JOAMR

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JSAMR
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Editor-in-Chief

Maria Charalambides

Final year medical student, University of Birmingham London

Maria is a final year medical student at the University of Birmingham and has been an active member of NSAMR since the start of medical school. She completed her intercalated BSc with First Class Honours at Imperial College, London and is a junior editor of the British Journal of Dermatology. Maria has presented research projects at National and International conferences. She began as an NSAMR student Ambassador and then was appointed as NSAMR Events lead and subsequently, General and Education editor of JSAMR. Maria's aim is to continue building a community of medical students interested in academia and encourage students to gain experience and skills in the world of publication that they can carry forwards to their careers.

Editor in Chief

Catrin Sohrabi

4^{th} year medical student, Barts and the London

Catrin is a medical student at Barts and The London passionate about combining her interests in medicine and scientific research with a future academically-focused career. Catrin continues to be involved with clinical and lab-based research, peer review and manuscript publishing, and endeavours to educate and inspire interest in clinical academia during her time at JSAMR. Outside of medicine she is a budding cyclist.

Deputy Editor
Brian Wang
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Dr Brian Wang is a final year MBBS/PhD student at Imperial College London. After receiving his Bachelors and Masters from the University of Cambridge in 2015, he transferred to Imperial College London for the clinical years of his medical studies and a PhD at the National Heart and Lung Institute funded by the British Heart Foundation. His areas of interest are in Cardiology, Stem Cell Technology and Medical Education. He has over a dozen peer-reviewed publications and has presented his research in the US, Europe and Asia.

Deputy Editor
Anamika Banerjee
5^{th} year medical student, Imperial College London

Anamika Banerjee is a fifth year medical student at Imperial College London. She has completed her BSc degree in Pharmacology with First Class Honours during the intercalation year and conducted various audits and clinical quality improvement projects during medical school training. Anamika is passionate about medical education and teaching and aims to increase opportunities for fellow students. She has led and developed lectures, workshops and tutorials through academic university societies, alongside developing educational resources whilst holding the position of JSAMR's Education Editor 2018-19. She became Deputy Editor of JSAMR in academic year of 2019-20 and hopes to share her experiences and visions for further development of the journal and continue to contribute to medical education.

Deputy Editor and Education Lead
Samantha Green
3^{rd} year medical student, University of Aberdeen

Samantha is currently a 3rd year medical student in Aberdeen after completing a BSc and MSc in Neuropsychology. She spent time working on clinical trials and in medical research and has a variety of research interests including neurology and rheumatology. She aspires to work as a clinical academic with an interest in medical education.

Education Lead

Jessica Craig

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

Jessica is a fourth year medical student at the University of Sheffield. She is currently completing a BMedSci, and am looking forward to continuing my involvement with research and education this year through being a part of the JSAMR team. Her interests lie in paediatrics, endocrinology, medical education and research, which she aspires to encompass in a career as a clinical academic. Outside of medicine, she enjoys running and yoga.

Copy and Layout Editor

Adrianna Wong

Final year medical student, Keele University

Adrianna is a final year medical student at Keele University having completed an intercalated MPhil on Clinical Neurosciences at the University of Cambridge. As an aspiring academic neurosurgeon, she hopes to combine the capabilities of academic research with clinical knowledge to enhance neurosurgical care. With the role of copy and layout co-editor, she looks forward to compiling issues of JSAMR that are easily engaging and accessible to all readers.

Copy and Layout Editor

Amber Knapp-Wilson

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

Amber is studying as a mature student at the University of Bristol, after completing a BSc and PhD in Biochemistry. She is excited to continue researching throughout her degree and aspires to make research opportunities more accessible for everyone. Her research interests include metabolic medicine, genetics and medical education. Amber is also involved in the Bristol Medical Research and Education Society and is a tutor for the education charity The Brilliant Club.

Copy and Layout Editor

Burleen Chhatwal

3^{rd} year medical student, University College London

Burleen is going into her third year of medicine at UCL, where she will be undertaking an intercalated BSc in Neuroscience. She has a range of interests, including academic medicine, neurology and medical education. She has been involved in research projects in the past and, with her involvement in the JSAMR, she hopes to make research more accessible for medical students, as well as furthering her own interest in academia.

Copy and Layout Editor

Adam Alin

Physiology Student, Kings College London

Adam is currently undertaking a BSc in Physiology at King's, with a keen interest in how the body responds to stresses at the extremes of human endurance. Despite his choice of degree, Adam hopes to pursue a career in surgery. One day, he hopes to integrate his love for sport with a career in orthopaedics.

Copy and Layout Editor

Gabriela de Scenza

Final year medical student, St George's University London

Gabriela is a final year at St George's University London. She 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.

Submission Editor

Alice Watson

4^{th} year medical student, University of Bristol

Alice is a 4th year medical student at Bristol. She completed her intercalated degree in Surgery and Anaesthetics at Imperial College London, where she undertook a clinical research project investigating the use of transcranial magnetic stimulation as an intra-operative monitor of spinal cord function. She then presented this research at 'Neuroscience 2018' in San Diego. Alice has been involved with many societies during medical school and is passionate about medical education and promoting research amongst students. Alice has a special interest in acute care, as well as sport and expedition medicine. She hopes to pursue a career in Anaesthetics.

Submission Editor

Jessica Xie

5^{th} year medical student, Imperial College London

Jessica is a fifth-year medical student at University College London (UCL). In her third year, she intercalated in Medical Sciences with Primary Health Care. Her research interests lie in the areas of Medical Education and Nutrition. She has worked with the UCL Primary Health Care Team to develop the Culinary Medicine teaching at UCL, which is the first Medical School in Europe to make such teaching mandatory in undergraduate medical training. She has presented her research at regional and national conferences and has won awards. Her passion for improving Nutrition Education in UK Medical School curricula have led her to work with reputable organisations, such as Culinary Medicine UK and Nutritank. Jessica is an aspiring Paediatrician and looks forward to working as a co-Submissions Editor of JSAMR to oversee the peer review process!

Submission Editor

Anca Vasilica

2^{nd} year medical student, University College London

Anca is a UCL medical student, having just finished her first year. After competing internationally in Neuroscience, she was determined to pursue a medical career, with an ambition to specialise in Neurosurgery. She became involved with research early in my medical student career by working in the research group of a neurosurgeon at NHNN. She also gained experience in working as part of a journal's editorial board by being a Junior Editor for the "International Youth Neuroscience Association" Journal. During her first year of medical school, she has been involved in the university life by being an academic representative for one of the modules and she has recently started to develop an interest in medical education, beginning to work as part of a newly founded online learning platform – LearnMed. She has recently been elected to be part of the committees for UCL Anatomy and UCL MedTech Societies. She is truly excited to start working as the JSAMR submissions editor for the upcoming year and she hopes to be able to bring her own contribution to the activity of the journal whilst further developing her interest in research and academia.

Publishing Editor

Amy Haeffner

4^{th} year medical student, University of Exeter

Amy is a 4th Year Medical Student at the University of Exeter. She has been interested and actively involved in research since the beginning of her degree and has been privileged to have some amazing opportunities to work on both translational, laboratory-based projects and clinical studies during her career. She is really excited to be part of the diverse and dynamic team that makes up the Journal of the National Student Association of Medical Research and have the good fortune to help many other medical students express their ideas and grow their skills in research and publication! Outside of medicine, she enjoys tutoring GCSE and A Level students, running, volunteering in schools teaching sex education (it's not as scary as it sounds), and – of course – sharing her time with some amazing friends. She is looking forward to this year of fantastic submissions and publications from fellow students!

Publishing Editor

Lewis O'Brien

Physiology student, University of Glasgow

Lewis is a Scottish Physiology student at the University of Glasgow and is interested in cardiovascular research.

IT Editor

Ian Tan

Final year medical student, University College London

Ian is a medical student at UCL who is in his final year. He completed an intercalated degree in neuroscience and is particularly interested in neurology and infectious diseases. In his spare time, he enjoys running and reading.

Social Media Editor

Ella Marson

Intercalating medical student, University College London

Ella is a medical student at the University of Birmingham, currently intercalating in Women's Health. She is yet to decide on her favourite speciality but particularly enjoys medical research, having been involved in several projects over the last year. Outside of medicine, she loves long-distance running, netball and baking.

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JSAMR

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Issue summary: Volume 4, Issue 1

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We are delighted to present our fourth issue of JSAMR, albeit in the most unprecedented times. The coronavirus disease (COVID-19) has undoubtedly posed countless challenges for everybody on a global scale. COVID-19 has not only taught us the importance of community, social responsibility and benevolence, but also adaptability. The COVID-19 crisis has highlighted the importance of accurate and swift science communication, emphasising the JSAMR's mission to foster scientific writing, peer review and critical appraisal skills amongst medical students so that they can become well-equipped with all the necessary skills required as future clinicians and academics.

Despite these uncertain times, the JSAMR team has worked endlessly via a collaborative effort; the level of dedication towards our journal and teamwork has been inspiring. Therefore, we would like to firstly thank all of our Editorial Team for their hard work in putting together this fascinating issue.

We would also like to sincerely thank all the authors for their excellent submissions, which we hope you will enjoy reading. The first research piece by Dosani et al. is focused on deciphering the underlying mechanisms of osteoarthritic pathology alongside potential therapeutic avenues for exploration. Thereafter, Rees et al. perform a comprehensive literature review on the pathophysiology behind age-related macular degeneration, whilst Kyriacou et al. conduct a fascinating primary care audit on the number of patients with chronic kidney disease prescribed non-steroidal anti-inflammatory drugs to evaluate their complex association.

Later in this issue, Wallner et al. present a detailed study investigating the effects of prenatal maternal stress on the neurodevelopment of offspring opioid receptors, whilst Oliver et al. conduct an intriguing retrospective study on the reliability of Garden and Pauwel's systems for intracapsular neck of femur fracture classification. Thereafter, an extensive and thought provoking literature review on the role of ketogenic diet and medium-chain triglyceride therapies on Alzheimer's disease (AD) by Inyand et al. is presented, whilst Patel et al. comments on neurodegeneration in epilepsy, tau treatments in AD, and aphasias in patients using British Sign Language after left hemisphere stroke.

Finally, an abstract comparing endovascular versus open repair for abdominal aortic aneurysms, and posters on changes in olfactory function and preclinical neurodegenerative disease by Winchester et al., and gender and behavioural traits in children with conduct disorder by Raja et al., are presented.

Our Education editors, Samantha Green, Jessica Carig and Maria Charalambides have produced a brilliant article on 'How to Write an Academic Paper', offering you all their invaluable tips stemming from their own experiences in academia. The value of effective scientific writing, data presentation and analysis cannot be undermined and is vital for producing high-quality research.

