for cancer immunotherapy. Using Nr4a3-Tocky technology, which tracks T cell receptor (TCR) signalling, we investigated PD1 expression and regulation of T cell activation.

Method: Splenocytes from OTI Nr4a3-Tocky mice were used as a source of T cells. These cells use a fluorescent Timer protein to trace TCR signalling over time. Splenocytes were stimulated with ova peptides of varying affinity and incubated to investigate PD1 expression over time. Murine PDL1-Fc-IgG was used to ligate PD1. PD1 and T cell activation markers were measured by flow cytometry.

Results: In activated T cells, PD1 expression was correlated closely with T cell activation. Increasing the incubation time, stimulant peptide dose and affinity increased the percentage PD1 expression, and the PD1 mean fluorescence intensity. When ligated by PDL1, the proportion of immature TCR-induced Timer protein was reduced, suggesting attenuation in TCR signalling.

Conclusions: Nr4a3-Tocky proved to be effective for investigating the interaction between PD1 and PDL1 in TCR signalling. The close coupling of PD1 expression to T cell activation suggests PD1 functions as a T cell activation marker. Ligation by PDL1 attenuates TCR signalling. Future work will investigate whether these findings are supported in murine tumour models, to further understand the role of PD1 and PDL1 in cancer development and the impact of immunotherapy, and to understand the role of PD1 on other T cell effector functions.

1 Introduction

Programmed cell death protein 1 (PD1) is a cell-surface ligand expressed on several types of immune cells, particularly T cells (Ishida, Agata, Shibahara, & Honjo, 1992). By ligating PD1 with one of two ligands, PDL1 or PDL2, it is possible to reduce the functional response of T cells to antigens presented on major histocompatibility complex (MHC) molecules by antigen-presenting cells (Freeman et al., 2000; Latchman et al., 2001).

Ligation of PD1 triggers an inhibitory signal through activation of SHP1 and SHP2 phosphatases (see Figure 1). These phosphatases de-phosphorylate and inhibit signalling proteins of TCR signalling pathways, namely CK2, ZAP70, PKC8 and PI3K, and stimulate BATF (Kanehisa Laboratories, 2019). This results in negative regulation of the effector functions of T cells, thus regulating peripheral immune tolerance by controlling immune recognition of presented antigens (Nishimura, Nose, Hiai, Minato, & Honjo, 1999).

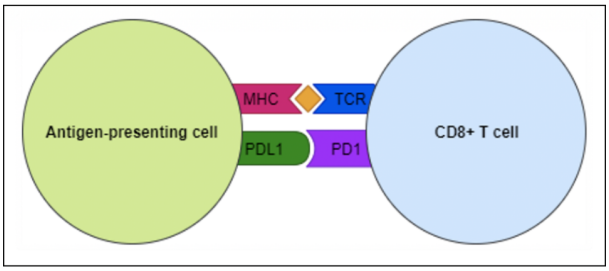

Figure 1 – PD1 and PDL1 interaction in vivo An inhibitory signal from PD1 ligation when an antigen is presented to a T cell results in suppression of T cell growth and survival (Arasanz et al., 2017; Freeman et al., 2000).

The immunomodulatory function of PD1 ligation is heavily implicated in cancer biology. It is well known that in cancers such as melanoma, non-small cell lung cancer, and some Epstein-Barr virus associated cancers, PDL1 is upregulated by cancer tissue compared to surrounding normal tissue. It is thought that upregulating PDL1 allows tumour cells to suppress the function of T cells, thus enabling immune evasion and allowing the tumour to continue to grow (Iwai et al., 2002).

The biological significance of PD1 ligation has resulted in the development of several monoclonal antibody-based therapies to target both PD1 and PDL1. In the United Kingdom, nivolumab and pembrolizumab, which are monoclonal antibodies targeting PD1, are currently licensed to treat several cancer types (British National Formulary, 2019c, 2019d). However, patients can also access atezolizumab and durvalumab, which target PDL1, via the Cancer Drugs Fund (see Table 1) (British National Formulary, 2019a, 2019b). Based on evidence from key randomized controlled trials demonstrating the efficacy of these therapies for improving overall survival in several cancers, PD1 and PDL1 immunotherapy are now widely utilised in clinical practice (Ascierto et al., 2019; Horn et al., 2017; Rittmeyer et al., 2017).

Drug name	Target protein	Indications
Nivolumab	PD1	Melanoma, non-small cell lung cancer, urothelial carcinoma, squamous cell cancer of the head and neck, classical Hodgkin lymphoma
Pembrolizumab	PD1	
Atezolizumab	PDL1	Urothelial carcinoma, non-small and small cell lung cancer, breast cancer
Durvalumab	PDL1	Non-small cell lung cancer

Table 1 – PD1 and PDL1 immunotherapies used in the UK Table details each licensed immunotherapy, their target proteins and their indications (British National Formulary, 2019a, 2019b, 2019c, 2019d).

However, there are several unanswered questions surrounding the use of PD1 and PDL1 immunotherapy, including the determinants of efficacy and the causes of side effects experienced by many patients. Given the highly specific molecular basis of this therapy, it is likely that the answers to these questions can be uncovered by investigating the precise impact of PD1 ligation and blockade on T cell functionality.

Transcriptomic technology and immunophenotyping are well established as robust methods for assessing the functionality of cells, as often the viability and functionality of cells correlates with their protein expression. Although there have been advances in these fields to study changes in protein expression by individual cells at a single point in time, there has been relatively little progress in studying changes in cell properties over a continued period. As the activity of cells under study is heavily influenced by the timing of sampling and analysis, to truly understand the sustained impact of courses of PD1 ligation and blockade on the human immune system, it is vital to utilise methods of studying cells that allow tracking of changes in signalling dynamics over time.

Timer of cell kinetics and activity, commonly known as Tocky, is a murine fluorescent protein technology developed by Bending et al, which can be used to resolve some of the limitations of transcriptomic and immunophenotyping technologies (Bending et al., 2018). In this system, ligating and activating a T cell via a T cell receptor (TCR) initiates fluorescent Timer protein transcription from the TCR signalling activated Nr4a3 transgene. This Timer protein matures over time, with an initial blue form, which has a half-life of four hours, before spontaneous maturation to a terminal stable red form. The system can thus capture T cell signalling dynamics in the time frames of hours compared to days with previous methods (see Figure 2) (Bending et al., 2018). As a highly faithful and sensitive system to signalling events occurring in vivo, this technology is a powerful tool for answering many questions about factors affecting T cell activation and signalling over time.

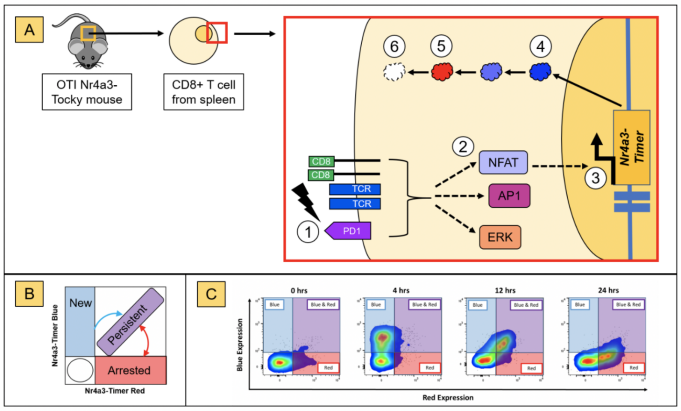

Figure 2 – Tocky system A) A schematic of the initiation of transcription of Timer protein and the maturation of protein in vitro: 1 – TCR signal, 2) Initiation of NFAT pathway, 3) Initiation of Nr4a3 transgene via NFAT pathway (Jennings et al., 2020), 4) Formation of blue Timer protein, 5) Maturation of Timer protein, causing change of fluorescence to blue-red then red, 6) Decay of Timer protein; B) An example flow cytometry plot to show how the maturation of FlowJo can be plotted to illustrate different stages of T lymphocyte activation and Timer protein production; C) Examples of real FlowJo plots showing the stages of maturation of Timer protein in vitro.

Integrating this novel technology with current limitations of understanding of PD1 ligation and blockade, we sought

to use the murine OT1-Tocky model to investigate PD1 expression and the impact of ligation by PDL1 on CD8+ T lymphocyte signalling dynamics over time. It is hoped that the subtext of these findings serves as proof of the potential of the Tocky system to be used in future clinical research surrounding understanding PD1 and PDL1 immunotherapy.

2 Materials and Methods

2.1 Mice

The breeding and properties of OTI Great Smart-17A (Price, Reinhardt, Liang, & Locksley, 2012) Nr4a3-Tocky mice has been described previously (Bending et al., 2018; Jennings et al., 2020). OTI mice are TCR transgenic for ovalbumin peptide, while Nr4a3 Great Smart-17A report IFNγ through yellow fluorescent protein (YFP) and IL-17A through human nerve growth factor receptor. Nr4a3-Tocky Great Smart 17A mice were initially bred to homozygous OT1 mice (Charles River Laboratories) to generate F1 OT1 Nr4a3-Tocky Great Smart 17A mice. In the OT1 Nr4a3-Tocky Great Smart 17A mice, the T cell repertoire is heavily skewed such that >95 % of T cells are specific for ova peptide. The OT1 Nr4a3-Tocky Great Smart 17A mice was the only mouse strain used because this is the only system with both a transgenic TCR to respond to ova peptide and the Nr4a3-Tocky system to report TCR signalling in vitro, as determined by staining for the specific OTI TCR V2 and V5.1/5.2 chains (Jennings et al., 2020). All animal experiments were performed in accordance with the local Animal Welfare and Ethical Review Body at the University of Birmingham, and under the authority of a Home Office project license (P18A892E0A).

2.2 Pre-coated plates

In experiments investigating the impact of PD1 ligation by PDL1, murine PDL1-Fc-IgG (BioLegend, UK) was used to pre-coat the plate. PDL1 was coated at the following concentrations: 2μg/ml, 10μg/ml, 50μg/ml, 200μg/ml. Plates were incubated with PDL1 for three hours before removing the PDL1 solution and plating.

2.3 In vitro cultures

Using a medium containing 45ml RPMI and penicillin-streptomycin (Life Technologies), and 5ml FBS (Sigma, UK), spleens from OTI Nr4a3-Tocky mice were forced through 70μm cell strainers (BD Biosciences) to obtain a splenocyte suspension. The initial suspension was centrifuged to obtain a pellet. Waste supernatant was removed, and the pellet was resuspended in medium. A red blood cell lysis solution (eBioscience) was added for one minute while the suspension was on ice. Medium was added to stop the red blood cell lysis reaction, the suspension was centrifuged again, and waste supernatant was removed. The pellet was resuspended in 10ml of medium for cell counting, then diluted or concentrated as appropriate to ensure wells would be seeded at 1 million cells per well. A 96 well U-bottom plate (Corning CoStar) was created for each time point to be investigated. 50μl of the cell suspension, 50μl of stimulant peptide or medium, and a further 100μl of medium was added to each well.

2.4 Stimulants

Three types of stimulant peptides were used, which had different affinities for the TCR receptor depending on the amino acid in the fourth position from the N-terminus. These were N4 (Cambridge Bioscience, UK), Q4 and V4 (GL Biochem Shanghai, China) (see Table 2). These peptides were tested at the following concentrations: 0.001μM, 0.01μM, 0.1μM, 1.0μM, 10.0μM.

Peptide	Sequence	Affinity for T lymphocyte receptor
N4	SIINFELK	High
Q4	SIIQFEKL	Intermediate
V4	SIIVFELK	Low

Table 2 – Stimulant peptides and associated amino acid sequences and affinities

2.5 Incubation of cells

For experiments solely investigating the impact of PD1 expression and T lymphocyte activation, the three plates were incubated at 37oC for 4, 16 and 28 hours respectively. Three time points were used in these experiments to explore the changes in PD1 expression and TCR signalling over an extended period. For experiments investigating the impact of PD1 ligation by PDL1, the seeded pre-coated plate was incubated at 37^{o}C for 24 hours. Only one time point was used for these experiments to limit experimental variables; 24 hours was deemed the most appropriate time point based on investigating the time taken for PD1 expression to occur in the PD1 expression experiments.