We are very grateful to our readers, editors, authors and peer-reviewers who have joined us in our journey and vision, helping JSAMR grow and promote academia and evidence-based medicine amongst the doctors of tomorrow.

Author statements

Conflicts of interest statement

Maria Charalambides and Catrin Sohrabi, the current Editors-in-Chief of the Journal of the National Student Association of Medical Research. Maria has previously held role as Education Editor 2019-20.

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 fi-

nal version, and accept responsibility for information published in this article.

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Contents

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How to Write an Academic Poster: Top tips for medical students

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Abstract

Academic posters are a creative and useful method of displaying work at conferences. Posters are a summary of research projects, which aim to dissipate advancement amongst the scientific community and showcase the importance and relevance of new work in the field. Successful posters stimulate discussion, encourage collaboration and generate further ideas for research. In this article, we present our advice on creating effective posters, including preparation and layout tips.

1 What is the purpose of an academic poster?

Academic posters give you the opportunity to summarise your project into a concise and aesthetically pleasing format to share with the scientific community (Gundogan, Koshy, Kurar, & Whitehurst, 2016). Posters enable you to present your research at conferences in a clear and simple manner whilst highlighting key points from your work.

2 What do I need to know before I start?

Ensure that you know who your audience at the conference consists of, so you can pitch your presentation to the appropriate level and decide the most salient points to include on your poster. Read the author guidelines issued by the conference and check whether there is a specified size (usually A0 or A1) and orientation (landscape or portrait) (Gundogan et al., 2016). It is important to set aside an adequate amount of time to prepare your poster and incorporate feedback from your supervisor and research team (Goodhand et al., 2011).

3 How should I prepare my poster?

Microsoft PowerPoint is the most commonly used application for creating posters as you can set the page to the required poster size ready for printing (Gundogan et al., 2016). Check your university intranet for ready-made templates that you can adapt for ease. Programmes such as Canva, Adobe InDesign and Adobe Illustrator can also be used if you want to be more creative or create highly specific illustrations to convey information. Choose a logical structure for your poster, begin with aims and objectives, flowing downwards to methods, results and conclusions (Shelledy, 2004; Taggart & Arslanian, 2000; Tasker, 2013). Most readers tend to read the poster from top to bottom and left to right.

To begin, plan the information that you want to include within the poster and highlight key points (it may help to speak to a supervisor to identify these). When writing, you should use the active tense ("We did this") and only consider using medical abbreviations where appropriate and easy to understand (Tasker, 2013). Also, try to avoid long sentences and large chunks of writing (Gundogan et al., 2016). To make titles stand out and allow readers further away to follow the

structure, you can make them bold. Many readers will quickly read over your poster from a few feet away and it is important they are still able to read your key points (Tasker, 2013). You should ensure that text size is appropriate, with the main title above 85 point and a size of 24-34 for the main text (Tasker, 2013), additionally, use a clear font such as Arial or Calibri. If possible, substitute words for images and graphics as these are more appealing and concise, and provide a visual means of presenting results and drawing conclusions (Shelledy, 2004; **?**). Continue to edit your poster and ask for feedback from peers and your supervisor until you are satisfied that all text is useful and relevant to your overall message.

Try to avoid overly embellishing your poster with formatting and pictures (Gundogan et al., 2016). Select a simple colour system with contrasting colours, but remember that light backgrounds are reader friendly and print better (Goodhand et al., 2011; Gundogan et al., 2016; Shelledy, 2004; **?**). It is best to use 2-3 colours and avoid using patterned backgrounds. Avoid using colours that colour blind readers are unable to see (most commonly it is difficult to distinguish red from green) (Tasker, 2013). Ensure that you insert logos for the location and institution where research was conducted and, if needed, include logos from sponsors. It is also worth checking with your supervisor whether your host organisation can aid with printing costs.

You may wish to print several copies of your poster in A4 for those interested in taking a handout home with them. It is important to ensure your contact details are available to the reader on your poster (an e-mail address) and included on any handouts so they can contact you with any future questions.

4 How should I organise my poster?

Title

Use a large font size (largest size on your poster) and keep it short and focused, between 10-12 words maximum is suggested (Tasker, 2013). You may wish to include the type of research within the title, for example: 'A randomised control trial investigating the use of aspirin as a preventative treatment for heart disease' or 'The effect of the coronavirus pandemic on medical students' mental health - a qualitative study'. All authors, including their affiliations, should be listed below the title, with the most senior author at the end (Gundogan et al., 2016).

Introduction

Describe the background to your project, including the rationale and project objectives (Gundogan et al., 2016). Explain how your work is novel in the field and explain how it differs to existing literature.

Methods

Identify your target sample, setting, duration of the study, inclusion/exclusion criteria, statistical techniques used and primary and important secondary outcomes measured. A flowchart or diagram can be helpful in presenting the methodology visually. Bullet point lists can be used to reduce words if you are struggling for space.

Results

Include any important raw results which address the objectives stated and statistical test summary values (Gundogan et al., 2016). Use graphs, tables, images and diagrams to summarise and present the data. Ensure any images are high resolution files such as JPEG (.jpg). Use appropriate captions and figure legends to explain your tables and diagrams. Remember to include units and label axes on graphs clearly. Avoid grid lines on graphs as this detracts from the plotted points.

Conclusions

Your conclusions should be supported by the data presented in the results section and answer the objectives of the project. This should be summarised in a few sentences (Gundogan et al., 2016). It is insightful to acknowledge the limitations of your research, suggest areas for improvement and implications on practise. It is also useful to identify areas for possible future research to explore.

References

To finish, include a textbox with your reference list citing key sources of information (around five references is ideal) (Tasker, 2013). These are written in a smaller, but still readable font, often using a numerical in-text referencing system to reduce space used. Acknowledge any funding or support received for your research.

See appendix A and appendix B for templates of portrait and landscape posters.

5 How should I prepare for my presentation?

Practise, practise, practise! Present to your friends (medical and non-medical) and ask for feedback. Try and schedule a time to present to your supervisor too. Ask everyone you practice with if they have any questions about your research and think of possible questions yourself.

Presentations tend to last under 10 minutes, with some time for questions. Ensure you stick to the set timing which should be available on the conference website. Guide your reader through the poster in a chronological order and set the scene well. Keep the presentation focused and highlight key points and results. Explain how your work is relevant to current practice and the contribution it has made. It is also important to acknowledge limitations of your work and how further research could build on your findings in the future.

Make sure you understand all of the information on your poster and that you have checked for any spelling and grammatical errors. This is easily done by using the inbuilt spell check function on programmes such as PowerPoint.

6 What will happen on the day?

Ensure that you arrive on time and bring pins, tape or other items to help stick the poster up. Pin a plastic wallet to the board to hold the A4 handouts of your poster for your readers to take away with them. Often poster presentations occur during breaks at conferences, so ensure you are at your poster and ready to engage with other conference attendees.

You must arrange to be by your poster when the judges inspect the poster so you can present and answer their questions. Dress smart and look the part, smile and enjoy!

7 What if my presentation is virtual?

Since the COVID-19 pandemic began, the popularity of virtual conferences has increased rapidly. Virtual conferences have proved popular as they have enabled scientists to continue to share new data on a global scale whilst remaining at home (Richards, 2020).

Once you sign up to present a poster, you will be able to view specifications such as poster format and upload deadlines. Many virtual conferences require posters to be submitted a few days in advance in a pdf format so posters can be uploaded onto a web-page accessible to conference attendees. As the poster will be viewed on a screen, vertical format is best as it enables readers to scroll through your poster easily. Although you should keep text to a minimum, readers will be able to zoom into your poster allowing them to see the details of your work better. This can be particularly beneficial when interpreting graphs and data. Additionally, since your work is being viewed online, it is possible to include links and QR codes to transfer readers to additional resources.

Alongside your poster, some conferences may ask you to upload a recording of your poster presentation in audio or video format. Once attendees have had the chance to see your poster and presentation, there may be a time allocated to you which enables other conference goers to ask questions about your work via a video conference.

8 Summary

- Academic posters are a visual way to showcase work
- Stick to a simple and clear format to aid readers
- Check for any spelling or grammatical errors
- Prepare and practise in advance
- Think about any questions you may be asked

Author statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

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Ethics statement

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

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The review process for this manuscript was double blind, where authors and peer reviewers were blinded to each others identity and institution.

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Goodhand, J., Giles, C., Wahed, M., Irving, P., Langmead, L., & Rampton, D. (2011, April). Poster presentations at medical conferences: an effective way of disseminating research? *Clinical Medicine*, *11*(2), 138–141. Retrieved 2020-11-04, from https://www.rcpjournals.org/lookup/doi/10.7861/clinmedicine.11-2-138 doi: 10.7861/clinmedicine.11-2-138

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Tasker, F. (2013). How to make a scientific poster. *BMJ*, *346*. Retrieved from https://www.bmj.com/content/346/sbmj.f297 doi: 10.1136/sbmj.f297