2.6 Flow cytometry analysis

Once incubated, the plates were centrifuged, and the supernatant was removed. Fluorescent antibodies and viability dye were made up into a solution with 2 % FBS PBS and used to stain the wells for 20 minutes at 4oC. A single stain panel was also included to enable compensation on analysis. Cells were stained with a viability dye (eFluor780, eBiosciences), CD8 BUV395 (clone S3-6.7, BD Biosciences), TCR V alpha 2 PerCPcy5.5 (clone B20.1, BioLegend), CD69 AF700 (clone HI.2F3, BioLegend, UK), PD1 APC (clone 29F.1A12, BioLegend, UK). 160ml 2 % FBS PBS was used to wash the cells after 20 minutes. The plates were again centrifuged, supernatant was removed, and the cells were resuspended in 150ml 2 % FBS PBS. The Fortessa flow cytometer (BD Biosciences) was run on a medium setting. Most experiments collected between 5,000 to 10,000 events.

2.7 Statistical analysis and data visualisation

Initial flow cytometry data were compensated, gated and analysed in FlowJo. Data from repeat experiments were analysed in GraphPad Prism 5 or 8. A two-way ANOVA with multiple

comparisons test was used to determine significant effects of PD1 ligation. Key measures analysed include percentage expression of PD1 and Timer protein and mean fluorescence intensity of PD1 and Timer protein. The mean fluorescence intensity is a measure of the average amount of fluorescence emitted from cells, which reflects the average expression of a marker by any one cell in the sample.

3 Results

Timer protein expression increases with time, increasing dose and increasing stimulant peptide affinity in CD8+ T lymphocytes

Analysing percentage expression revealed that Timer protein expression increases in OTI CD8+ T lymphocytes over time when stimulated with ova peptides. The affinity of the stimulant peptide was found to affect the probability of Timer protein expression. Cells stimulated with the low affinity peptide were consistently associated with lower Timer protein expression, particularly at the earliest time point and with lower doses of stimulant peptide. The impact of peptide affinity on Timer protein expression can be seen in Figure 3, in which cells stimulated with low affinity peptide have less blue-red protein (represented by the upper-left quadrants) compared to the intermediate and high affinity peptides at all doses across both time points.

Similarly, the dose of stimulant peptide affected the probability of Timer expression. Increasing the dose of all three stimulant peptides increased the percentage Timer expression observed at all three time points. This can be seen in Figure 3, in which increasing the dose of stimulant peptide increased the proportion of cells expressing blue protein (represented by the upper-left quadrants) at all doses across all peptides and time points. Furthermore, the greater proportion of cells not expressing Timer protein was associated with the lowest doses, and, in the case of the 0.001μM V4 dose, was analogous to the unstimulated sample. Cells stimulated with the lowest affinity peptide were particularly susceptible to this trend.

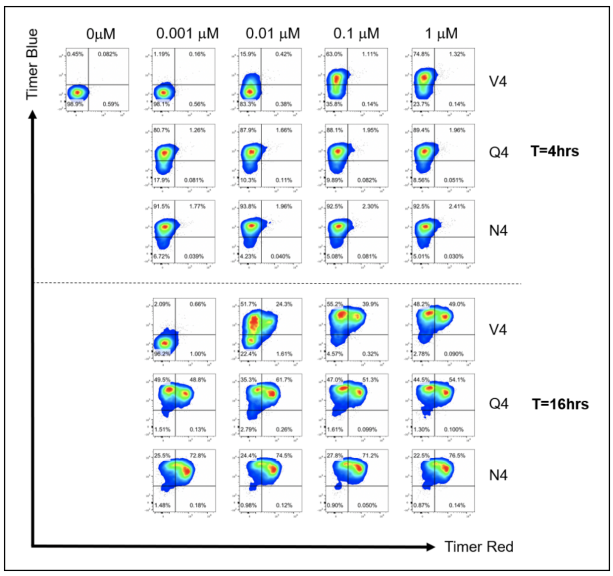

Figure 3 - Flow cytometry plots from one complete experiment The plots show Nr4a3-Blue versus Nr4a3-Red Timer expression at 4 and 16 in OTI T cells when stimulated with V4, Q4 and N4 peptides at 0.001μM, 0.01μM, 0.1μM, and 1.0μm doses. At the same time point, a consistently higher percentage Timer protein expression was observed with the higher dose. (n = 1).

The affinity of stimulant peptide was also found to affect the rate of Timer protein expression; intermediate and high affinity peptides were associated with a faster rate of Timer protein expression, compared to the low affinity peptide. This is best evidenced in Figure 4A. At the 4-hour time point, the low affinity peptide stimulated Timer protein expression between 20 – 70 % but had achieved nearly 100 % expression at the higher doses between 16 – 28 hours. By comparison, the intermediate and high affinity peptides induced Timer protein expression between 80 – 100 % at all three time points.

The dose of stimulant peptide also affected the rate of Timer protein expression. Higher doses of stimulant peptide consistently induced greater Timer protein expression at all time points; this is best visualised in Figure 4B. Comparing the 0.001μM dose to the 1.0μM dose, at all three time points, the percentage Timer expression was greater with the 1.0μM. To achieve this higher percentage of expression at the same time points, these data suggest that Timer protein expression is faster with the 1.0μM dose of stimulant peptide compared to the 0.001μM dose.

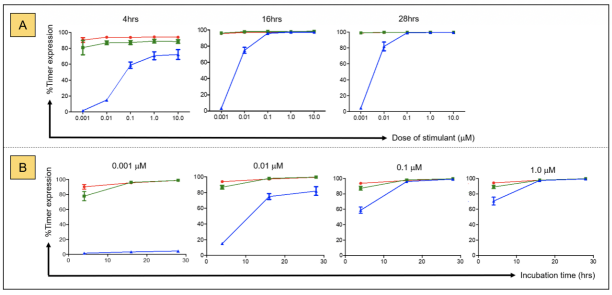

Figure 4 – Percentage expression of Timer protein by time and dose of stimulant A) Percentage expression of Timer protein at 4, 16 and 28 hours as a function of stimulant dose. Increasing Timer protein expression was associated with increasing dose and affinity of stimulant peptide; B) Percentage expression of Timer protein at 0.001μM, 0.01μM, 0.1μM, and 1.0μM doses as a function of incubation time. Key: Red = N4, Green = Q4, Blue = V4. (n = 2 for N4 at 4hrs, n = 3 for Q4 and V4 at 4hrs, n = 1 for N4, Q4 and V4 at 16hrs, n = 2 for N4 at 28hrs, n = 3 for Q4 and V4 at 28hrs).
Because Timer protein production only occurs as a result of TCR activation, the increase in percentage expression of Timer protein over time, with increasing dose and increasing affinity of stimulant peptide suggests that TCR activation is determined by these three factors.

3.1 PD1 percentage expression and median fluorescence intensity increases with time, dose and stimulant peptide affinity among Timer-positive CD8+ T lymphocytes

In the same cultures, percentage expression of PD1 was found to increase in a similar way to percentage expression of Timer protein. The affinity and dose of stimulant peptide were found to affect the percentage expression of PD1; this is best observed in Figure 5A. At all three time points, the low affinity peptide was associated with a lower percentage PD1 expression compared to the intermediate and high affinity peptides. This was particularly noticeable at the lowest doses, 0.001μM and 0.01μM. Furthermore, the affinity and dose of stimulant peptide were also found to affect the rate of PD1 expression, as seen in Figure 5B. Cells stimulated with the low affinity peptide tended to reach maximal PD1 expression at a much slower rate than the intermediate and high affinity peptide. This is evidenced by the fact that, at the 4-hour time point, the intermediate and high affinity peptides yielded between 70 – 90 % PD1 expression and subsequently 100 % expression at 16 hours. In comparison, at the 4-hour time point, the low affinity peptide yielded between 30 – 70 % percentage PD1 expression, and the lowest doses of this peptide never yielded maximal expression at the time points studied.

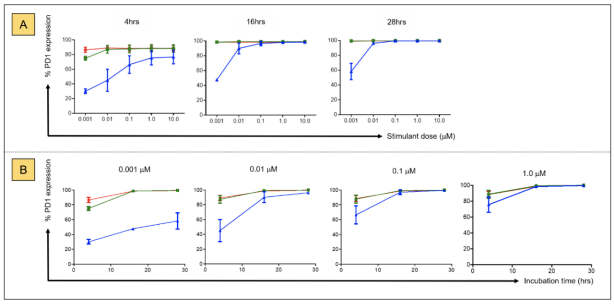

Figure 5 – Percentage PD1 expression by time and stimulant dose A) Percentage expression of PD1 at 4, 16 and 28 hours as a function of stimulant dose. Increasing PD1 expression was associated with increasing dose and affinity of stimulant peptide; B) Percentage expression of PD1 at 0.001μM, 0.01μM, 0.1μM, and 1.0μM doses as a function of incubation time. Key: Red = N4, Green = Q4, Blue = V4. (n = 2 for N4 at 4hrs, n = 3 for Q4 and V4 at 4hrs, n = 1 for N4, Q4 and V4 at 16hrs, n = 2 for N4 at 28hrs, n = 3 for Q4 and V4 at 28hrs).

The similarity in the rate and probability of activation to the findings observed with Timer protein expression suggests that TCR signalling drives PD1 expression, and that Nr4a3 and PD1 may both be similarly regulated by NFAT/ERK pathways (Jennings et al., 2020).

3.2 Ligation of PD1 with PDL1 decreases the ratio of fluorescent blue:red Timer protein

The ratio of blue to red Timer proteins is an important indicator of the dynamics of TCR signaling. A reduction in TCR signalling will cause a reduction in newly made blue protein, and thus a decrease in blue-to-red (blue:red) protein ratio. Ligating PD1 on cells stimulated with 0.1μM of stimulant peptide reduced the ratio of expression of blue:red Timer protein, as seen in Figure 6. This suggests that ligating PD1 attenuates the TCR signal, as measured by Nr4a3 activity. The degree of attenuation, as represented by the reduction in blue:red Timer protein ratio, correlated with increasing dose of PDL1. This suggests PD1 signalling can have an analogue effect on TCR signaling, as opposed to binary on/off control.

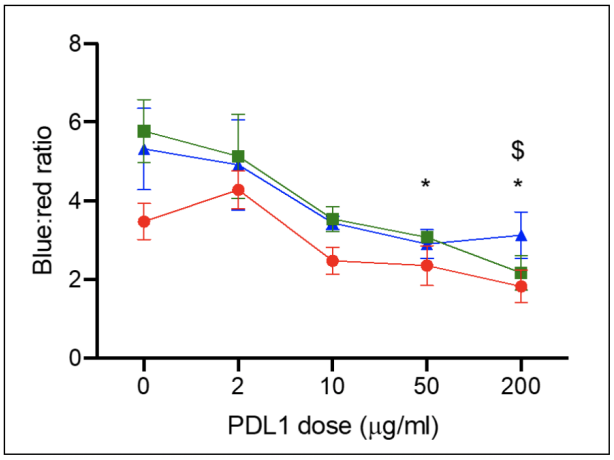

Figure 6 – Change in blue:red Timer protein expression ratio with increasing PDL1-Fc-IgG dose Higher doses of PDL1 were associated with lower blue:red ratios, suggesting a greater degree of attenuation. * = statistically significant ($p<0.05$) results with Q4 by two-way ANOVA compared to 0μg/ml PDL1 dose. * = statistically significant ($p<0.05$) results with Q4 by two-way ANOVA compared to 2g/ml PDL1 dose. Key: Red = N4, Green = Q4, Blue = V4. (n = 2 at 200g/ml PDL1, n = 3 at all other PDL1 doses).

4 Discussion

To our knowledge, this study is the first to investigate PD1 and PDL1-related signalling dynamics using the Tocky model. The results from experiments investigating PD1 expression suggest that PD1 expression is tightly coupled to Nr4a3 expression and distal TCR signalling. The affinity and dose of stimulant peptide were both found to impact the probability and rate of TCR activation. PD1 ligation was found to attenuate TCR signalling; the degree of attenuation correlated with the dose of PDL1. This latter finding is in line with previously observed clinical findings from histological and immunohistochemical examination of patient tumour biopsies, which attribute upregulation of PDL1 by tumour cells compared to surrounding normal tissue as essential to suppressing the function of tumour-infiltrating T lymphocytes effectively (Konishi et al., 2004).