9 Appendix

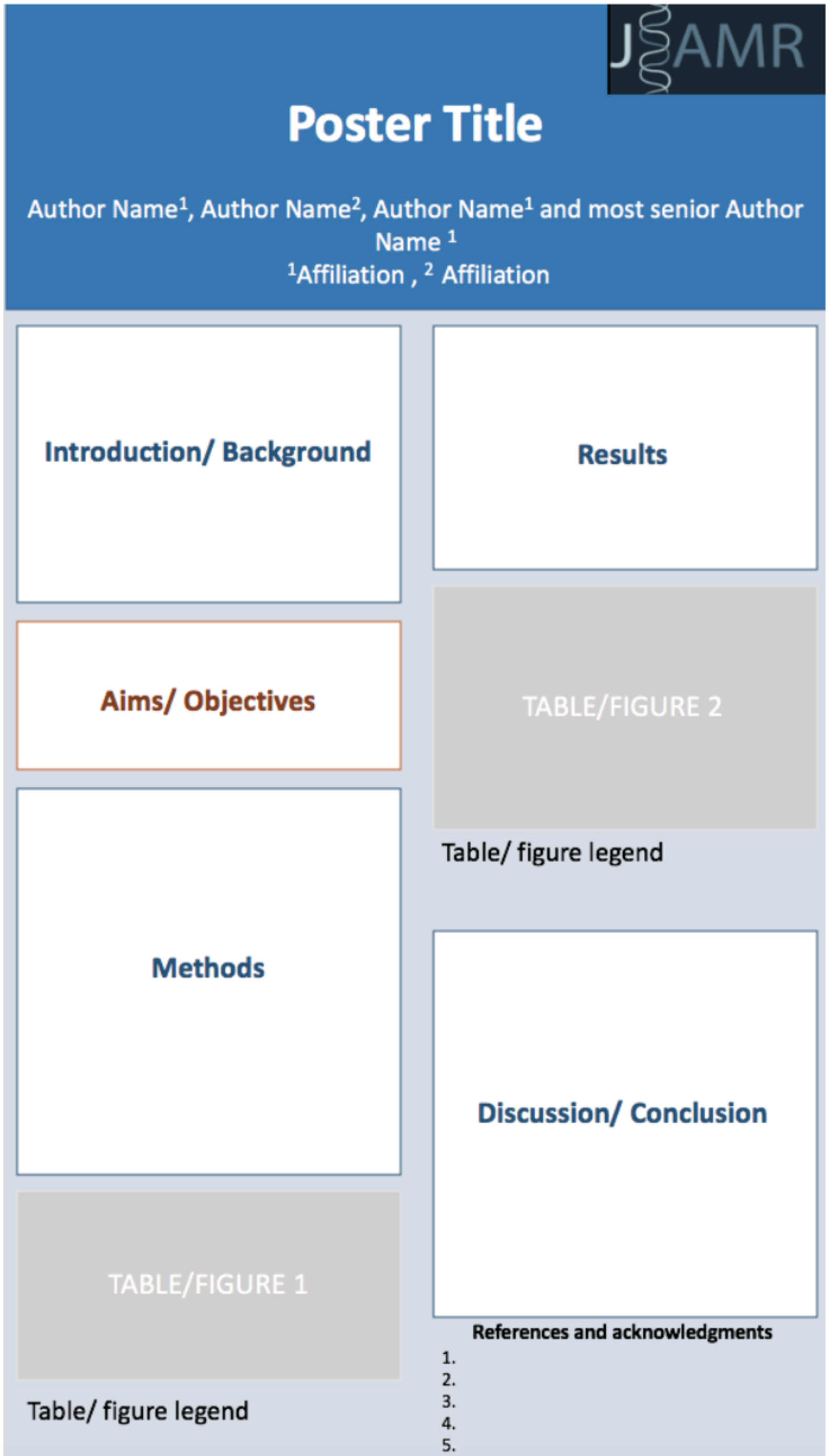

Figure A0: Example layout for a portrait poster

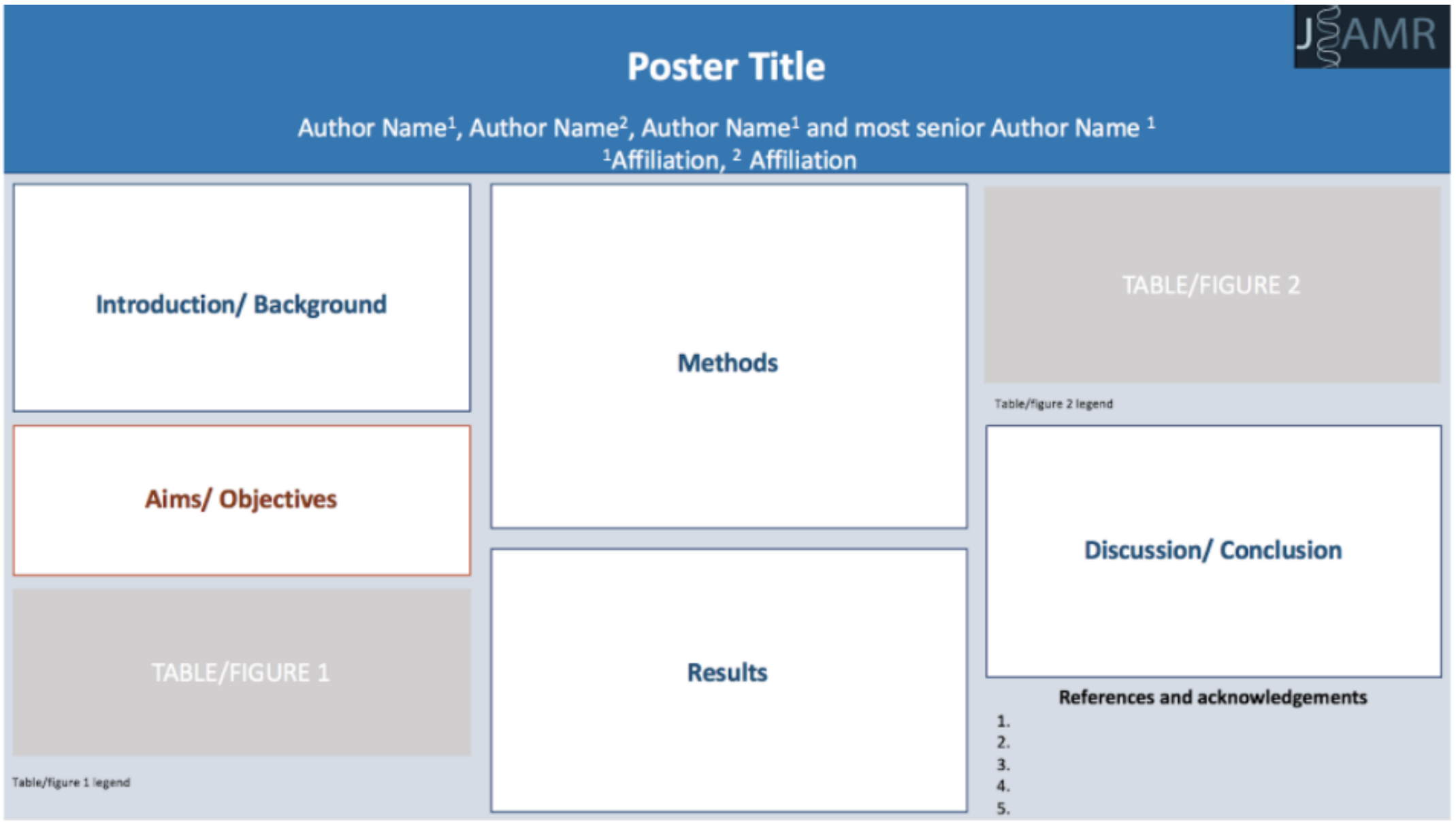

Figure B0: Example layout for a landscape poster

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Are Tau Therapies a Treatment Possibility in Alzheimer's?

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Abstract

Introduction:

Currently, Alzheimer's disease has two main hallmarks, amyloid plaque formation which proceeds the presence of neurofibrillary tau tangles. Amyloid is the main therapeutic target in clinical trials to improve cognition in Alzheimer's disease. Despite promising outcomes in animal models amyloid targeting therapies failed to reduce cognitive impairment in humans. Why exactly this is remains unclear although there are fundamental problems with the amyloid cascade hypothesis, the trial designs, particularly primary outcome measures and how animal models relate to human disease. It is possible that tau is a bigger driver of toxicity than previously believed, with several trials in tau targeting therapies now underway hoping to bring positive results. For this to be successful these trials must overcome the shortcomings of amyloid targeting trials to create appropriate targets with meaningful outcomes for patients. This article explores the notion that tau-targeting therapies could hit the same barriers as the amyloid hypothesis.

1 Introduction

Alzheimer's disease (AD) is a global health crisis with an increasing incidence arising from the ageing population. Symptomatic therapies exist however the prognosis remains unchanged despite ongoing research. The continued development of future disease modifying therapies is based on the two main features of AD; histopathological accumulation of amyloid plaques and hyperphosphorylated neurofibrillary tangles (NFTs) eventually leading to neuronal death and brain atrophy.

It was widely accepted that amyloid plaque formation is the driving force of AD, which precipitates NFT formation and toxicity later in the disease although the exact interactions between amyloid and tau are unknown. This was supported by familial variants of AD in which proteins involved in the amyloid processing cascade contain mutations, with the APOE4 allele being the single biggest risk factor for AD. Reasonably, there have been many attempts to reduce or 'detoxify' amyloid plaque as a therapeutic target. Despite promising outcomes in mice models and phase 1 testing, amyloid targeting therapies including Bapinezumab and Solanezumab monoclonal antibodies have failed to reduce cognitive impairment in humans (Huang, Chao, & Hu, 2020; van Dyck, 2018).

To understand why the trials have failed one must ask several questions:

1) Is amyloid truly the correct target? Indeed, amyloid is present in normal ageing demonstrating a disconnect between load and clinical symptoms and is greatly conserved over an

evolutionary timescale suggesting an unknown function.

2) Is the intervention too late? Trials recruit patients already displaying signs of mild cognitive impairment (MCI) and a patient presenting in clinic has already laid down all of the plaque they are going to produce and are now experiencing atrophy.

3) Are the primary outcomes in clinical trials appropriate? If the patient has cerebral atrophy, is it reasonable to expect cognition to improve after therapeutic administration, or is cessation/slowing of decline more appropriate? (Whitehouse, 2014).

2 Tau

Tau is a microtubule-associated protein implicated in microtubule stability and dynamic regulation of the axonal transport apparatus (Medina, Hernández, & Avila, 2016). Tau has been proposed to mediate AD pathology via intracellular and extracellular mechanisms. Intracellular mechanisms include the aggregation of tau by hyperphosphorylation, producing NFTs detected on histology at autopsy in AD as well as other tauopathies such as progressive supranuclear palsy (PSP). Secondly, three repeat (3R) and four repeat (4R) tau isoforms are balanced physiologically. The switch to an excess of 4R tau has been implicated in disease. Extracellular tau oligomers may escape the cell independently of cell death which is associated with synaptic decline in AD and tau spread throughout the brain (Sebastián-Serrano, Diego-García, & Díaz-Hernández, 2018).

Given the lack of success with amyloid targeting therapies there is now a discussion on whether tau is a bigger driver of toxicity, with trials in tau targeting therapies such as Tideglusib, Methylene blue and Davunetide now underway (Congdon & Sigurdsson, 2018). Will research in tau-targeting therapies hit the same barriers as amyloid-targeting ones? This is analysed by addressing the implication of tau in AD at the level of: topographical distribution; relationship with atrophy; tau in normal ageing and cognitive decline and the outcomes of tau therapies.

3 Methods

Articles cited have been obtained from PubMed using syntax: Alzheimer's disease, therapy, tau, amyloid-beta. Inclusion criteria included: peer reviewed; clinical diagnosis of AD or MCI in human studies; primary research data and reviews. Exclusion criteria included dissertations, abstracts, editorials and unpublished comments. 26 papers were chosen in an initial search with 16 fitting this criteria.

4 Results and discussion: Tau as a therapeutic target

4.1 Tau in AD, MCI and normal ageing

Tau in the cerebrospinal fluid (CSF) may not be more predictive of AD progression than amyloid, with increased correlations with cognitive impairment (Brier et al., 2016; Kandimalla et al., 2013). A drawback from the amyloid hypothesis is its presence in normal ageing. A study by Lowe et al., (2018), studied widespread association in tau with ageing, MCI and AD using positron emission tomography (PET)(Lowe et al., 2018). Similarly, to amyloid, the authors found tau globally present in cognitively unimpaired individuals as part of a normal ageing process, however there was elevated tau in patients with MCI and AD in comparison to cognitively unimpaired individuals. Other studies support this, finding globally elevated tau with MCI and AD, with a higher load in AD (Sabbagh et al., 2010). Although presence of tau in normal ageing demonstrates it is unlikely tau infers toxicity alone, these studies suggests a potential NFT burden accumulating prior to clinical symptoms. Indeed, in the Lowe et al., (2018) study cognitively impaired individuals had a higher load of NFTs at a younger age further supporting this notion (Lowe et al., 2018). Tau is therefore a logical target at this stage as it accumulates before MCI and is more predictive than amyloid for progression.

4.2 Topographical distribution and atrophy

Learning and memory are the most affected aspects of cognition in AD which is performed by medial temporal lobe structures such as the hippocampal cortex and the entorhinal cortex. These are most affected by atrophy in AD. Therefore, it would be reasonable to expect pathological mechanisms to be taking place here. There is also global damage for example in occipitotemporal regions (reduced ability to read and write) and in frontal lobes (social behaviour and decision making) (Fitzgerald M.J.T, Gregory Gruener, 2012).

Although tau is found globally in normal ageing, differing distribution may be a driver in disease and be more closely linked to clinical symptoms than amyloid. Indeed amyloid deposition appears to develop in the isocortex, with lower effects on subcortical structures (Lane, Hardy, & Schott, 2018). The topographical distribution of tau throughout the brain can be classified into Braak staging (Braak & Braak, 1991; Braak, Alafuzoff, Arzberger, Kretzschmar, & Tredici, 2006).

Table 1.

Stage	Brain regions involved
Stage I and II: Transentorhinal Clinically silent	Entorhinal and transentorhinal cortex, moving towards the hippocampus
Stage III and IV: Limbic MCI	Severe involvement of the entorhinal cortex. Extension to neocortex. Insular cortex becomes affected in stage IV. Some temporal lobe involvement.
Stage V and VI: Isocortical AD	Large neocortical involvement across temporal, occipital and frontal lobes.

Braak staging of tau in AD. Moving through the stages shows a spread from the entorhinal region later affecting many areas of the cortex which give rise to memory loss followed by sensory, motor and higher function debilitation in later disease. Figure adjusted from H. Braak Braak, (1991) (Braak & Braak, 1991)

These stages describe a sequentially 'predictable' pattern of NFT spread from the entorhinal cortex and hippocampus towards the limbic and isocortical regions throughout AD progression. However, there is now mounting evidence that tau does not always accumulate and spread in this manner.

Interestingly, Sabbagh et al., (2010) found tangle accumulation had a stronger correlation with cognitive impairment in the neocortex than with the hippocampus and entorhinal cortex (Sabbagh et al., 2010). This correlation with the neocortex was more positive in patients with AD over MCI. Comparing this with amyloid, correlations were stronger with NFTs than amyloid plaques in entorhinal and hippocampal regions in the AD patient group. Other studies have found tau accumulation predominantly in the frontal regions with atrophy only in later stages (Harrison et al., 2019). Moreover, Lowe et al., (2018), have found higher Braak staging present in cognitively unimpaired individuals (Lowe et al., 2018). An explanation for this is the hippocampus and entorhinal cortex are more susceptible to toxic effects of tau accumulation and therefore succumbs to neurodegeneration at a lower threshold than the frontal regions of the brain, or, that tau is under-represented as atrophy has already occurred.

A 2 year study by Harrison et al., (2019), compared tau-PET signalling with atrophy later in the disease course and found that atrophy followed the pattern of tau deposition and new tau seeded between scans (Harrison et al., 2019). Longitudinal studies tracking tau accumulation and neurodegeneration can show whether tau directly antecedes atrophy, although timescales in current studies remain too low and record data once a diagnosis has already been established. Measuring the degenerating brain is not wholly appropriate as if the neuron is gone, so are the pathological proteins that were present.

Tau may be a more appropriate target than amyloid as it is better correlated to clinical symptoms, has a higher presence in the hippocampus and entorhinal cortex and is also present before onset of symptoms. However, both are present in normal ageing and are not always correlated with atrophy hence the clinical picture is unlikely attributable to tau and amyloid alone. The next question is whether there has been success with tau therapies in AD.

4.3 Tau therapies

Therapies target multiple ways in which tau could propagate toxicity such as post translational modifications, aggregation, proteolytic cleavage, truncation and turnover. Specific therapies with either terminated or ongoing trials are discussed here.

4.4 LMTX

LMTX is a more stable form of methylene blue which prevents aggregation in vitro and improves cognitive function in mild and moderate AD patients after fifty weeks of treatment. Despite rescued learning impairment in two mouse models of AD, LMTX failed to rescue cognitive impairment in humans. The authors suggest a dosing issue and another trial with altered dose will finish in 2020. Indeed, lower concentrations of methylene blue were more beneficial than the higher dose tested in the initial trial (Huang et al., 2020; Congdon & Sigurdsson, 2018). As previously discussed, aggregates or oligomers could incur toxicity. By inhibiting aggregation, tau will remain in oligomeric form, although this has the capacity to be more toxic as both aggregates and oligomers have been linked to pathogenesis. The fact that mice models showed promise is not necessarily reliable as oligomers may not have a negative effect on cognition in mice as AD does not occur naturally in these animals.

4.5 Davunetide

Davunetide is an inhibitor of activity dependent neuroprotective protein (ADNP) which has an interesting although incomplete link with tauopathies, with a deficiency in mouse models causing disease. It has been postulated that davunetide is neuroprotective by microtubule stabilisation and reducing tau-hyperphosphorylation. Although the drug is safe and well tolerated in a phase II study there has been no improvement in cognition in a trial in PSP and there are no further trials planned for the drug (Huang et al., 2020; Bakota & Brandt, 2016).

4.6 Tideglusib

Tideglusib is an irreversible inhibitor of GSK3 which phosphorylates tau. In animal models tideglusib reduces tau phosphorylation, amyloid burden and improves cognition in AD patients in a pilot study. In a larger cohort benefit was seen in patients with mild AD in isolation but there was no significant improvement when the entire cohort was taken together (Congdon & Sigurdsson, 2018). This could demonstrate the need for stage targeting of AD. Separating patients into mild, moderate and severe groups might be beneficial to find a drug that targets mild symptoms and therefore delays progression.

4.7 C2N-8E12 (AbbV-8E12)

Immunotherapies are in very early stages of development predominantly going through safety trials with minimal data on outcomes around cognition. One example is passive immunisation with C2N-8E12 antibody which has been shown to prevent NFT aggregation, hyperphosphorylation and improved cognition in mice. In a phase I trial humans were given three doses over twelve weeks, with twenty-nine of thirty participants producing IgGs, however some adverse effects such as microhaemorrhage and infection were reported, a larger study is now underway to evaluate safety (Huang et al., 2020).

5 Conclusion

The trials for tau targeting therapies have not yielded positive results so far, with many trials failing to meet primary outcomes. So is the field heading in the same direction as amyloid?

Is tau the correct target? From the evidence given here, tau could be a reasonable target but has many pitfalls. The presence of tau in normal ageing is concerning particularly if higher Braak stages are present in cognitively unimpaired individuals. This is complicated further by the fact that most of the drugs in current trials are not primarily tau targeting (instead targeting a tau kinase or stabilise microtubules) and so cannot give a true correlation between presence of tau and decline in cognition. Moreover, microtubule instability may be a 'loss of function' mechanism in AD, but attention should also be given to gain of toxic functions of tau. Lastly, tau does not always correlate with atrophy which gives rise to clinical symptoms hence there may be a limit to what a successful tau targeting therapy could achieve.

Are interventions timely with appropriate outcome measures? As in amyloid, tau therapies are tested in patients with existing disease. With the field in such early stages, it is necessary to do this before looking at prophylaxis, however the outcome measures continue to be unreasonable, looking at improvement in cognitive function despite established atrophy and subsequent presence of clinical symptoms. Some therapies have shown promise in patients with MCI and studies presented here demonstrate atrophy occurring after accumulation of tau (Harrison et al., 2019). Therefore, using cessation of decline as oppose to improvement may be beneficial.

There are many similarities between tau and amyloid therapeutic trials. Research must address the pitfalls of the amyloid hypothesis in order to produce meaningful trials and results providing patients with acceptable treatments. Changing outcome measures, smarter targets and longitudinal trials will be necessary to do this.

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No conflicts of interest have been declared by any authors.

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Anatomical Changes to the Retinal Region in Normal Ageing and Age-Related Macular Degeneration; Implications for Age-Related Macular Degeneration Pathophysiology

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Abstract

Purpose:

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness and research has shown that ageing is the most significant risk factor. Despite the increasing prevalence of AMD, its pathophysiology remains poorly understood. This review evaluates anatomical changes that take place in the retinal region (RR) during normal ageing (NA) and in AMD to improve current understanding of AMD pathophysiology.

Materials and Methods:

A comprehensive literature review of peer reviewed publications related to anatomical changes in the retinal region in NA and AMD was performed using the PubMed® database to identify relevant research articles.

Results:

Reductions in Bruch's membrane permeability are crucial in the general pathophysiology of AMD and macular thickening facilitates hard drusen accumulation. Extracellular deposits modulate AMD pathophysiology; breakdown of hard drusen and coalescing basal linear deposits preferentially predispose to atrophic AMD and neovascular AMD, respectively. Patterns of retinal pigment epithelial cell loss are similar in NA (principally modulated by lipofuscin) and atrophic AMD, both demonstrating foveal sparing. Choroidal neovascularisation has a predilection for the fovea and the foveal choriocapillaris also undergoes the greatest degree of degeneration during NA, compared to other regions. Choriocapillaris degeneration appears to be the primary insult in neovascular AMD pathophysiology.

Conclusions:

Enhanced lipofuscin accumulation and subsequent retinal pigment epithelial cell loss during senescence is more likely to result in atrophic AMD when combined with decreases in macular Bruch's membrane permeability and subsequent hard drusen formation. In contrast, accelerated loss of the choriocapillaris during ageing probably predisposes to neovascular AMD. However, further research is required to elucidate the exact mechanics of this process.