Although the results presented in this study are preliminary, they serve as proof of the sensitivity of the Tocky system to PD1-mediated signalling in CD8+ T lymphocytes. Thus, Tocky has potential to be used in future immunological and clinical research to further our understanding of PD1 and PDL1 immunotherapy and its impact on the immune system. Given the current clinical interest in better stratifying patients to receive this immunotherapy, analysing the molecular impact of PD1 and PDL1 immunotherapeutic agents via this system may yield patterns of cell changes that correlate with clinical

effectiveness. This approach to combining molecular biology and clinical medicine has been applied among melanoma patients; Riaz et al recently used whole genome exome sequencing to identify changes in patient transcriptomes and tumour mutational burden among melanoma patients receiving nivolumab that were predictive of response to nivolumab (Riaz et al., 2017). The Tocky system may improve methodology of similar studies by increasing the capacity to study the dynamic impact of this therapy on cell transcriptome profile. Interestingly, a recent study has explored the effects of PD1 on the global transcriptome (Shimizu et al., 2020). Here the authors show that different genes have different sensitivities to PD1 signalling, and that PD1 may preferentially inhibit T cell acquisition of effector functions. Intriguingly the authors suggest that Nr4a3 shows only a modest sensitivity to PD1 signalling, which is in keeping with our findings that PD1:PDL1 interactions resulted in a gradual reduction in TCR signalling. Thus, one read out from Nr4a3 alone may not capture the full spectrum of the effects of PD1 immunotherapy, and a combination of read outs may be preferable for understanding its effect on effector functions, particularly in in vivo settings.

4.1 Limitations

Our work is not without its limitations. As these were largely pilot studies, a small sample size of mice was used to conduct these experiments; typically, splenocytes were derived from the spleen of a single mouse in each experiment. Although the Tocky system is known to report TCR signalling faithfully, more biological replicates should be analysed to increase power for statistical analyses.

Furthermore, the data presented is solely derived from analysis of CD8+ T cells. Although initial experiments using CD4+ T cells from the Nr4a3-Tocky lines yielded highly similar results to those obtained using CD8+ T lymphocytes, it remains to be confirmed how PD1 signalling regulates Nr4a3 expression, and therefore TCR signalling, in CD4+ T cells. For instance, Nr4a3 is a gene that is controlled heavily by the NFAT and ERK signalling pathways (Jennings et al., 2020). TCR signalling is channelled through many hubs, including AP1, and NFB transcription factors. Therefore, it is important to realise that in this study we largely assessed the effects of PD1 signalling on the NFAT and/or ERK pathways that mediate downstream of TCR signalling, which may not reflect the total impact of PD1-mediated signalling via other branches of the TCR signalling cascade.

Finally, all data was derived from analysis of splenocytes from murine models alone. Mouse models are well established in the scientific community for the study of human related conditions, due to the similarity in their anatomy and physiology, the presence of many orthologous genes, and the ease at which transgenic species can be bred. However, until investigated, it is not possible to determine the fidelity of this model to the human immune system, which affects the generalisability of these findings to human research.

4.2 Future work

Despite these limitations, the OTI Tocky model proved useful for investigating the fundamental impact of PD1-PDL1 interaction on TCR signalling. Future work will be focused on establishing the functional impact of PD1 expression and ligation on the cytotoxic function of CD8+ T lymphocytes, which were previously unclear from our experiments. However, Nr4a3 could be used as a promising read out for studying human T cell responses to immunotherapeutic agents, such as anti-PD1 or anti-PDL1.
Furthermore, experiments conducted to generate these data are to be repeated in murine tumour models to further our understanding of the in vivo relevance of these findings to tumour biology.

5 Conclusions

To conclude, this study has demonstrated that PD1 is expressed as a T lymphocyte activation marker in response to stimulation, and ligation by PDL1 modestly attenuates TCR-mediated signalling as measured by Nr4a3 activation. In doing so, the findings of this study prove the potential for Tocky models to be used to study PD1 and PDL1 interactions in mice.

Author statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at `http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html`. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Research into Intermediate Syndrome

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Received: 30th January 2020
Revised: 19th March 2020
Accepted: 19th March 2020

Keywords: toxicology poisons

Organophosphate (OP) poisoning is an important worldwide clinical problem. The World Health Organisation (WHO) identifies pesticide poisoning as a commonly used method to commit suicide globally (Ajdacic-Gross et al., 2008). The staggering number of intentional deaths caused by pesticides was calculated in a systematic review conducted by Gunnell (Gunnell, Eddleston, Phillips, & Konradsen, 2007). They estimated that there could be up to 400,000 pesticide related suicides globally each year. It is thought that Organophosphate (OP) pesticides – containing OPs and solvent co-formulants - are responsible for roughly 200,000 of these deaths. The number of fatalities caused by accidental exposure to OPs is far fewer, but still a problem in regions where highly toxic OP pesticides are used. OP compounds are also of global public health concern as they can and have been used as chemical weapons.

A key contributor to the ill health and death caused by OP poisoning is intermediate syndrome (IMS). IMS typically develops 24–96 hours after acute OP poisoning. The syndrome presents with weakness and paralysis of the proximal limb muscles, neck flexors, muscles supplied by the cranial nerves, and muscles of respiration. Paralysis of the respiratory muscles results in respiratory failure, which is the most common cause of death in patients with acute OP poisoning. At present, the only way to effectively manage IMS in the clinic is via mechanical ventilation. This places considerable demands on limited intensive care resources across Asia. A greater understanding of the pathological mechanisms underlying IMS can help identify individuals at risk of developing respiratory failure and help guide the development of an effective therapy. An effective therapy would reduce the mortality rate associated with OP poisoning and allow better allocation of sparse medical resources.

One possible mechanism by which IMS may occur is through the inhibition of acetylcholinesterase (AChE). OPs inhibit AChE by phosphorylating the serine hydroxyl group in its active site. This leads to accumulation of acetylcholine (ACh), and subsequent over-activation of nicotinic acetylcholine receptors (nAChRs) at the neuromuscular junction (NMJ). Over-stimulation of post-synaptic nAChRs leads to a depolarising block, where the voltage-gated Na+ channels needed to generate an action potential (AP) are inactivated. Generally, muscular fasciculations precede the paralysis associated with a depolarising block. In 2003, John studied 25 patients with acute OP poisoning (John, Oommen, & Zachariah, 2003). They found that fasciculations occurred in 70

Nonetheless, recovery from IMS takes 1 to 2 weeks, whilst AChE activity is restored within a couple of days via synthesis of new AChE enzymes and sometimes spontaneous dephosphorylation. These contrasting time courses suggest that other parts of the NMJ must be affected in IMS. There is clinical evidence to back this hypothesis. By analysing data from multiple randomised controlled trials (RCTs), Buckley (Buckley, Eddleston, Li, Bevan, & Robertson, 2011) found that oximes - AChE-reactivating drugs - offered little benefit to patients suffering from OP poisoning. This suggests that something other than AChE enzymes are affected in IMS. However, there is the possibility that the oximes were unable to reactivate AChE sufficiently; for example, if the oximes were given at too late a time in the RCTs, or if OPs were so abundant in the patients that they could re-inhibit any reactivated AChE enzymes.

Another possibility is that a secondary consequence of AChE inhibition is motor end-plate myopathy due to ACh accumulation at the NMJ. Fenichel (Fenichel, Dettbarn, & Newman, 1974) found that an excessive amount of ACh at the NMJ led to myopathy occurring. They showed that enhancing ACh release via daily injections of guanidine rapidly resulted in a severe myopathy. They also demonstrated that a prior nerve section that prevented ACh release could inhibit this myopathy. Furthermore, OP poisoning has been associated

with end-plate myopathy. Ariëns (AriËns, Meeter, Wolthuis, & van Benthem, 1969) found that a single intravenous infusion of an OP metabolite, paraoxon, caused necrosis at the motor end-plate region in rats. This all suggests that IMS may be due to end-plate myopathy. Hughes (Hughes, Knight, Brown, & Marrs, 1991) investigated the effects of sub-lethal doses of sarin (an OP) on the mouse diaphragm. They found that muscle fibre degeneration occurred in the vicinity of the end-plate by 24 hours, and that the fibres started to recover within 7 days. This time course fits with that of IMS, further lending support to the theory of OP induced motor end-plate myopathy.

Additionally, there is evidence that Ca2+ homeostasis dysregulation is responsible for motor end-plate myopathy in OP poisoning. Inns (Inns, Tuckwell, Bright, & Marrs, 1990) histochemically demonstrated that Ca2+ accumulated in muscle fibres following OP administration and accumulation was confined to the end-plate. Leonard and Saltpeter (Leonard & Salpeter, 1979) found that removing Ca2+ from a muscle incubation medium with EGTA (a Ca2+ chelating agent) could prevent myopathy. These lines of evidence suggest that myopathy occurs due to excess Ca2+ accumulation within the endplate. It could be that prolonged ACh-nAChR interaction causes Ca2+ levels to remain higher than at resting levels for a longer duration. Alternatively, OP pesticides and their metabolites could inhibit VGKCs and/or CAKCs, which delays repolarisation and the decline in the Ca2+ level.

Laskowski (Laskowskil & Dettbarn, 1977) noted that the susceptibility of different muscles to necrosis following OP administration varied, with the diaphragm being the most severely affected of the muscles studied. This could explain why respiratory failure is a common occurrence in patients with IMS. However, the study did not explore the rationale behind the different susceptibilities. Some possibilities for the increased susceptibility of a certain muscle are: it is used more, it contains isoforms of target proteins that are more vulnerable to OP action, it possesses a lower number of OP target proteins or it has target proteins with lower functionality.

Another possible mechanism that could explain IMS is that the OP inhibits AChE and a solvent co-formulant, used to improve the agricultural usability of OP pesticides, pathologically affects another part of the NMJ. Eddleston (Eddleston et al., 2012), examined the role of solvent co-formulants in OP poisoning in a minipig model. They found that dimethoate (the OP used in this experiment) and cyclohexanone (the major solvent) individually failed to cause the toxicity characteristic of pesticide poisoning. However, combining the two chemicals caused the same loss of NMJ function as that of an OP pesticide as measured by mechanomyography. These results indicate that solvents may have a critical role in OP poisoning. Future research in this field should examine how solvents and OPs interact to cause a worse phenotypic effect.

Other parts of the NMJ that may be affected include nAChRs. NAChRs are a potential candidate as congenital myasthenic syndrome (an inherited neuromuscular disorder) has been associated with mutations in the genes encoding the receptor (Shen, Brengman, Edvardson, Sine, & Engel, 2012). Furthermore, there are reports that the administration of rocuronium (a nAChR antagonist) just before or within 2 hours after simulated ingestion of an OP pesticide protects the function of the NMJ in pigs (Eddleston et al., 2012). This indicates that the nAChR is necessary for OP-induced pathology. Additionally, Witzemann (Witzemann et al., 1996) found that mice that possessed nAChRs containing a -subunit instead of an - subunit had impaired neuromuscular transmission, muscle weakness, and muscle atrophy. This suggests compounds that could drive this subunit switch might enhance myopathy. However, it is difficult to draw any definitive conclusions about the effects of the subunit switch from this experiment, as there were fewer nAChRs in the motor end-plate region of mutant mice compared to wild-type mice. Nevertheless, it is an interesting theory that merits further investigation. AChRs may also be affected by the Ca2+ homeostasis dysregulation described earlier. There is evidence that an increase in cytosolic Ca2+ level activates proteases, phospholipases and endonucleases that contribute to cell death (Orrenius, Zhivotovsky, & Nicotera, 2003). Ca2+-activated proteases can cleave the proteins involved in anchoring the nAChRs to the cytoskeleton, such as rapsyn. Untethered nAChRs can move away from the crests of the post-junctional folds to the troughs, where they can be endocytosed. A decrease in the number of nAChRs at the motor endplate may reduce the size of the EPP to an extent where it no longer reaches the threshold potential to trigger an AP. This could be another reason for continued muscle weakness following the restoration of AChE activity. In addition, the activation of phospholipase A2 - a Ca2+ and calmodulin-dependent enzyme – could enhance the breakdown of phopsholipids present in the sarcolemma. This would affect many proteins associated with the sarcolemma and could lead to synaptic instability.