1 List of Abbreviations

AMD - Age-related Macular Degeneration
RR - Retinal Region
NA - Normal Ageing
GA - Geographic Atrophy
CNV - Choroidal Neovascularisation
RPE - Retinal Pigment Epithelium
BrM - Bruch's Membrane
PR - Photoreceptor
RP - Rod Photoreceptor
CP - Cone Photoreceptor
FAF - Fundus Autofluorescence
OCTA - Optical Coherence Tomography Angiography
aAMD - Atrophic Age-related Macular Degeneration
nAMD - Neovascular Age-related Macular Degeneration
BLinD - Basal Linear Deposit
OCT - Optical Coherence Tomography
VEGF - Vascular Endothelial Growth Factor

2 Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in adults over 50 years old (Pennington & DeAngelis, 2016). Age is believed to be the most significant risk factor for AMD development (Chen et al., 2008). In a cross continental study, AMD prevalence was 0.2% in people aged 55 to 64 years old, rising exponentially to 13% in those older than 85 years (Smith et al., 2001). At the same time, people are living longer than ever before. In 2001, for the first time in history, there were more persons over 60 years of age than children in the UK (Office for National Statistics (ONS), 2019). Given this association, it is unsurprising that by 2020, the number of people with AMD globally is expected to be around 200 million, increasing to nearly 300 million by 2040 (Wong et al., 2014). Despite the irrefutable epidemiological links between ageing and AMD, there is scant research into the clinical association between these processes which has contributed to limited knowledge regarding the natural history of this condition compared to many others.

AMD is a degenerative disease in people of 50 years old or above, which involves distinct anatomical changes to the retinal region (RR) visible on fundus examination (Bird et al., 1995). Such anatomical changes observed in AMD include debris accumulation, geographic atrophy of cells (GA) and choroidal neovascularisation (CNV) (Ferris et al., 2013). Virtually every measure of our visual function, including visual acuity, contrast sensitivity, field sensitivity and dark-adaptation threshold, shows a functional decline during normal ageing (NA) (Salvi, Akhtar, & Currie, 2006). Degeneration of retinal function during NA is considered a key factor in visual deterioration and anatomical changes to the RR play a major role in this process; debris accumulation, loss of retinal cellular populations and changes to the choroidal circulatory complex have all been implicated in the NA process (Delori, Goger, & Dorey, 2001; S. H. Sarks, Arnold, Killingsworth, & Sarks, 1999; Gao & Hollyfield, 1992; Ramrattan et al., 1994).

Three overarching concepts of anatomical changes therefore appear to pertain to both NA and AMD development; debris accumulation, loss of cellular populations and vascular changes to the choroidal complex. However, whilst such anatomical changes have previously been investigated, it has tended to be on an individual basis. As a result establishing the relative contributions of each to the NA and AMD processes and providing a comprehensive critical review of the range of anatomical changes documented, has not been possible. The majority of anatomical changes to the RR in NA and AMD appear to affect the outer aspects of the RR comprising the photoreceptor layer, retinal pigment epithelium (RPE), Bruch's membrane (BrM) and the underlying choroidal complex (Grossniklaus, Nickerson, Edelhauser, Bergman, & Berglin, 2012; Salvi et al., 2006; Bonilha, 2008). Understanding the structural and functional anatomy of this aspect of the RR is therefore of paramount importance in exploring the association between NA and AMD to investigate its pathophysiology. With this in mind, the review will focus on exploring the outer retinal anatomy followed by changes to the anatomy in these regions during both NA and AMD, respectively.

To date, very few papers have directly compared NA and AMD, and a much smaller minority have used anatomical changes that occur in each of the processes as a means of comparison to investigate AMD pathophysiology. Considering the obvious epidemiological link between NA and AMD, this review will be in the unique position of utilising a comparative anatomical approach with the aim of consolidating understanding of AMD's complex pathophysiology. Moreover, differentiating between anatomical changes in NA and AMD has important implications for clinical practice; establishing key anatomical differences between these processes may lead to earlier AMD diagnosis and clinical intervention. Comparative analysis between the anatomical changes of the RR in the senescent process and AMD should improve current understanding of AMD pathophysiology.

3 Methods

A comprehensive literature search of the PubMed® database was used to identify relevant research articles. The literature search was confined to the English language. Additional articles were selected from a review of the references of the original articles identified in the search. The following key words and combinations of these words were used in compiling the search: age-related macular degeneration; retinal pigment epithelium; ageing; aging; senescence; anatomy; drusen; lipofuscin, basal linear deposits; geographic atrophy; choroidal neovascularisation; atrophic; neovascular.

4 Anatomical Changes to the RR during NA

Ageing can be broadly defined as the chronological deterioration of the physiological functions that are necessary for survival and fecundity, affecting all the individuals of a species (Gilbert, 2000). Whilst the ageing process continues in both those with and without AMD, this review will only consider

individuals whom do not show clinical hallmarks, and are therefore not diagnosed with AMD, to have undergone NA. One of the most important considerations when discussing ageing is its different associations with dividing and non-dividing cellular populations (Marshall, 1987). By virtue of the high degree of differentiation exhibited by cellular populations of the RR, they are accordingly extremely limited in regenerative ability. This has profound consequences for the anatomical changes taking place during the senescent process.

5 Accumulation of Lipofuscin

Lipofuscin is a complex material that can be found in many metabolically active post 144 mitotic cells (such as RPE cells) and a key anatomical change occurring in the RR during the NA process. RPE lipofuscin is mostly composed of lipids with proteinaceous content comprising less than 2% (K.-P. et al., 2008). Evidence that a significant proportion of RPE cell lipofuscin precursors are found in the photoreceptor (PR) outer segments have been demonstrated whereby in rodent models, RPE cells unable to phagocytose PR outer segments, have a much-diminished level of intracellular lipofuscin (Katz, Drea, Eldred, Hess, & Robison, 1986). It is therefore widely acknowledged that lipofuscinogenesis results chiefly from RPE mediated incomplete phagolysosome degradation of PR outer segments throughout life. Regulated by both an innate circadian rhythm and environmental diurnal cycle, outer segment shedding of rod photoreceptors (RPs) occurs at a much faster rate than cone photoreceptors (CPs) (Boulton, Dontsov, Jarvis-Evans, Ostrovsky, & Svistunenko, 1993). Consequently, the accumulation of lipofuscin should occur at a faster rate in regions where there is a high density of RPs.

Signal intensity from fundus autofluorescence (FAF) imaging is used as an index of RPE lipofuscin content (Lois, Hatfyard, Bird, & Fitzke, 2000). In the largest study to date, Delori (2001) found that RPE lipofuscin accumulated quasi-linearly between 20 to 70 years using FAF imaging. Feeney-Burns (1984) also showed that there was an 11% increase in the volume of RPE cells occupied by lipofuscin between the ages of 40 to 80 years. FAF intensity peaked at 11° temporally and 7° nasally; both are regions whereby RP density is reaching maximal levels (Delori et al., 2001). FAF intensity was significantly lower in the fovea (Delori et al., 2001). These data are supported by other FAF and histological studies (Wing, Blanchard, & Weiter, 1978; Ach et al., 2014). Ach et al., (2014) found that the topography of FAF intensity is extremely similar to RP topography as FAF intensity was lowest in the foveal centre and peaked in the perifoveal annulus. However, outside of the macula region, FAF intensity has been noted to decrease more rapidly at increasing eccentricities than RP density (Ach et al., 2014; Delori et al., 2001). This may be partly explained by the reduction in length of RP outer segments with increasing eccentricity (Hendrickson & Drucker, 1992). Nevertheless, it is clear from these data that the accumulation of lipofuscin is extremely closely correlated with RP topographical densities and appears to result from the greater rate of outer segment shedding of RPs compared to CPs.

6 Extracellular Deposits

Whilst there is little contention regarding mechanism of formation and spatial distribution of lipofuscin, there is a degree of discord in literature regarding the implications of RPE cell lipofuscin accumulation. Yasakawa et al. (2007) proposes that lipofuscin accumulation principally acts to congest RPE cell cytoplasm which may result in progressive impedance to bimodal RPE metabolic flux. As a result, there may be sub-RPE accumulations of retinal debris such as drusen or basal laminar deposits. Basal laminar deposits are excrescences that are located between the basolateral plasma membrane of the RPE and RPE basal lamina (van der Schaft, de Bruijnz, Mooy, & de Jong, 1993a). Drusen are focal deposits, over 40% of which are comprised of lipids with a greater proteinaceous content than lipofuscin (L. Wang et al., 2010). They accumulate between the basal lamina of the RPE and the inner collagenous layer of BrM ((Spaide & Curcio, 2010). Hard (hyalinised) drusen are particularly associated with senescence and are generally <63m in diameter (S. H. Sarks et al., 1999).

In a histological analysis of aged eyes, Sarks et al. (1999) concludes that there are two processes for drusen formation; entrapment sites in BrM that may enlarge during nodular accumulation of debris, and also pathological drusen formation through accumulation of debris only. Hard drusen formation during NA therefore appears to chiefly result from structural changes to BrM which create entrapment sites for hard drusen accumulation during senescence. Structural changes to BrM during NA are already well documented in medical literature and therefore substantiate the theory of Sarks et al. (1999). In one study, BrM was observed to increase in thickness from 2m in the second decade to 4.7m in the tenth decade (Ramrattan et al., 1994).

Moreover, increasing elastin fibre calcification and collagen fibre cross-linking have both been observed during ageing of the BrM (Booij, Baas, Beisekeeva, Gorgels, & Bergen, 2010). Accumulation of advanced glycation end-products have also been observed to accumulate within BrM during NA (Booij et al., 2010). Advanced glycation is a feature of BrM thickening and, combined with focal lipid-based hard drusen, reduces BrM permeability during NA (Moore, Hussain, & Marshall, 1995). Moore (1995) also found that hydraulic conductivity of macular BrM halves every 9.5 years, compared to every 19 years in the periphery. Although the reduction in permeability may reduce macromolar exchange to a greater extent that smaller metabolic solutes, impaired metabolic exchange between the choriocapillaris and RPE is likely to occur in addition to impedance of fluid dynamics (Moore & Clover, 2001).

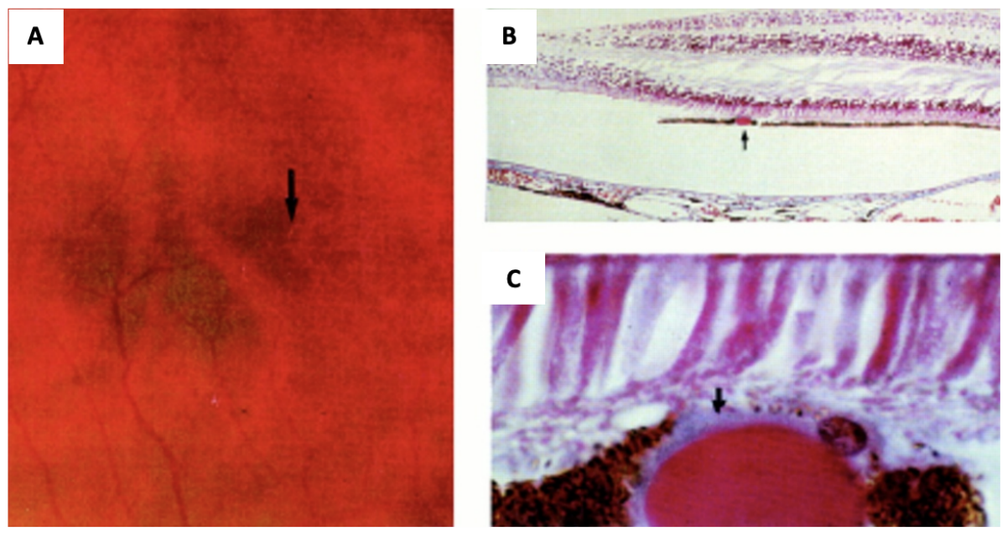

Figure 1. (From Sarks *et al.,* 1999). **Fundoscopic and Histological Imaging of Hard Drusen *in situ*.**

Figure 1A: Fundoscopic image of the smallest clinically detectable hard drusen (arrow). Figure 1B: Light microscopy of histological section (x75 magnification) through fovea showing a hard druse (arrow). Figure 1C: Light microscopy (x500 magnification) of druse in figure 1B, highlighting displacement and attenuation of the RPE layer above.

Although isolated small hard drusen are a common observation in normal, aged eyes occurring in around 93.6% of people between 43 to 86 years old (R. Klein, Klein, & Linton, 1992). Rudolf et al. (2008) reports finding a loss of RPE coverage and associated focal atrophy in regions where hard drusen were present. Whilst a limitation of this study is that the retinas used had been previously diagnosed with age-related maculopathy and therefore not truly comparable to NA, such findings have also been documented by other groups. Johnson et al. (2003) also reported finding structural abnormalities in retinal cells, including PRs, overlying regions where hard drusen were present.

Even small, subclinical drusen were found to compromise regional anatomy. In the large Beaver Dam Eye Study, those with large numbers (8) of hard drusen were found to have an increased 15-year age-adjusted incidence of soft drusen formation and pigmentary abnormalities of 16.3% and 10.6%, respectively, compared to 4.7% and 2.7% for those with <8 hard drusen (R. Klein et al., 2007). It seems that hard drusen can lead to significant changes to retinal anatomy, although this is likely to be according to the number of drusen present. Figure 1 shows drusen of the hard, hyalinised type observed in the right eye of a 71-year-old male with visual acuity 6/6. Although displacement and a degree of attenuation of the RPE cell layer is evident,there is an absence of significant RPE atrophy.

7 RPE Density

Unlike Yasakawa et al. (2007), some authors advocate that lipofuscin principally increases oxidative stress of the RPE by acting as a photoinducible generator of reactive oxygen species (Boulton et al., 1993; Davies et al., 2001; Mazzitello, Arizmendi, Family, & Grossniklaus, 2009). Cumulative exposure to ultraviolet irradiance throughout life creates an ideal oxidative environment to procure reactive oxygen species, which can induce apoptosis of the RPE cell (Boulton et al., 1993). Lipofuscin loaded RPE cells have demonstrated an increased rate of reactive oxygen species generation and reduced viability when they are exposed to visible light, supporting the notion that lipofuscin acts to induce RPE cell death by increasing oxidative stress (Wihlmark, Wrigstad, Roberg, Nilsson, & Brunk, 1997; Davies et al., 2001). Regions of the retina with a higher RP density appear to have increased lipofuscin content and therefore, should have more profound RPE cell loss also.

The concept of changing RPE cell densities to some degree during NA is fairly well supported in the literature. Gao (1992) examined foveal and equatorial (defined as 13mm/45° eccentricity from foveal centre) RRs from pan-retinal tangential sections of 35 eyes from 35 donors, spanning from the second to ninth decade of life using light microscopy. The density of foveal RPE cells did not significantly change during NA ($P>0.2$) (Gao & Hollyfield, 1992). In the equatorial RR however, the density of the RPE cell population decreased uniformly from the second to the ninth decades at 14 cells/mm2/year ($P<0.03$), supporting the above hypothesis (Gao & Hollyfield, 1992). Panda Jonas (1996) also found that there is a marked reduction in RPE cell density during NA in the mid-periphery, a region around 13-35° eccentricity with high RP density and low CP density as shown by figure 1 previously. Interestingly, a similar trend was noted for the foveal RPE cell population. The mean RPE density across the retina was found to decrease by an average of 0.3% per year (Panda-Jonas, Jonas, & Jakobczyk-Zmija, 1996).

Unlike Gao (1992) and Panda-Jonas (1996), Dorey et al. (1989) reported no significant decrease in equatorial RPE cell density in a similar study analysing 30 eyes aged between the first and ninth decades. A more general age-related loss of RPE cell density was still observed, however. Differences can partly be explained by the nature of the analyses. Cross-sectional analysis permits investigation of a restricted cellular population whereas tangential pan-retinal sections and whole mount preparations used by Gao (1992) and Panda-Jonas (1996), allow analysis of a greater proportion of the cellular population. Quantification of RPE cells is also likely to be made more difficult by the disruption to the regular, hexagonal pattern with adoption of a more heterogenous morphology during NA (et al. Forrester, 2002).

One histological study proposes that there is no decline in RPE cell numbers during NA and rather, changes in RPE densities in NA are probably due to retinal area fluctuations (Harman, Fleming, Hoskins, & Moore, 1997). However, biochemical studies have proved that increased apoptosis of RPE cells does occur with advancing age (Del Priore, Kuo, & Tezel, 2002; Dunaief, Dentchev, Ying, & Milam, 2002). Del Priore (2002) used TUNEL labelling to investigate RPE apoptosis and report that apoptotic RPE cells were principally confined to the macula region in aged eyes. No significant change in density was found in the central 3mm diameter region (containing the fovea) supporting the observations of Gao (1992). Whilst the highest rates of apoptosis were also found in this region, whether the rates were higher in the foveal or parafoveal regions were not detailed. The proportion of apoptotic cells increased significantly in the periphery (>12.5 mm from the foveal centre) during NA, although remained lower than the central zone. Del Priore (2002) calculated RPE

cell loss of 2.3% per decade which is similar to the reduction in density of 0.3% per year (3% per decade) calculated by Panda-Jonas (1996). Del Priore (2002) postulate that migration of peripheral RPE cells from the periphery to the macula may compensate for macula cell loss in NA (Del Priore et al., 2002). The results of Del Priore (2002) offer some support to the theory that RPE lipofuscin-induced apoptosis results in a reduction in RPE density that roughly correlates with RP and lipofuscin topography. However, a parallel correlation is difficult to ascertain as pleomorphism and dynamic shifting of the RPE mosaic are complicating factors (Del Priore et al., 2002).

8 PR Density

Closely opposed to the RPE, PRs have been reported to undergo specific age related changes by several studies. Panda-Jonas (1995) found that outside of the foveal centre, the PR density decreased significantly during NA. Both RP and CP cell losses were greatest at an eccentricity of 5-8mm (17.4-27.8°), the region in which the density of RPs is maximal (?, ?). More specifically, the decline of PR density was calculated to be 0.37% and 0.18% per annum for RPs and CPs, respectively. Panda-Jonas (1995) therefore conclude that RPs decrease in density at a faster rate than CPs during NA. Foveal CP density changes as a function of NA could not be evaluated due to technical reasons. Curcio (2001) reported that the density of foveal CPs remains static during NA. In contrast, para-foveal RP densities significantly decreased by 30% over adulthood (Curcio, 2001). Gao (1992) found that there were no significant changes in foveal CP density during NA, supporting the findings of Curcio (2001). However, whilst Curcio (2001) found that there was an undetectable reduction in RP density at 8.4mm eccentricity (29.2°), Gao (1992) recorded significant RP (and CP) density losses in the equatorial retina during NA. Although other studies have shown significant decreases in foveal CP densities during NA, the concept of preferential vulnerability of RPs compared to CPs in NA prevails through medical literature (Song, Chui, Zhong, Elsner, & Burns, 2011; Gao & Hollyfield, 1992; Curcio, 2001; Panda-Jonas, Jonas, & Jakobczyk-Zmija, 1995).

It is estimated one RPE cell provides trophic support for thirty to fifty PRs superiorly (Zinn, K. M. and Marmor, 1979). There is less literature available regarding PR apoptosis in NA compared to the equivalent RPE. Despite this, given the metabolic reliance of PRs on a well-functioning RPE, it can be deduced that losses of RPE cells should promote apoptosis of the superiorly located PRs. Indeed, PRs are known to undergo cell death by apoptosis when separated from the RPE during retinal detachment (Lo, Woo, Wong, & Wong, 2011). The theory that regions of RPE cell loss correlate with regions of PR loss is supported by two of the three main studies investigating PR changes during NA (Panda-Jonas et al., 1995; Gao & Hollyfield, 1992). The only study to investigate PR and RPE changes together, Gao (1992) explicitly conclude that PRs and RPEs show parallel losses during NA.

Such anatomical changes during NA generally concur with observed functional changes. The loss of scotopic sensitivity and dark adaptation (accorded by RPs) throughout the senescent process is greater than the equivalent loss of photopic sensitivity (accorded by CPs) (Jackson, Owsley, & Curcio, 2002). Elliott (1987) established that decreases in the contrast sensitivity of aged individuals were chiefly due to neural changes instead of optical factors such as pupillary miosis and lens photo absorption. Some aspects of visual function changes are not as readily explained by the aforementioned anatomical changes. Central visual acuity facilitated by CPs is stable until 44 years, after which there is a slow deterioration (Sjöstrand, Laatikainen, Hirvelä, Popovic, & Jonsson, 2011). A 0.3 LogMAR reduction (equivalent to Snellen reduction from 6/6 to 6/12) occurs between 44 and 88 years, equating to a yearly reduction of 1.7% (Sjöstrand et al., 2011). Curcio (2001) calculated that there must be a loss of 75% of CPs to cause a corresponding decrease in visual acuity from 6/6 to 6/12, and such profound losses have not been documented during NA. The reasons behind this remain to be determined but may involve other changes to the neurosensory retina such as ganglion cells (Gao & Hollyfield, 1992).

9 Choriocapillaris

Wang et al. (2016) reported that choriocapillaris density was not significantly associated with age (Q. Wang et al., 2016). However, the study method was not designed to analyse the relationship between choriocapillaris density and NA. Moreover, the spectral domain optical coherence tomography angiography (OCTA) device used to measure choriocapillaris density in the study is believed to have limited capability in penetrating the RPE layer, thus is likely to lead to a major underestimation of choriocapillaris thinning. Contrary to the findings reported by Wang et al. (2016),Ramrattan et al. (1994) reported a decrease in choriocapillaris density of 45% and a reduction in overall choroidal thickness of 57%, over 10 decades. A limitation of this study is that the analysis was of donor eyes and therefore, in vivo factors were not accounted for. However, these histological findings also correlate with changes observed in vivo in a cross-sectional study involving 72 healthy participants aged 20 to 80 years (mean 47.4 years) (Sacconi et al., 2019).