In conclusion, little is known about the pathological mechanisms underlying NMJ dysfunction in IMS. Although there is evidence indicating that the pathogenicity of IMS goes beyond AChE inhibition, further research is required to validate the targets of the OPs, the solvents used in pesticides, and the metabolites of both types of compounds. If further research is not carried out, the only way to currently stem the number of OP-related deaths may be to ban the compounds.

Author statements

Conflicts of interest statement

The author declares no conflicts of interest.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at `http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html`. All authors have read and approved the final version, and accept responsibility for information published in this article.

Ethics statement

Authors declare that no ethical approval was required for this article.

Editorial and peer review statement

This article passed through the external peer review process.

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Challenge Of The Current Local Protocols For Peripheral Venous Cannulation In Secondary Care

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Received: 2nd February 2020
Accepted: 3rd November 2018

Keywords: peripheral venous cannulation | phlebitis

Abstract

Aim: To establish if present recommended guidelines for peripheral venous cannulas (PVCs) are being followed and if not, to assess whether this affects the complication rate with regard to developing phlebitis.

Standard: The current PVC standard guidelines for Sheffield teaching hospitals are as follows: Phlebitis score must be recorded at least once every 24hrs and should be recorded 8hrly if the cannula is in situ beyond 72hrs. If not used for 24hrs the cannula should be removed. Cannulas should be removed at 72 hours, unless reason stated in medical notes – 120 hour maximum. Cannula site to be observed for 72 hours after removal.(Mortimer, 2013)

Questions to be Answered:

- Are cannulas being correctly documented – insertion, monitoring and removal?
- Are cannulas being removed within 72-96hrs as recommended by local guidance?
- Is there an increase in complication rate in patients where protocol has not been followed?

1 Introduction

The National guidelines state that peripheral venous catheters (PVCs) should not be in situ for longer that 72-96 hours after routine insertion (Mortimer, 2013. Bates, 2017). Since the risk of infection was thought to increase with the length of time the cannula remains in situ (Dougherty and Lister, 2008), the guidelines were compiled to take this into account. In 2015, however, this guidance was challenged (Webster et al, 2015). It was argued that a cannula should remain in situ for as long as it was needed, until there are two or more clinical signs indicative of phlebitis or if it malfunctioned (Jackson, 1998). In 2019, Webster et al, carried out a review comparing clinically indicated and routine replacement of PVCs and found that there was no significant difference in risk of infection. Their advice following this study was for healthcare providers to consider changing their local policy.In addition to the length of time that a cannula should be in place, current guidelines also state that the date, time and reason for insertion and removal of the PVC should be documented within the patient's notes along with an assessment of the site and allocation of a phlebitis score (McCallum and Higgins 2012). The Royal College of Nursing (RCN, 2012) also recommend that PVC sites are checked at least on a daily basis.

2 Methods

A seven-day long audit was carried out in which patients in a 27-bed ward were assessed each morning. Cannulation sites were visibly checked for signs of phlebitis (scored according to the phlebitis score - Jackson, 2003) and insertion/removal

dates were recorded.

3 Results

Over the course of the audit, a total of 57 PVCs were assessed. Only 12% (n=7) PVC insertions and 0% (n=0) PVC removals were documented. Furthermore, of the cannula insertions not documented, 14% (n=7) failed to have a date on the cannulation site. In addition, 3.5% (n=2) were documented to be monitored on one occasion, 0% (n=0) monitored daily and 96.5% (n=55) not monitored at all over the course of the audit. 23% of cannulations were removed according to local guidelines, whilst 47% remained in situ longer than the recommended 72-96hrs and 30% of cases had undocumented insertion dates. Phlebitis scores increased (>0) in 11% of cases where the cannulation had been left in situ longer than the recommended 72-96hrs, whilst of those removed in accordance to the guidelines 15% had an increased phlebitis score (>0).

4 Discussion

It is clear from the data described that general insertion, monitoring and removal documentation according to standard protocol is poor. The lack of documentation with respect to insertion of a cannula renders current protocols regarding its removal impossible to follow. Similarly, lack of monitoring for signs of phlebitis makes it difficult to assess whether the cannula was replaced/removed in a timely manner.

5 Conclusion

Results from this audit are in support of theories that cannula duration (>72-96hrs) does not increase the risk of phlebitis. However, the audit sample size was small and may not be representative, but the findings do warrant further discussion with regard to the length of single site peripheral cannulation.

Author statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at `http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html`. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Who Is Your Consultant? An Audit Of Surgical Patients' Ability To Correctly Identify Their Named Consultant On An Upper Gastrointestinal Ward

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Received: 31st January 2020
Accepted: 1st February 2020

Keywords: francis inquiry report | recommendation 236 | accountability | informed patient | named consultant

Abstract

Introduction The Francis Inquiry report (2013) states that hospitals should review and reinstate identification of a named consultant and nurse (recommendation 236). This reiterates emphasis of the report on accountability and communication. The audit assessed the number of patients able to correctly name the consultant and nurse responsible for their care, to ensure that clinical practice is in line with the standards outlined in the Francis inquiry report.

Methods 45 patients were included in the baseline audit, through use of a questionnaire. Thereafter, Plan Do Study Act (PDSA) cycle 1 was introduced, educating junior doctors. They were responsible for verbally informing patients who their named consultant was, and ensuring accurate head-board information. A subsequent re-audit using the same questionnaire included 32 patients.

Results Only 44% of patients could correctly name their consultant at baseline, increasing to 56% after PDSA1. Of the emergency patients included, 0% could name their consultant at baseline, increasing to 25% after PDSA1. 60% of elective patients could name their consultant at baseline, increasing to 88% after PDSA1. 40% of headboards were correct at baseline, increasing to 56% after PDSA1. There was a strong correlation between correct headboard information and the ability to name the responsible consultant.

Conclusions Significant improvements were made with the introduction of PDSA1, but the hospital is still significantly underperforming in line with recommendation 236. PDSA2 will utilise a top-down management approach, with the consultant surgeons driving the change, to improve motivation amongst junior doctors. PDSA3 will introduce an 'admission card' given to all patients, stating their named consultant and nurse.

Author statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at `http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html`. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Anatomy of a Critical Appraisal

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Received: 25th April 2020
Accepted: 25th April 2020

Keywords: critical appraisal review

Abstract

Critically appraising a document is central to understanding its contribution to the field of research. It is a systematic approach used to identify the strengths and weaknesses of research, so the validity of the findings can be established. Some key steps in the critical appraisal process include evaluating the appropriateness of the chosen study design for the specific research question, assessing the rigour of the methodology and suitability of statistical methods for the data and subsequent interpretation, ensuring that the conclusions are indeed supported by the data and have not been extrapolated. Addressing any conflicts of interest is also important. Subsequently, the extent to which the study influences clinical practice and its overall importance should be established. Considering the significance of an evidence-based approach to research studies, we present a guide to aid in evaluating research papers so that their true contribution to the medical field can be assessed.

1 Introduction

A critical appraisal is a process of assessing the validity and relevance of a research article, to inform and potentially integrate the findings into clinical practice (Al-Jundi & Sakka, 2017). Therefore, a critical appraisal enables healthcare professionals to assess the quality of the evidence and choose treatment options accordingly. The importance of critical appraisal is highlighted by Andrew Wakefield's case series publication that suggested a link between the measles, mumps and rubella (MMR) vaccine and autism (Sathyanarayana Rao & Andrade, 2011). Despite the several shortcomings of the paper, it was published in a high-impact journal, the Lancet, which inevitably led to Wakefield et al. 's work being widely publicised. Although following a rigorous review of the evidence, the paper was later retracted lot of damage was caused as MMR vaccination rates began to drop (Sathyanarayana Rao & Andrade, 2011) and continue to be affected to date. This example highlights the harm that can be caused by inappropriate presentation and interpretation of evidence. Critical appraisal is one tool that may help mitigate the publication and utilisation of evidence that is poor or has been misinterpreted. The assessment of a research article includes an evaluation of the study design in accordance with the research question, the appropriateness of the methodology and statistical analysis, along with identification of any conflict of interest. This article provides a guide on how to conduct a critical appraisal.

2 Aims

We aim to discuss the importance of critical appraisal and provide a guide to facilitate the process of conducting and writing a critical appraisal. We also recommend external sources to supplement readers' learning.

3 Methods

A non-systematic literature search on PubMed and Google Scholar was conducted to identify articles relevant to conducting a critical appraisal.

4 Appraising the journal

The critical appraisal begins with the journal that the article is published in. It is important to ascertain the credibility of the journal, to determine whether the research is being presented by a reliable and trustworthy source.

4.1 Predatory Journals

Some publishers publish articles, made available through open access, upon receipt of evaluation and publication fees. These publications are known as predatory journals. In such instances, the peer review process can often be absent or minimal, since the motivation of these publishers is to make money rather than present valuable research (Bartholomew, 2014). Therefore, the material presented by these journals is unevaluated and needs to be carefully considered.

The inclusion of articles beyond the journal specialty, grammatical errors, lack of descriptions of the manuscript handling process, promises of rapid publication and use of a non-professional contact email address (Beall, 2015) are some of the factors that hint towards a potential predatory journal. The Beall's criteria for determining predatory open-access publishers (Beall, 2015) provides a guide to assist with the identification of predatory journals, to avoid submitting your own work and to carefully approach the existing published material. The criteria identifies documents published by the committee on publication ethics and describes some of the practices of a predatory journal (Beall, 2015). A detailed guide of the peer review process is also available through a previous article in this series, titled Anatomy of a Peer Review (Banerjee, Harvey, Rees, Jackson, & Byrne, 2019).

4.2 Impact factor

The impact factor measures the number of times a journal has been cited in a given year (*Measuring Your Impact: Impact Factor, Citation Analysis, and other Metrics: Journal Impact Factor (IF)*, 2016). The impact factor is calculated over 2 years, by dividing the number of citations by the number of published articles.

For example, to calculate the 2010 impact factor (IF) of a journal:

A = the number of times articles published in 2008 and 2009 were cited by indexed journals during 2010. B = the total number of citable items published in 2008 and 2009.

A/B = 2010 IF

IF has some limitations as a measure of a journal's credibility. The calculation of the IF is an average, therefore, it can be skewed by a few highly cited papers. A paper may be heavily cited because it presents compelling evidence or it consists of serious flaws such as a biased methodology, non-reproducible results, etc. Therefore, a higher IF may not necessarily equate to a higher quality of work. Review articles, editorials, letters, abstracts, etc., may also increase the IF.

5 Appraising the paper

5.1 Background

This section of the research article should have an appropriate summary of what is known about the topic of research. There should also be a clear explanation of the relevance of the current study, in relation to existing literature, to allow a clearer understanding of whether the research is original or adds anything of value to previous research. The similarities and differences in this research compared to existing studies should be clearly outlined.

5.2 Research Question

Research questions should be well thought out. A popular and suitable framework in scientific literature, especially clinical questions is PICO: population/ problem, intervention, comparison and outcome (Fig. 1).

P	Among newborns, what is the effect of
I	10 dB exposure to noise during gestation versus
C	10 dB incremental increase on
O	Postnatal hearing impairment.

Fig.1. Demonstrates an example of a research question designed using the PICO framework (Morgan, Whaley, Thayer, & Schünemann, 2018)

A well-written question does not have much value if the question being asked is not relevant or the clinical issue in question is not important. Patient and public involvement provides a valuable indicator to assess the quality and relevance of the research. This is because their active contribution in the discussions and decision-making, throughout all stages of the research process, often positively influences the quality and end-result of the research.

5.3 Hypothesis statement

A hypothesis is a useful statement as it gives a clear idea of what the study expects to find and whether this is justified. The hypothesis is based on theory and developed prior to conducting any form of practical research. A common example is the null hypothesis, which states that there is no statistically significant difference between the observed and expected results and any difference can be attributed to chance. For example, for a research question that investigates whether teens are better at maths than adults, the null hypothesis would state that age has no effect on mathematical ability (Helmenstine, 2019). The most widely used value to denote statistical significance is $p<0.05$. Therefore, the

null hypothesis is rejected if the p-value is <0.05 because the results are said to be statistically significant at this level. However, it is important to note that researchers may set statistical significance at different values, such as $p<0.05$, $p<0.01$, $p<0.001$, depending on the statistical calculations and data type.