Sacconi et al. (2018) found a significant negative correlation between choriocapillaris perfusion density and age for foveal, perifoveal and parafoveal regions, respectively ($p<0.001$ for each region). Foveal perfusion density was observed to decrease from a mean of 77% in participants aged 20-29 to 70% in those aged 70-80 years old. Perfusion density decreased the most in the foveal region, followed by parafoveal and then perifoveal; this is similar to the findings of another recent swept source OCTA study (Nassisi et al., 2018). Sacconi et al. (2018) postulates that the reductionnin choriocapillaris perfusion density during NA results from a reduction in choriocapillaris vessel calibre instead of a reduction in number, as vessel diameter also showed a strong negative correlation with age ($p<0.001$). Ramrattan et al. (1994) substantiates this, arguing that the reduction of choriocapillaris density during senescence is secondary to a reduction in choriocapillaris vessel diameter. Reduced perfusion of the RR due to morphological

changes to the choriocapillaris may cause an increased level of oxidative stress in the outer RR and contribute to the loss of cellular populations and other senescent changes (Bhatt, Groeger, McDermott, & Cotter, 2010; Boulton et al., 1993; Davies et al., 2001; Friedman, Smith, & Kuwabara, 1963).

10 Anatomical Changes to the RR during AMD

10.1 Extracellular Deposits

There are several forms of extracellular deposits that are linked to AMD pathophysiology. Soft drusen and basal linear deposits (BLinD), (collectively referred to as membranous debris) are recognised as specific anatomical changes of AMD (S. Sarks, Cherepanoff, Killingsworth, & Sarks, 2007). Other AMD associated extracellular deposits include basal laminar deposits and sub-retinal drusenoid deposits. basal laminar deposits correlate with AMD only when they thicken or incorporate vesicular structures and can occur during NA as described in previously. Figure 2 shows that basal laminar deposits are located between the RPE basement membrane (innermost layer of Bruch's membrane) and the RPE plasma membrane (Van Der Schaft, Mooy, De Bruijn, & De Jong, 1993b). Basal linear deposits (BLinDs) by contrast are found between the RPE basement membrane and the inner collagenous zone of Bruch's membrane (BrM) also shown by Figure 2 (S. H. Sarks, Van Driel, Maxwell, & Killingsworth, 1980).

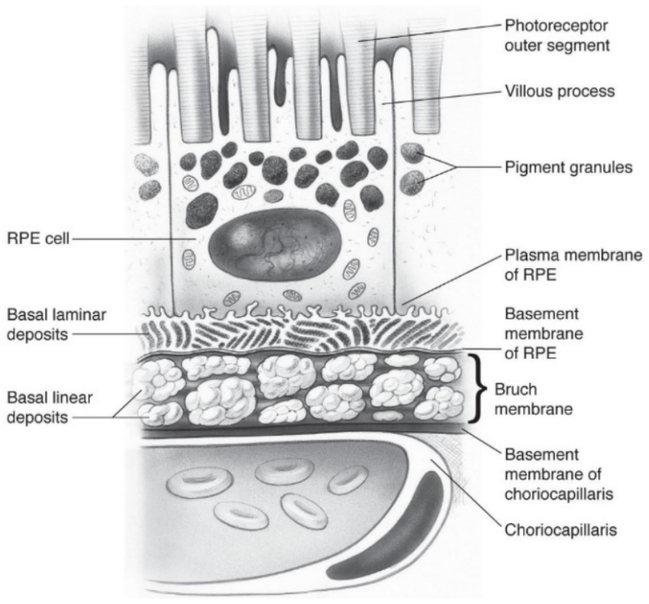

Figure 2. (From American Academy of Ophthalmology, 2019). **Anatomy of basal laminar deposits and BLinDs.**

Schematic depicting the relative anatomical locations of basal laminar deposit and BLinD in the RR.

Although BLinDs are often diffusely spread through BrM, they very commonly coalesce to create focal mounds manifesting as soft drusen (J. P. Sarks, Sarks, & Killingsworth, 1994). Soft drusen appear as yellow excrescences beneath the RPE layer but unlike hard drusen, tend to be larger and have amorphous borders (Kanski, 2003). More anatomically distinct is another subtype of extracellular deposit, called sub-retinal drusenoid deposits (also known as reticular drusen). Sub-retinal drusenoid deposits aggregate between the apical surface of the RPE and overlying PRs (Huisingh et al., 2016). The Beckman Classification is a commonly used method to define chronological AMD stages based on anatomical changes to the RR with particular emphasis on the aforementioned extracellular deposits (Ferris et al., 2013). Table 1 below depicts the Beckman Classification, illustrating the differences between extracellular deposit morphology and association with NA and AMD.

Table 1. (From Ferris *et al.,* 2013).

Diagnosis and Staging of AMD Based on Anatomical Features Associated with the RR.

Stage	Anatomical Features
Normal (aged eyes)	Small, hard drusen <63μm
Early AMD	Medium (soft) drusen 63-125μm
Intermediate AMD	Extensive medium soft drusen *Or* ≥1 large druse(n) >125μm *Or* Both
Late AMD	Signs of geographic atrophy (GA) *Or* Signs of choroidal neovascularisation (CNV) *Or* Both

During histopathological analysis of 41 human eyes, Curcio (1999) found that eyes with AMD were 24 times more likely to have BLinDs or large drusen when compared to age-matched controls (p=0.002) (Curcio & Millican, 1999). Curcio (1999) reached the conclusion that membranous debris may be significant for the progression to the late stages of the disease (Curcio & Millican, 1999). Sub-retinal drusenoid deposits have also been found to increase the risk of AMD development by 2.24 times when compared to controls and are associated with AMD progression to both GA and CNV (Huisingh et al., 2016). The mechanism of soft drusen formation is complex. Based on analysis of clinico-pathological cases, it has been mooted that there are two main mechanisms leading to the formation of soft drusen (J. P. Sarks et al., 1994). Firstly, soft drusen may form from the aggregation and fusion of multiple small, hard drusen in a process described as 'hard drusen clustering'. Subsequent breakdown of these drusen clusters results in a varied degree of morphological softening and is thought to predispose to GA. Secondly, accumulation of diffuse extracellular debris in BrM (BLinD in particular) can create soft drusen, a method which is more associated with onset of CNV (J. P. Sarks et al., 1994).According to these findings, soft drusen pathogenesis seems highly important in determining the course and manner of AMD progression.

There is an association between anatomical topography of the retina and the formation of extracellular lesions. Indeed, soft drusen tend to form in regions of the retina where the concentration of CPs is higher, such as the fovea (Curcio, 2018; J. Sarks, Arnold, Ho, Sarks, & Killingsworth, 2011). Figure 3 illustrates through various ophthalmic imaging techniques, the appearance, morphology and anatomical locations of typical soft drusen. Drusen appear concentrated and enlarged in the macula compared to the peripheral retina; it is relatively uncommon for soft drusen to develop in the retinal periphery (Ardeljan & Chan, 2013). Such morphological findings concur with current evidence regarding functional implications of the

deposits. Frennesson (1995) noted that the mean colour contrast sensitivity in patients with soft drusen was significantly lower than that measured in age-matched controls ($p<0.0002$ for each of the three CP types) (Frennesson, Nilsson, & Nilsson, 1995).

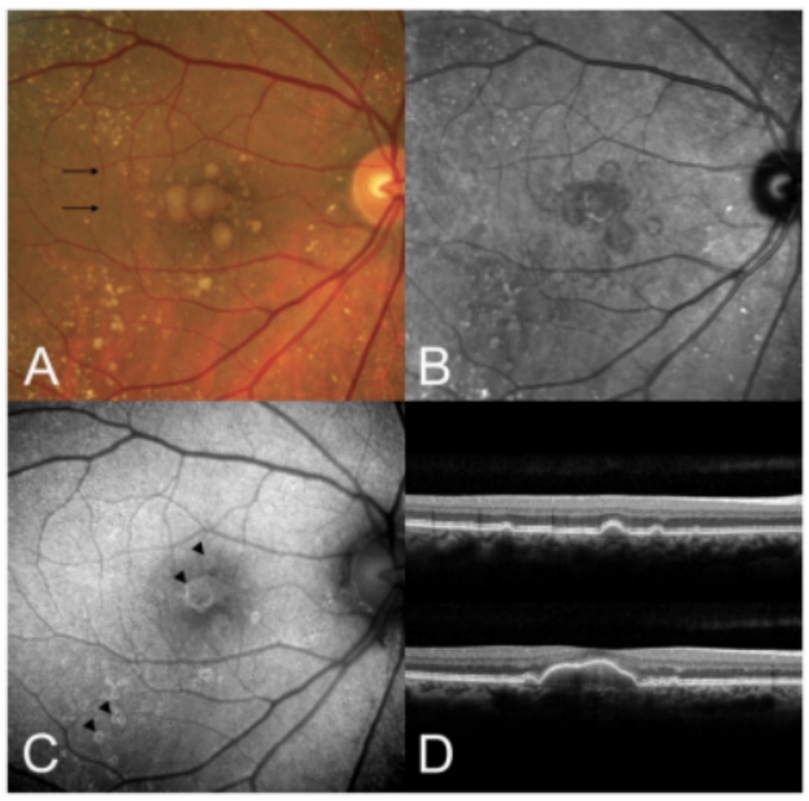

Figure 3. (From Spaide, 2010). **Anatomy of Soft Drusen.** 458 Figure 3A: *En face* colour fundus photography showing extensive large soft drusen 459 in macula and much less, in peripheral retina. Arrows indicate scan lines for 460 transverse section optical coherence tomography (OCT) in figure 3D. Figure 3B: 461 scanning laser ophthalmoscopic image with darkened areas at soft drusen sites. 462 Figure 3C: Scanning laser ophthalmoscope image illustrating hyperfluorescence at 463 soft drusen boundaries indicated by the arrowheads. Figure 3D: Transverse section 464 OCT at sites identified in 6A. There is clear accumulation of membranous debris 465 under the RPE, with main accumulations indicated by arrows.