5.4 Design

The hierarchy of evidence, illustrated by Fig. 2, forms the foundation of evidence-based practice and stratifies study types into ranks, based on the rigour of their research methods (*Evidence-Based Practice in Health: Hierarchy of Evidence*, 2019). The consensus is that the higher the ranking of the study, the more rigorous the methodology and the more likely it is to reduce the effects of bias on the results. Systematic reviews with or without meta-analysis are generally considered to provide the highest level of evidence. However, a large, well-conducted randomised controlled trial (RCT) may provide better evidence than a systematic review of smaller RCTs. Therefore, the position of studies in the hierarchy is not as rigid as traditionally thought but rather a fluid structure that is subject to change, depending on the context in which the research was conducted and evidence obtained.

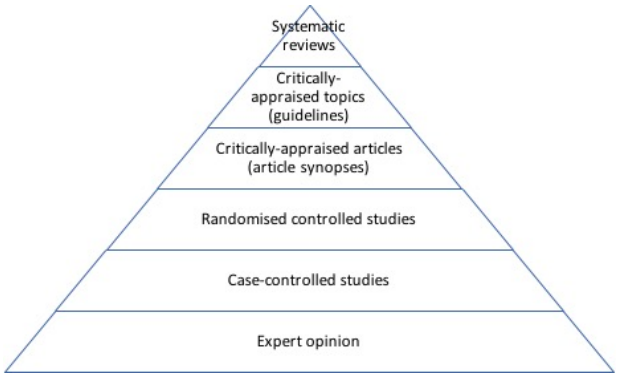

Fig. 2. Hierarchy of evidence ("Evidence-Based Practice in Health: Hierarchy of Evidence", 2019).

It is also important to determine whether the chosen study design is appropriate for the type of research question being investigated. Therefore, the research article should clearly justify their choice of study. For example, although a randomised control trial (RCT) provides a high quality of evidence, it may be ethically inappropriate to conduct a RCT in some situations. An example of such an instance is a study that tests novel pharmaceuticals in pregnant women with potential teratogenic side effects.

The information provided below outlines the different types of studies and questions to consider when assessing the robustness of the chosen study design.

A. SYSTEMATIC REVIEWS AND META-ANALYSES

This type of study follows a protocol to identify and critically appraise all the studies that are relevant to the chosen topic (Young & Solomon, 2009). A subsequent statistical analysis may be conducted to produce a single pooled result, giving rise to a meta-analysis. Tools like the Quality of Reporting of Meta-analyses (QUORUM) frameworks and Assessment of Multiple Systematic Reviews (AMSTAR) assessment tool/Critical Appraisal Skills Program (CASP) checklist can be used to assess the quality of the meta-analysis or systematic review that has already been conducted (Young & Solomon, 2009). Systematic reviews are at risk of bias from multiple sources. Therefore, a clear protocol should be developed and adhered to, in order to avoid evidence selection bias, resulting from a failure to identify all available data on the topic and publication bias that preferentially publishes data from statistically significant studies (Drucker, Fleming, & Chan, 2016). Additionally, any bias arising in primary studies included in the systematic review is also likely to affect the overall results and so these studies should be critically appraised individually.

The questions below highlight some of the important aspects to consider whilst appraising systematic reviews and meta-analyses (Young & Solomon, 2009):

- Does the study include all relevant articles?
- Was the data search and extraction conducted by two independent reviewers?
- Does the study provide an adequate level of detail about the included primary studies?
- Did the study assess the quality of the primary studies?
- Was the appropriateness of combining the results for a summary measure assessed?

B. RANDOMISED CONTROLLED TRIALS

This type of study accounts for confounding factors by randomly allocating the participants into the treatment and control group. Therefore, the outcomes of the study can be attributed to the intervention. RCTs also implement blinding such that the group allocation is concealed from one or more individuals involved in the research study (Karanicolas, Farrokhyar, & Bhandari, 2020). In the absence of blinding, participants' behaviours and response to the outcome measures may be affected, such that they might provide biased assessments of the effectiveness of the intervention (Karanicolas et al., 2020). Additionally, participants who are aware of receiving a placebo or the current best protocol may be less compliant with the trial protocol or seek additional treatment. Therefore, blinding aims to limit the bias in clinical trials.

The Consolidated Standards of Reporting Trials (CONSORT) statement flow chart fulfils a similar purpose as the QUORUM framework mentioned above. The questions below highlight some of the important aspects to consider, whilst appraising randomised controlled trials (Young & Solomon, 2009):

- Was there any bias in the selection of participants or the allocation of treatments?
- Was the treatment allocation truly random, e.g. use of a computer algorithm for allocation?
- Did the study implement blinding of participants (single-blind) or researchers (single-blind) or both (double-blind)?
- Was the assessment of the outcomes objective?
- Did the study implement an intention to treat analysis?

C. COHORT STUDIES

This type of study follows a group of individuals to observe who develops the outcome of interest, such as a disease (Young & Solomon, 2009). This can take the form of a prospective or retrospective study, as shown in Fig.3. For example, if a study aims to identify the risk factors for developing lung cancer, a group of smokers and non-smokers can be longitudinally followed in a prospective study. However, this type of study is not useful for studying rare diseases because a large study group would be required to find sufficient disease cases.

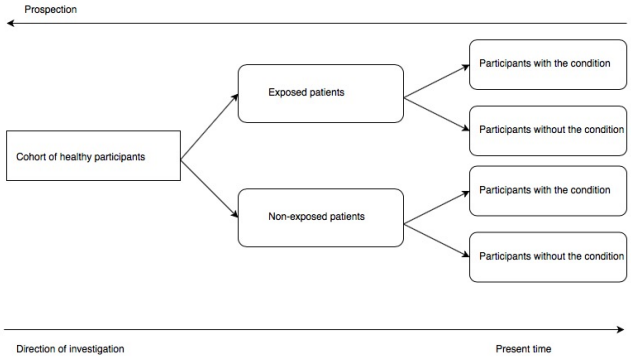

Fig. 3: Design of a retrospective cohort study (Suchmacher Geller, 2012).

The questions below highlight some of the important aspects to consider, whilst appraising cohort studies (Young Solomon, 2009):
- Is there selection bias?
- Is the cohort representative?
- Did the study identify all of the important confounding factors?
- Were all the participants appropriately followed up i.e. is there missing data?
- Were the participants followed up for an appropriate amount of time?

D. CASE-CONTROL STUDIES

These types of studies are always retrospective and participants are selected on the basis that they have already developed the outcome of interest (e.g. a disease) (Young Solomon, 2009). The cases are compared with controls that have not developed the outcome of interest to identify factors that differ and may have contributed towards the development of the outcome of interest. Case-controlled studies are ideal for studying risk factors for rare outcomes.

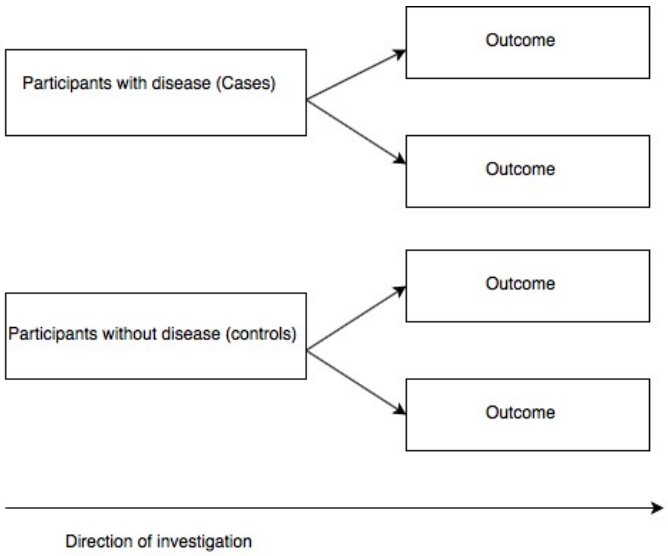

Fig. 4: Design of a case-controlled study (Levin, 2006).

The questions below highlight some of the important aspects to consider whilst appraising randomised case-control studies (Young Solomon, 2009):
- Were the cases clearly outlined and justified in terms of being representative of a defined population?
- How did the study select the controls?
- Were similar study measures used for cases and controls?
- Were the measures objective and subjective i.e. is there a likelihood of recall bias?

E. CROSS-SECTIONAL STUDIES

This type of study provides information about a population at a specific point in time, such as a one-off survey or audits of a practice (Young Solomon, 2009). For example, a cross-sectional study on bullying amongst students can be conducted by providing a questionnaire for students to compete. The questions (Young Solomon, 2009) below highlight some of the important aspects to consider whilst appraising randomised cross-sectional studies:
- Was the study sample clearly defined and representative?
- Were all relevant exposures, potential confounding factors and outcomes measured accurately?
- Were patients with a wide range of severity of the disease assessed?

F. CASE REPORT/ SERIES

Case series provide low-level evidence about the effectiveness of an intervention but are commonly used in medical literature (Young Solomon, 2009). The questions below highlight some of the important aspects to consider, whilst appraising case report/series (Young Solomon, 2009):
- Were cases identified prospectively or retrospectively and are they representative?
- Were all relevant exposures, potential confounding factors and outcomes measured accurately?

5.5 Study Methodology

A study's methods should be clearly outlined. The aim of the study, independent variable, dependent variable and controls should be clearly defined. Additionally, the study should also include information about the expected outcomes, including primary and secondary endpoints, and the methods used to measure these. It is important to assess whether the method was appropriate for the outcomes measured and whether an objective approach was adopted. Therefore, a critical appraisal requires the reviewer to look up different methodological techniques in similar research, to determine whether alternative methods may have been better or if the best approach was chosen. Additionally, determine whether the follow-up was complete, to assess the accuracy of the presented results. Furthermore, identify any potential biases, illustrated by Fig.5, that may influence the study results, such as selection bias, performance bias, exclusion bias and detection bias.

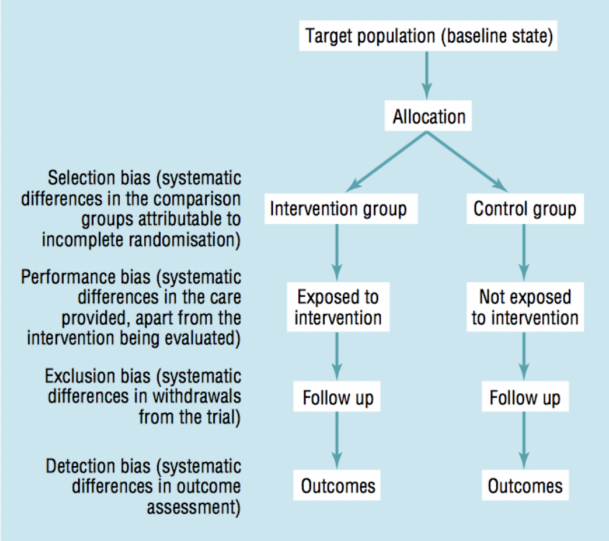

Fig. 5: Source of bias to check for in a RCT (Greenhalgh, 1997).

5.6 Statistics

The science of statistics deals with the collection, organisation and analysis of data and the use of samples to infer results across an entire population (Ali Bhaskar, 2016). Statistics are increasingly complicated and the statistical methods used vary according to the type of data collected and research conducted. It is common to not be able to fully understand the statistics, since a statistician is required to truly analyse the data. However, it is important to have an idea about the basic statistical methods used to conduct a research study.

Assess whether the study uses statistical tests to analyse the data and if so, the justification for the use of a specific test. For example, in a randomised control trial, a statistical test that is commonly used is an intention to treat (ITT) analysis or per-protocol analysis. The ITT analyses patients according to their originally assigned groups; for example, even if some patients in the non-treatment group, subsequently receive treatment, they are still analysed as being part of the non-treatment arm (McCoy, 2017). This allows for randomisation to be maintained so that the two treatment groups are still comparable at baseline. However, this form of analysis is no longer appropriate if a significant proportion of the participants are non-adherent, drop out or switch treatments. In this instance, per protocol analysis may be used. This statistical method analyses participants based on the treatment that has been administered (McCoy, 2017). However, this form of analysis increases the risk of selection bias and ideally shouldn't be used as a standalone primary analysis but rather as an adjunct to ITT analysis. Additionally, ascertain whether the authors have appropriately calculated and interpreted the p-value, confidence interval, relative risk reduction, absolute risk reduction, the number needed to treat and odds ratio (see glossary).

5.7 Results

The results should be complete and clearly presented, whilst accounting for any missing data. Assess whether the author has correctly interpreted the processed data and the quality of the presentation of the data, such as figures, graphs, etc. For studies that make a causal inference, use the criteria shown by Fig. 6 to assess the accuracy of this interpretation. Additionally, assess whether the study analyses the outliers from the obtained data.

Tests for causation[4]

- Is there evidence from true experiments in humans?
- Is the association strong?
- Is the association consistent from study to study?
- Is the temporal relation appropriate (did the postulated cause precede the postulated effect)?
- Is there a dose-response gradient (does more of the postulated effect follow more of the postulated cause)?
- Does the association make epidemiological sense?
- Does the association make biological sense?
- Is the association specific?
- Is the association analogous to a previously proved causal association?

Fig. 6: Outlines some of the criteria, originally developed by Sir Austin Bradford Hill that should be met before assuming causation (Greenhalgh, 1997).

5.8 Discussion

In this section, the results should be critically discussed and justify the conclusion. Assess whether the conclusion addresses and answers the original question. In addition, the clinical usefulness of the data should also be ascertained. A good research article would also set out the context, describing how the findings relate to existing literature. Finally, any conflicts of interest, such as a positional or financial influence should be clearly outlined.

5.9 Conflicts

A conflict of interest (COI) is defined as a situation in which professional judgements regarding a primary interest may be influenced by secondary interests, such as financial or personal

gain (Romain, 2015). For example, an individual involved in the study may have significant shares in a company that can be affected by the outcome of the research study. This can introduce a bias in the manner in which the research is conducted and presented, posing an issue for professional, patient and public trust in the research. The disclosure is considered to increase transparency and hence, enhance trust in the research. Disclosures of COI are managed by strategies that can be broadly categorised into three domains: regulation of the individual, design and regulation of the research process and critical assessment of the research product. Furthermore, journals such as the BMJ prohibit authors with a relevant COI from writing certain articles. The peer review process is also considered to confer some protection against COIs (Romain, 2015). Therefore, critical appraisals should identify and assess any COI and the strategies implemented to manage these.

6 Presenting the findings of the critical appraisal

Key points of critical appraisal (Young & Solomon, 2009).

a. Is the study question relevant?
b. Does the study add anything new?
c. What type of research question is being asked?
d. Was the study design appropriate for the research question?
e. Did the study methods address the most important potential sources of bias?
f. Was the study performed according to the original protocol?
g. Does the study test a stated hypothesis?
h. Were the statistical analyses performed correctly?
i. Do the data justify the conclusions?
j. Are there any conflicts of interest?

7 Glossary

- Absolute risk reduction: The absolute risk of events in the control group minus the absolute risk of events in the treatment group.
- Confidence interval: An estimated range of values we are fairly certain (expressed as a percentage) that the true value lies within.
- Number needed to treat: The number of patients that require treatment to prevent one additional adverse outcome.
- Odds ratio: The odds that an outcome will occur in the presence of a particular exposure, compared to odds that an outcome will occur in the absence of that exposure.
- P-value: Probability that the observed result or a more extreme result could be obtained if the null hypothesis is true.
- Relative risk reduction: Relative decrease in the risk of an adverse event in the exposed compared to the unexposed group.

Author statements

Conflicts of interest statement

Rhea Lopes is the Education Editor for JSAMR and Maria Charalambides is a General/Education Editor for JSAMR.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

The Effect of Perception of Adequate Study Preparation on Test Anxiety

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Received: 29th May 2019
Accepted: 29th December 2019
Not peer-reviewed

Keywords: letter perception anxiety

Dear Editor

We read with great interest the study by Yusefzadeh et al. (Yusefzadeh, Iranagh, & Nabilou, 2019) exploring the effect of study preparation on test anxiety and performance. As medical students at Imperial College London, We would like to offer our perspective on this subject matter.

We agree that study preparation reduces levels of test anxiety and improves test performance. What remains to be explored is the perception of study preparation – how prepared does one believe themselves to be for the test? As briefly mentioned by the authors, there are studies that show despite study preparation, a highly evaluative environment can lead to increased test anxiety. (Hancock, 2001) In addition to this studies also show that females are more likely to engage in study preparation than males. (Buchmann, Condron, & Education, 2010) However, females are still more likely to suffer from higher rates of test anxiety, especially in evaluative situations. (Hembree, 1988; Kurt, Balci, & Kose, 2014; Rana, 2010) This suggests that other factors, such as beliefs and self-efficacy are important influencers of test anxiety. (Cassady & Johnson, 2002; Hembree, 1988; Onyeizugbo, 2010) For these reasons, perhaps student perceptions along with actual preparation would be the more salient point to discuss. We recommend a questionnaire measuring student perceptions of study preparation before and after the intervention, alongside the measurements of test anxiety and exam performance; this would provide added value to the study.

The importance of perceptions in relation to anxiety is highlighted by the Cognitive Behavioural Therapy technique. (Beck, n.d.) This involves helping people become aware of negative thoughts that impact their feelings and how they manage problems. This is a highly effective therapy and has been shown to reduce anxiety. (Zhu, Zhang, & Jiang, 2014)

The impact of test anxiety is great, especially in practical exams such as OSCEs and PACES where students see patients consecutively for hours and there is limited time to recuperate. Personally, we have witnessed this have a direct negative effect on student exam performance. Perhaps, study preparation along with perceptions can be explored in this practical exam context as this is of higher yield regarding test anxiety.

We greatly value the study by Yusefzadeh et al. which sheds light on the effect of study preparation on test anxiety and performance. (Yusefzadeh et al., 2019) However, perhaps beliefs and perceptions of adequate preparation are where the discussion lies. As medical students, alongside active learning, we encourage challenging negative thoughts, creating a study schedule and forming a study group for support as methods of decreasing test anxiety.

Author statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Emotional Intelligence and Leadership for Healthcare

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Received: 11th December 2019
Accepted: 13th December 2019

Keywords: opinion | leadership | emotional intelligence

The Healthcare Leadership Academy hosted its annual conference this year on 12th December 2019. In the run up to the conference, The HLA and Medics Academy have come together to produce a series of blog posts, some in collaboration with student publications from across the country. This publication has been authored by Anamika Banerjee, from Journal for the National Student Association of Medical Research (JSAMR). JSAMR is an open access, online only journal that is authored, reviewed, and edited by medical students.

1 Introduction

Healthcare is an exciting, varied and dynamic field, but is also full of challenges, demanding workloads and risks of complications, particularly as people's lives literally depend on us! With an ageing population and increasing number of patients with various comorbidities, the multi-disciplinary approach is more important than ever.

In order to ensure the smooth running of such a complex team in healthcare, good team-working and leadership is vital. But as the saying goes: 'with great power, comes great responsibility'(Lee, 1962) and often with great responsibility comes physical, psychological and emotional stress. One of the first things I notice in a good leader is how they appreciate, befriend and respect all members of the team. More often than not, when individuals feel comfortable with their leader, the team spirit, enthusiasm and productivity all increase naturally. A calm level-headed approach alongside features described in the GMC guidelines such as: accountability and responsibility for the team, good organisation and management skills and of course good interpersonal and communication skills, are all required in a good leader.(General Medical Council, n.d.-b)

The term 'Emotional Intelligence' (EI) has been increasingly used over recent years to describe these attributes. EI is a relatively broad concept and it is generally sub-divided under the following main themes (Goleman, 1996):

- Self-awareness and control
- Appropriate expression
- Emotional interpretation and response

Although some inspirational leaders appear to have these skills naturally, EI is still something everyone can learn and develop. Through understanding, self-reflection and determination, individuals can become equipped with the tools they need to thrive in a leadership role.

2 Self-awareness

Every individual is unique and each of us interpret, react and cope with situations differently. A lot of how we cope with stress depends on our EI. The notion that emotions cloud our judgement isn't necessarily true if we use emotions correctly. Self-awareness and the art of reflection is encouraged throughout our careers to improve and develop our skills. Emotions are immensely important in how we interpret and react to a situation and in turn influence our capacity to manage it effectively. If something goes wrong, how many of us actually reflect and acknowledge how we are feeling in that instance? Instead, emotions are often side-lined in favour of the 'practical brain' in an attempt to power through to get the job done. But emotions can re-emerge later, or if suppressed,

can accumulate until we crumble. Alongside causing suffering to the individual, lack of acknowledging our emotions can weaken team spirit and impair productivity. Being aware of how we feel and how this influences our behaviour is crucial to being able to recognise one's limitations, only after which we can improve. Greater insight into the self allows people to better interpret and appreciate other people's emotions too.

3 Emotional expression

After understanding our own emotions, we need to release them in an appropriate, accurate and controlled manner. Some people struggle with this – perhaps due to lack of self-awareness or fear of 'showing weakness.' Emotional expression is integral to developing trust, another key feature described in the GMC's 'Good Medical Practice'.(General Medical Council, n.d.-a) Through sharing our feelings, we can gain better insight to others and ourselves. But emotions need to be conveyed appropriately. Excessive suppression is known to be harmful to oneself and team. Conversely, over-expression can be inappropriate and potentially disrupt the smooth running of work. This is why it is important to lead by example. When a team leader appropriately expresses their emotions to their team, positive praise provides encouragement to continue the good work and negative emotions help problems to be identified and resolved. Emotional expression increases approachability of a leader if the team are ever in need. This in turn may help leaders to understand the strengths and weaknesses of team members and hence allow better delegation of roles.

4 Interpreting other people's emotions

Empathy and the ability to recognise emotional cues are key traits required to treat patients in a holistic manner. But sometimes in a busy, time-pressured schedule in 21st century medicine, it is easy to shift the balance to a more mechanical and less emotive approach. As a patient, I remember and appreciate doctors who showed they genuinely care.

Skills like active listening and responding appropriately to emotional cues increase patient comfort and may facilitate a smoother consultation. This is equally important in team working and leadership. Some of the best leaders I've observed make genuine efforts to know and understand each member of the team. As a medical student, it is easy to feel out of place in the medical team, intimidated by new environments and new people – especially when put amidst seniors. The best clinical placements have always been those where doctors notice this discomfort and rather than declaring us 'incompetent students' instead encourage us to become actively involved. In a supportive and active experience, not only do we as students gain better learning opportunities, but we become greater assets to the medical team. Through the process of reflecting on various experiences, analysing my strengths and weaknesses and acknowledging feedback from peers, I have learnt a great deal about myself as a leader and team-player. Now more than ever, I have come to appreciate the importance of EI in leadership and in healthcare.

Author statements

Conflicts of interest statement

Anamika Banerjee is the Deputy Editor-in-Chief for JSAMR.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

An Irish Packet Arriving

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Received: 30th January 2020
Accepted: 15th February 2020
Not peer-reviewed

Keywords: reflection | medical humanities | art

'Calais Pier: An English Packet Arriving' by Joseph Mallord William Turner is an oil canvas painting on display in the National Gallery, London. The painting depicts Turner's own experience of his first trip abroad via Calais in 1802 (Watson, 1992). The painting demonstrates that crossing the Channel wasn't always pleasant, due to adverse weather, near collisions with other ships and the abundance of people packed like sardines onto one vessel.

I like this artwork because I can empathise with Turner on the turmoil of going somewhere new with people I don't know, as I have done with university, and how fear set in when I was met with the unknown. My confidence and self-assurance was battered and bruised having received no offers from my initial applications. It was not until 'A level' results day with the weight of four rejections on my shoulders and the immeasurable stress of whatever was in the brown envelope defining me for the rest of my life, did I get a lifeline. Little did I know that less than forty eight hours later, including two plane flights, four train journeys and several 'meal deals', that I would none the less achieve my goal in securing a place to read medicine and a new chapter would dawn in my life. However, with a new beginning comes a fresh slate; one that new adventures, friendships and experiences can stem from.

Turner's piece shows that a new situation may appear frightening at first but with time, the clouds will part, presenting new, character building experiences. In writing this piece, it's the first opportunity when I've reflected upon my own experiences and acknowledged my different thoughts and feelings of rejection, low self- esteem and worthlessness. Confessing them here has shown me how I still allow them to influence my journey, as I constantly reflect on how fortunate I am to study my vocation. As an aspiring doctor, I will meet new situations on a daily basis and it is important for me to reflect on them to help me grow as a person and a professional.

As a viewer, I notice the prominent, whirlpool-like waves in the foreground. This demonstrates the harshness of nature and it makes me feel nauseous at the thought of the boat rocking from side to side. I also feel anger and fear when I look at it which perhaps stems from my need for regimental organisation, which Turner's harsh strokes and free-flowing movements disagree with. These emotions might also come from my fear of the unknown, as Turner creates an abyss like effect with the waves, triggering my sense of helplessness that sets in when I am unsure, often ensued by anger when I make a mistake. This is something I am learning to cope with since beginning medicine, as I realise it's impossible to know and remember everything.

Turner's use of reductionism, (an art movement emphasising simplification and clarity through the use of primary shapes and few colours) captures the realism (Kandel & Mack, 2003). The looming clouds and the intensity of the grey create a sense of foreboding. The ships in the distance disappear into the darkness, making them appear hopeless. I used to feel ambivalent to the idea of travel. I don't like being far from home and as Turner's work suggests, at times it creates more trouble than it's worth. This is emphasised by the white flag flying, possibly indicative of surrender. What I notice about the piece is that the colour and vibrancy comes from the people. They are the light amongst the darkness of the clouds. This artwork resonates with me as it makes me optimistic

Figure 1: Figure 1: 'Calais Pier: An English Packet Arriving, 1803'. "An English packet or mail boat, crowded with travellers, narrowly avoids collision with a French fishing boat as it attempts to land. This picture is based on Turner's experience of a stormy landing at Calais during his first trip abroad in 1802. Oil on Canvas." Collection of National Gallery, London 1856-NG472. Text by National Gallery of London and image is taken from their website.

for the future. It helps me to remember that when times are difficult, a conclusion will be met and a lesson can be learnt from my actions. I often let my shortcomings over shadow my triumphs but in reflecting upon challenging situations in my life, I realise it is more important to evaluate my strengths and weaknesses to allow holistic improvement. Furthermore, my experience of this painting draws comparison with medicine as it taught me not to look at the separate parts (the clouds, the ship or sea) but look at them all together to see Turner's message. This is the same in medicine as knowledge and experience must be integrated from different areas to get an overall understanding.

Sea painters like Turner trained in Holland in the 18th and 19th centuries. The 'crowded agitated sea scenes' were contemporary sources to learn new techniques and appreciate new mediums (Reynolds, 1970). To me, medicine (like art) is a vocation and in both we must travel to learn new skills, build upon of the work of others and then pass on what we have learnt to those who come after. This artwork projects the importance of where I study as what I see or learn may be unique to a certain place. It conveys to me how I must always take the opportunity as my own experiences will shape me as a professional.

The chaos and the lack of control is what struck me about this work. This is how I felt when I first started university a few months ago. But though times were tough, there was ultimate resolve and calm as Turner depicts through the parting clouds. After doing this task, I now appreciate medical school as a journey with limitless opportunities to take advantage of. I also see the painting as a tableau for the current healthcare system as even though there are bed shortages, increased waiting times and a deficit, noble work still prevails.

Prior to entering medical school, I held a cynical stance that the NHS was under-performing (as highlighted in the Mid-Staffordshire Enquiry) and those seeking asylum only overstretched an already failing system. However, now that I am training to be a healthcare professional, I appreciate that it is those who work for the health system that keep it afloat and stop at nothing to ensure the best possible health for patients. This is the first time I have used art to help me reflect. It is only through its use that I am able to see my prejudices and how I must change them to better my practice I have found art an extremely effective tool in drawing out my inner emotions. It has allowed me to feel proud of myself for how far I have come, from my home comforts of rural Ireland, to the fast-paced, no nonsense pace of the city. Having been extremely divided whether to take the opportunity and leave my home, family and friends, I see now that I made the right decision. I have been challenged mentally and socially, having left behind my introverted ways and regained my confidence that I felt I had lost. In writing this I see that the difficult and frightening decision was the right one, and am glad I sailed away from the harbour in search of a new adventure.

Author statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and

-contributors.html. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Editorial and peer review statement

This piece was reviewed internally by the JSAMR Editorial Board. As a reflection, it was not externally peer reviewed.

Open access and distribution statement

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Relationship between Selenium Status and Baseline Characteristics in Older Women with low Bone Mineral Density

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Received: 5th January 2020
Revised: 22nd February 2020
Accepted: 23rd February 2020
Keywords: selenium bone density

Abstract

Background In Europe, dietary selenium intake is low, and serum selenium is below the proposed optimum range (130 to 150 mcg/l) for all-cause mortality. Low serum selenium has been associated with low BMD and high bone turnover. The possible adverse effects of selenium excess are thyroid dysfunction and hyperglycaemia. Selenium status can be assessed by measurement of serum selenium.

Methods We recruited 65 postmenopausal women (aged 55-83, mean=66) with DXA BMD T-score between -1.0 and -3.0 at the lumbar spine or hip. Screening bloods (calcium, creatinine, TSH, glucose and HbA1C) and baseline serum selenium (BSe) levels were measured by Sheffield Teaching Hospitals clinical laboratories.

Results The distribution of BSe (mean=90.52mcg/l, SD=11.51) was consistent with the UK national NDNS data. BSe was inversely correlated with BMI (Pearson r=-0.342, p<0.005). There were no significant correlations between BSe and age, calcium, creatinine, TSH, glucose or HbA1C.

Discussion The participants have serum selenium below the proposed optimum range. There was no evidence of an association of BSe with thyroid function or blood glucose, but patients were recruited on the basis of normal tests. We found a significant inverse correlation between BSe and BMI and this has not previously been reported; the mechanism for this association is not yet known.

Author statements

Conflicts of interest statement

Thomas Franchi is the Publishing and IT Editor for JSAMR.

Authorship statement

Authors fulfill ICMJE authorship criteria, which can be accessed at `http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors`

Ethics statement

Authors declare that no ethical approval was required for this.

Open access and distribution statement

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

The University Of Sheffield.

The Mellanby Centre

Relationship between selenium status and baseline characteristics in older women with low bone mineral density

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Introduction

In Europe, dietary selenium intake is low, and serum selenium is below the proposed optimum range (130 to 150 mcg/l) for all-cause mortality. Low serum selenium has been associated with low BMD and high bone turnover[1], which increases the risk of fragility fracture. The possible adverse effects of selenium excess are thyroid dysfunction and hyperglycaemia. Selenium status can be assessed by measurement of serum selenium.

Aims

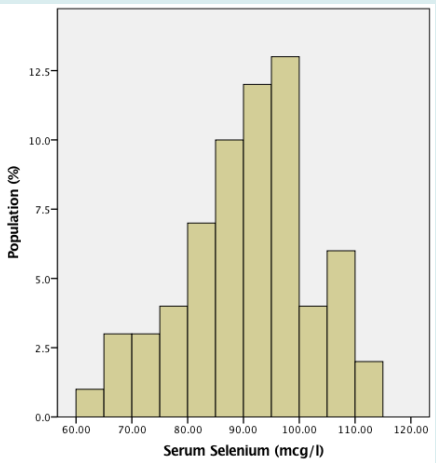
- To determine whether the selenium status of our patient set is representative for the population
- To determine the relationship between selenium status and baseline characteristics in postmenopausal women with osteopenia or osteoporosis

Methods

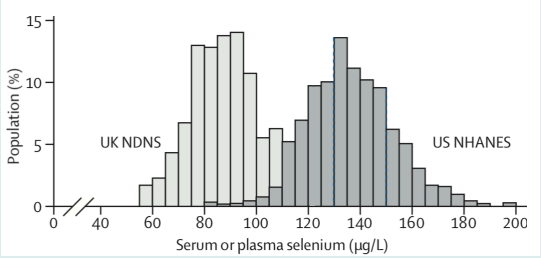
We recruited 65 postmenopausal women (aged 55-83, mean=66) with DXA BMD T-score between -1.0 and -3.0 at the lumbar spine or hip (Hologic). Screening bloods (calcium, creatinine, TSH, glucose and HbA1C) and baseline serum selenium (BSe) levels were measured by Sheffield Teaching Hospitals clinical laboratories.

Results

The distribution of BSe we found (mean=90.52mcg/l, SD=11.51) was consistent with the UK national NDNS data and that found by Rayman 2012[2]:

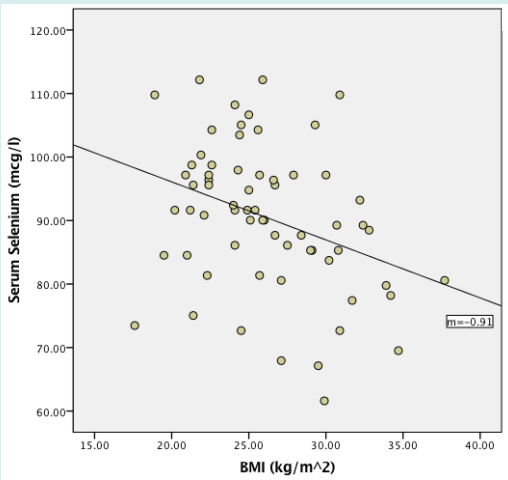


Right: Data from our patient set



Left: Figure taken from Rayman 2012[2]

There was a significant inverse correlation between BSe and BMI (Pearson's product-moment correlation coefficient r=-0.342, p<0.005):



We found no other significant correlations between BSe and age, calcium, creatinine, TSH, glucose or HbA1C:

	Selenium	
	r-value	p-value
Age	-0.208	0.097
BMI	-0.342	0.005
Calcium	-0.107	0.397
Creatinine	-0.116	0.356
TSH	0.037	0.773
Glucose	-0.136	0.280
HbA1C	-0.216	0.084

A limitation in this study is that fact that we did not present data on patient ethnicity, which is known to affect BMD.

Conclusion

The participants have serum selenium below the proposed optimum range and inline with current UK data. There was no evidence of an association of BSe with thyroid function or blood glucose, but patients were recruited on the basis of normal tests. We found a significant inverse correlation between BSe and BMI and this has not previously been reported; the mechanism for this association is not yet known.

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Acknowledgements Thanks to the staff of the Academic Unit of Bone Metabolism and of the Clinical Research Facility, NGH

NHS National Institute for Health Research

JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Improving General Practitioner Adherence to Prescribing Pathways (following an Audit of Prescribing for Overactive Bladder)

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Received: 5th January 2020
Revised: 22nd February 2020
Accepted: 23rd February 2020
Keywords: bladder | overactive | prescribing

Abstract

Background Use of prescribing pathways created by clinical commissioning groups is a key issue in General Practice (GP) as they curb unnecessary drug costs [Iacobucci, 2017] and ensure patient safety by eliminating drugs no longer recommended by NICE; but is under-researched. Following the results of an audit in a London GP practice on whether the correct prescribing pathways for Overactive Bladder (OAB) were followed, we carried out a study aiming to quantify use of prescribing pathways, understand opinions on the subject and propose solutions to the issue of under-use.

Methods We carried out an opinion survey of general practitioners (n=12) from 3 different NHS Trusts online, using a mixture of dichotomous determinant-choice, scaling and open questions. Subsequently, we quantified self-reported use of the pathways as well as collecting their opinions and ideas for solutions and worked with respondents to format proposed solutions into viable ideas.

Results Our initial OAB audit data showed that 10 % of practitioners followed pathways initially but following increased awareness there was a significant (+40 %) increase in use (CI 10.8-65.5 %, p<0.05). Our Opinion survey results highlighted a gap between the perceived importance of prescribing pathways (100 %) and the use in clinical practice (70 %) and described novel reasons as to why practitioners do not use pathways including a lack of time and hindrance to the consultation (25 %) or that they are too complex (25 %).

Conclusion We found that though practitioners on the whole view pathways as useful, they do not employ them in practice due to practical problems. Potential solutions which we suggest and describe in our poster include incorporating pathways into computer systems, simplifying pathways at the level of creation, weekly lecture sessions for awareness and more.

References Iacobucci, G. (2017). NHS will publish national list of "low value" drugs to curb GPs' prescribing costs. BMJ, j1613. doi: 10.1136/bmj.j1613

Author statements

Conflicts of interest statement

Catherine Dominic is a Submissions Editor for JSAMR.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at `http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-html`. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

Barts and The London
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Improving Practitioner Adherence to Prescribing Pathways following an Audit of Prescribing for Overactive Bladder

Introduction

- Overactive Bladder is defined by the International Continence Society as the presence of urgency (with or without urge incontinence) usually with nocturia and frequency[1]. It is estimated that three to six million in the UK are affected by Over Active Bladder (OAB)[2]
- We carried out an audit to establish whether the current OAB prescribing pathways were being used in clinical practice correctly, by evaluating patient records at a General Practise (GP) in East London (anonymised) amongst four salaried doctors and locums employed at this practise from January to July 2019. The audit was undertaken to integrate with the emerging clinical focus regarding management of incontinence and promote practices to conduct annual continence reviews by reviewing prescribing of all OAB in light of the WEL OAB pathway. It was hoped that the audit would work to improve prescribing of OAB medications and ensure that increasing compliance by the GPs would lead to cost efficiency and higher success rates of treatment which is the purpose of the prescribing pathway
- Following this audit and re-audit we conducted an opinion survey and generated solutions to Improving Practitioner Adherence to Prescribing Pathways

Methods

Identify patients using OAB code → Patients found = 6

Identify patients using drug search → Patients found=23

Identify using physiotherapy/ bladder clinic → Patients found = 1

→ Compared against the WEL OAB Pathway [3] and NICE Guidelines [4]

→ Results of doctors adhering correctly to WEL OAB

→ Re-audit 6 Months later

→ Results of doctors adhering correctly to WEL OAB*

The WEL OAB Pathway was split into 15 components and a binary adherence for each patient was used. Overall for the practise we used the following standards:

IDEAL = 100%
Good = 90%+
Moderate = 80%+
Poor = 60%+

→ Survey GP's in England about Prescribing Pathways and their attitudes and collect responses

* We then conducted a statistical test (Paired t-test) in order to compare the impact our intervention (a presentation to the GP's at the practise)

Results

100% *of GPs feel that it is important to use Prescribing Pathways when prescribing for patients*

70% *of GP's use the Prescribing Pathways for patients*

42% *of GP's think that that multistep prescribing pathways (MPP) are useful and helpful to stay up-to-date with clinical guidelines*

25% *of GP's think that that there is not a lack of time to check pathways or that they hinder patient care and the consultations*

25% *of GP's thought that MPP's are too complicated and confusing to use during short consultations*

17% *of GP's thought that MPP's are difficult to use due to constant change*

Results

Correct Use of Pathway	Audit	Re-audit	Change
	10%	50%	+40% (CI 10.8% to 65.5%, p<0.05)

The Research Question

After conducting the Audit and re-audit it led us to question whether changing the awareness and opinions of practitioners might be key to increasing adherence to the pathways and hence led us to pursue this research question –

'Can anything be done to improve the ease of use and application of prescribing pathways?'

- Use of the pathways is a key issue in General Practice as Prescribing Pathways to curbing unnecessary drug costs [5] and to ensure patient safety by eliminating drugs which are no longer recommended e.g. solifenacin rather than darifenacin (NICE Recommended)
- In an evaluation study of antibiotic prescribing for simple abscesses before and after the implementation of a pathway it was found that the use of unnecessary MRSA directed therapy dropped significantly, highlighting the importance of pathways in clinical practice [6].
- This research question was answered using an opinion survey of general practitioners (n=12) from 3 different NHS Trusts online, using a mixture of dichotomous determinant-choice, scaling and open questions. We quantified self-reported use of the pathways and collected ideas for solutions.

Proposed solutions to The Issue

As part of the survey we also asked doctors what they would like to see done to help them follow pathways better. We compiled and edited the responses into viable ideas that we have outlined in further detail.

➢ *Incorporating Prescribing Pathways into Computer Systems, Interactive Online Guidance*

Currently, when prescribing practitioners are able to see drug interactions and it would be most preferable if they also got a pop-up of the prescribing pathway for the provisional diagnosis alongside the patient records.

➢ *Simplification of the pathways*

Local or national clinical groups releasing the guidelines should make the pathways easier to follow for example just by reducing the text on the diagrams and by making the contra-indications and alternative medications easier to see.

➢ *Easier Availability of the Pathways during consultation*

Physical copies being stuck on walls for periods of time following the release of new guidelines is a cheaper alternative to a computer system. Though not as streamlined as integrating it into the computer system, this would save some time in that GPs are able to access them all in one place.

➢ *Lecture sessions by pharmacists*

Inviting pharmacists in to provide up-to-date information at weekly practice meetings is another viable option however the information retained from sessions would be lower and there is no guarantee of doctors attendance to these sessions.

➢ *More Choice in Treatment*

The introduction of alternative suggested drugs in each section taking into account individual patient factors e.g. by providing a drop down list of drug options rather than limiting them to one drug per step of the pathway.

Conclusion

- The audit and re-audit conducted clearly showed the lack of adherence to prescribing pathways and clear impact that the intervention had for this practise.
- It can be concluded from our study that though general practitioners on the whole view prescribing pathways as useful and providing the best guidance they also find they are difficult to use and that the time required to use them can hinder the patient consultation.
- We therefore proposed a number of solutions to this which were suggested by practitioners and which would be a viable option to address this issue and to ensure adherence to prescribing pathways which exist to ensure maximum cost efficiency and patient safety within prescribing, and will benefit the NHS and primary care.

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JSAMR
Journal of the National Student Association of Medical Research
Volume 3, Issue 1

Journey of a Journal: the use of a Student-Run Peer Reviewed Journal as a Learning Tool

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Received: 16th February 2020
Accepted: 16th February 2020
Not peer-reviewed

Keywords: conference abstract | poster presentation

Abstract

Background: Publication of peer-reviewed research is the cornerstone of the scientific process. The GMC's Outcomes for Graduates states: newly qualified doctors must be able to apply scientific method and approaches to medical research; with additional points for foundation programme applications awarded for publications with PubMed identifications. However, only 14% of medical students publish whilst at medical school. Major challenges include limited understanding of the research process in early years, limited opportunities for research projects with a good chance of publication and competition with established researchers for publication. Therefore current methods and opportunities for fostering the skills needed to publish research are inadequate.

Objectives: To provide opportunities for education, training and publication in a student-run, peer-reviewed journal. To lead by example in promoting open access publishing and open source software

Methods: Establishing the journal The Journal of the National Student Association of Medical Research (JSAMR) was set up in 2017 to provide a platform through which students can learn about and gain experience of the publication process. JSAMR provides:

- Authorship - opportunity to publish work not traditionally published in journals in a free, open-access peer-reviewed journal run by and for medical students.
- Education - Educational resources on skills needed to publish research, e.g. writing and peer review, are developed and published by the Education Editors of the journal.
- Editorial Board - recruitment and establishment of a core Editorial team who oversee the journal's publication processes.
- Peer review - Open recruitment and training for students to peer-review the research of others.

Results: As of February 2020, three issues have been published This includes 30 pieces from 52 authors Peer reviews have been sought from a pool of 229 reviewers In 2019, the website had a total of 79,455 hits from 1,136 unique users.

Conclusions: The infrastructure and policies of JSAMR have proven effective as a model for successful production of a student-run, peer-reviewed journal. Further research should: explore the impact of contributing to JSAMR on the professional development of students involved. determine whether educational resources are improving student understanding of research processes and academic skills

Author statements

Conflicts of interest statement

The authors are committee members of NSAMR and/or JSAMR.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at `http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html`. All authors have read and approved the final version, and accept responsibility for information published.

Ethics statement

Authors declare that no ethical approval was required for this article.

Open access and distribution statement

Journey of a Journal: the use of a student-run peer-reviewed journal as a learning tool

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Affiliations: National Student Association of Medical Research, United Kingdom

1.Background

- Publication of peer-reviewed research is the cornerstone of the scientific process.
- The GMC's 'Outcomes for Graduates' states 'newly qualified doctors must be able to apply scientific method and approaches to medical research'(1), with additional points for foundation programme applications awarded for publications with PubMed identifications.(2)
- However, only 14% of medical students publish whilst at medical school.(3)
- Major challenges include limited understanding of the research process in early years, limited opportunities for research projects with a good chance of publication and competition with established researchers for publication.
- Therefore current methods and opportunities for fostering the skills needed to publish research are inadequate.

2. Aims

- To provide opportunities for education, training and publication in a student-run, peer-reviewed journal.
- To lead by example in promoting open access publishing and open source software

3. Methods

3.1 Establishing the Journal

The Journal of the National Student Association of Medical Research (JSAMR) was set up in 2017 to provide a platform through which students can learn about and gain experience of the publication process. JSAMR provides:

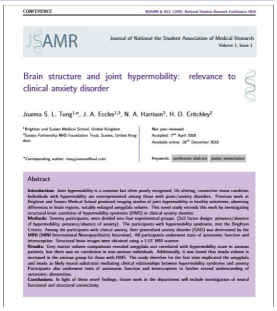
1. **Authorship** - opportunity to publish work not traditionally published in journals in a free, open-access peer-reviewed journal run by and for medical students.
2. **Education** - Educational resources on skills needed to publish research, e.g. writing and peer review, are developed and published by the Education Editors of the journal.
3. **Editorial Board** - recruitment and establishment of core Editorial team who oversee the journal's publication processes.
4. **Peer review** - Open recruitment and training for students to peer-review the research of others.

3.2 Publication Protocol

The publication and editing protocol of JSAMR is similar to that followed by established medical journals. Briefly, submitted works by authors undergo peer-review in a double-blind fashion and the Submissions Editor(s) regulate quality control checks, organising second reviews were appropriate. The Editor-in-Chief then finalises the decision to accept, reject or request the author to revise the work for publication.

3.3 Published Material

JSAMR aims to publish a variety of works across all medical fields and types of research conducted solely by medical students, and accepts work that is often done at medical school, but rarely suitable for publication in traditional journals, such as literature reviews, case studies and posters. Alongside this JSAMR publishes educational articles and guides designed for the readers by Education Editors. Figure (Fig.) 1 provides an example of a publication.


JSAMR Journal of National Student Association of Medical Research

Brain structure and joint hypermobility: relevance to clinical anxiety disorder

Figure 1. Example of published article format taken from Volume 1 Issue 1.

Key Message: Medical students are keen to learn about the publication process and need to be given the tools to do so. A student-led, open access, peer reviewed journal has been effective in filling a gap in the formal medical curriculum.

3.4 Software

- JSAMR uses customised Open Journal Systems (OJS) software, written in PHP on top of a PostgreSQL database, with a fully featured online submission, review and publication system.
- A customised public OJS frontend has been developed for JSAMR to allow modern, usable design, using HTML, CSS and preprocessors, and JavaScript. This will be offered back to the community as a plug-in.

3.5 Marketing and Publicity

To encourage student participation, our own website and various social media platforms have been used to publicise opportunities for submission and published issues.

Scan the QR code to see JSAMR's latest issue.

4. Results

- As of May 2019, two issues have been published
- This includes 30 pieces from 52 authors
- Peer reviews have been sought from a pool of 229 reviewers
- Since the journal's inception over 30 students have served on the editorial panel. Our 2019 recruitment drive had a 2:1 application to position ratio.

5. Conclusion

The infrastructure and policies of JSAMR have proven effective as a model for successful production of a student-run, peer-reviewed journal and encourages student participation in research and academia.

6. Further Study

Further research should:

- explore the impact of contributing to JSAMR on the professional development of students involved.
- determine whether educational resources are improving student understanding of research processes and academic skills.

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