Prevailing hypotheses have tended to place emphasis on drusen components being derived from either RPE cells or choroidal vasculature (Hageman et al., 2001; Penfold, Madigan, Gillies, & Provis, 2001). Crabb et al. (2002) proposes a more holistic mechanism of drusen formation. They suggest that RPE waste products and extravasation of choroidal vasculature components, together, provide abundant material for drusen formation. Over time, immunological involvement may lead to more diffuse expansion and accumulations. The findings of Johnson et al. (2000) suggest immune pathogenesis of RPE cells may indeed contribute to drusen formation. Immunoreactivity analysis revealed that immunoglobulin and complement components were concentrated in soft drusen and drusen-associated RPE cells. However, some of these measures of immunological involvement were also present in hard drusen and associated RPE cells. Although immunoreactivity of drusen has been described by other research groups (Mullins, Russell, Anderson, & Hageman, 2000), others have failed to detect any form of immunoglobulin component of drusen (Van Der Schaft et al., 1993b), thus the exact role of the immunological system in drusen pathogenesis remains unclear.

10.2 Geographic Atrophy

GA is the underlying process that occurs during aAMD. GA involves progressive and irreversible atrophy to the RPE layer, overlying PRs and underlying choriocapillaris epithelial cells (J. P. Sarks, Sarks, & Killingsworth, 1988; ?, ?; F.G., E.C., S., & M., 2014). aAMD is the most common form of late AMD accounting for around 90% of such cases (Morris, Imrie, Armbrecht, & Dhillon, 2007).

Extracellular deposits, soft drusen in particular, are strongly associated with development of AMD into the GA phase (M. L. Klein et al., 2008). The mechanism by which GA arises is an area of contention in current literature, split between those advocating apoptotic or necrotic pathways, respectively (Dunaief et al., 2002; Hanus, Anderson, & Wang, 2015). Using TUNEL immunochemistry to identify apoptotic cells, Dunaief et al. (2002) discovered that in eyes with late AMD, there was a significant increase in TUNEL-positive RPEs and PRs. Dunaief et al. (2002) concludes that GA is a sequela of cellular apoptosis. Limitations of this study however, included that AMD eyes analysed were affected by both GA and CNV thus the result is likely to be biased and a true correlation between apoptosis and GA difficult to establish.

In contrast, Hanus (2015) proposes that the process of GA is chiefly necroptotic. Figure 4 illustrates a possible simplified mechanism of GA pathogenesis as described by Hanus (2015). Hanus (2015) proposes that the formation of soft drusen leads to a progressive bidirectional impedance to membranous flux between the choriocapillaris and the RPE. Deprivation of trophic support may accentuate oxidative stresses experienced by the RPE cell, shown in figure 4A. As drusen accumulate, there is macroglial recruitment and involvement of the complement cascade with the RPE cells overlying drusen becoming oedematous and beginning to undergo necrotic and necroptotic cell death resulting in widespread GA, correlating to drusen distribution (figure 4C). Subsequently deprived of trophic support, the overlying PRs atrophy too.

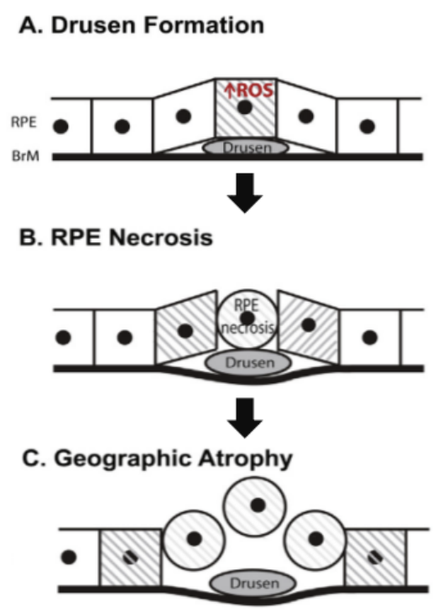

Figure 4. (Adapted from Hanus, 2015). **Possible Simplified Mechanism of GA Pathogenesis.**

Drusen formation amplifies oxidative stresses (figure 4A), causing involvement of inflammatory pathways and necroptosis/necrosis of the RPE (figure 4B), finally leading to regions of GA (figure 4C).

Drusen formation amplifies oxidative stresses (figure 4A), causing involvement of inflammatory pathways and necroptosis/necrosis of the RPE (figure 4B), finally leading to regions of GA (figure 4C). It seems that soft drusen formation plays a pivotal role in GA development; excessive diffusion distance from the choriocapillaris to the RPE and impedance to membranous flux due to the hydrophobic nature of the soft drusen are two theories that may cause primary insult to the RPE (Curcio, Zanzottera, Ach, Balaratnasingam, & Freund, 2017; Rudolf et al., 2008). Soft drusen in situ can be observed in figure 5C, in association with a typical GA pattern illustrated in figure 5A. Whilst apoptosis may be a supplementary mechanism for RPE cell death in aAMD, the inflammatory changes associated with GA highlight necroptosis and necrosis as potentially more significant means for cell death (Anderson, Mullins, Hageman, & Johnson, 2002). However, their relative contributions remain an area of intense research.

In areas of large drusen formation and focal atrophy of the RPE, there appears to be a corresponding RPE hypopigmentation, presumably due to RPE cell death and loss of the intracellular pigments such as melanin (Bressler, Silva, Bressler, Fine, & Green, 1994). Figure 5A shows a perifoveal RPE hypopigmentation indicating regions affected by GA. Focal hyperpigmentation is correlated with areas of hypertrophy of the RPE which possibly develops as a response to the initial RPE atrophy, although no overt hyper pigmentary response can be observed in figure 5A (Bressler et al., 1994). Although these findings are in keeping with current knowledge of GA processes, it must be noted that the aforementioned conclusions were reached from clinicopathological analysis of only 3 eyes from 2 patients (Bressler et al., 1994).

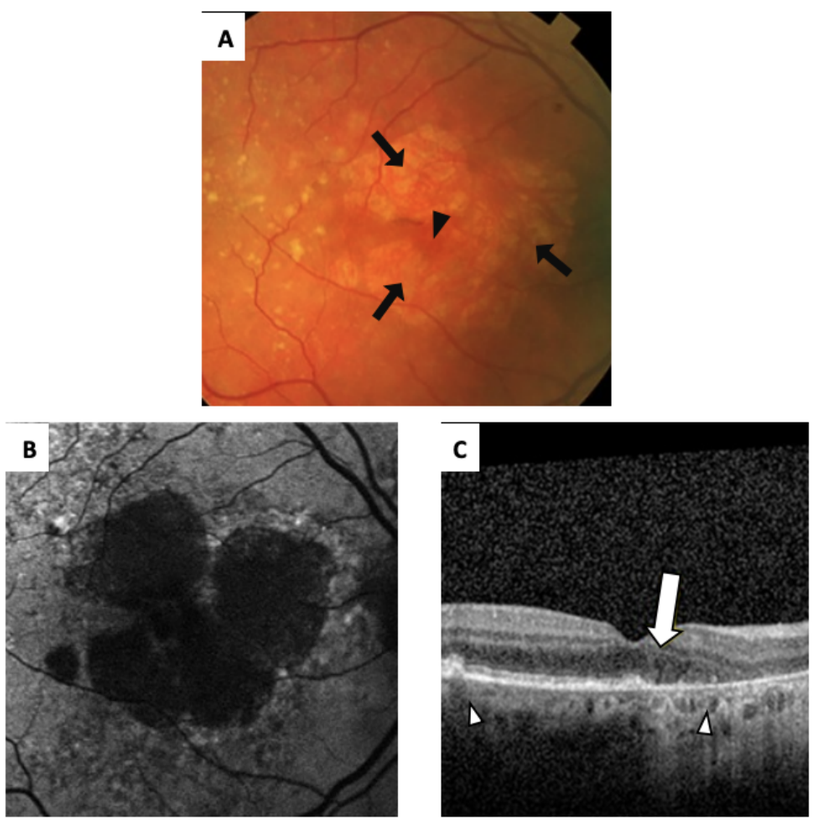

Figure 5. (Adapted from Danis, 2015). **Retinal Images of the Right Eye in a Patient with aAMD.**

Figure 5A: *En face* colour fundus photography with RPE hypopigmentation indicating regions of extensive GA (arrows) with foveal sparing (arrowhead). Figure 5B: FAF image showing clear demarcation between area of GA (dark) and lighter, functional RPE. Figure 5C: Transverse section OCT. Arrow shows demarcation between normal RPE (inferior and immediate left) and RPE affected by GA (inferior and immediate right). Soft drusen are also present under normal RPE (arrowheads).

Although GA is characterised by both RPE and choriocapillaris epithelial cell atrophy, RPE atrophy probably precedes choriocapillaris breakdown. An experimental study by Korte (1984) provided evidence that isolated destruction of the RPE subsequently leads to choriocapillaris atrophy. The choriocapillaris remained normal in areas where the RPE was preserved. Although the study was animal based, histological studies carried out on human tissue reach the same conclusion. McLeod et al.(2009) found that in regions of GA, there was well-defined sub-macular RPE atrophy and degeneration of the underlying choriocapillaris. External to the GA, RPE showed signs of pigmentary changes and drusen accumulations whilst choriocapillaris morphology appeared relatively normal. Mean capillary diameters also showed a reduction from 13.9m ($\pm$1.4m) in non-atrophic regions, to 10.34 ($\pm$0.7m) in border regions to 7.9 ($\pm$1.2m) in GA regions ($p<0.01$). There was no significant statistical difference between the diameters in non-atrophic regions of GA eyes and normal controls ($p=0.44$). These data substantiate the conclusion made by Bhutto (2012) that during GA, RPE atrophy is the primary insult and choriocapillaris atrophy is secondary to this.

Visual loss due to GA tends to be slow and progressive, taking around 5-10 years to result in legal blindness (Arnold & Heriot, 2007). GA progression rates show a large variation in the literature ranging from 0.53 mm2/year to 2.6mm2/year (median 1.78mm2 573 /year) (Batiolu, Ouz, Demirel, & Özmert, 2014; Sunness et al., 2007). A dual combination of both colour fundus photography and FAF have been reported as the optimal method to analyse GA progression patterns (Khanifar et al., 2012). Domalpally et al. (2016) analysed GA progression, reporting a GA enlargement rate of 1.44mm2 /year averaged across the two methods (colour fundus photography= 1.45mm2/year, FAF= 1.43mm2/year).

This figure approximates closely to the median 1.78mm2/year quoted previously, thus appears a reliable general estimate. There appears to be a degree of contention in the literature regarding the expansion of GA areas as a function of time. Some believe that GA lesions, with the exception of a few very small or very large lesions, show a relatively linear progression over time (Feuer et al., 2013). However, others suggest that the enlargement rates differ exponentially according to the size of the GA lesion. Sunness et al. (2007), found that GA lesions of size $<1.3mm^2$ and $8.3mm^2$, progressed at rates of 0.8 and $3.0mm^2$/year, respectively.

The theory of GA lesions expanding at different rates is further supported by the observations of Lindner et al. (2015) who noticed that progression of atrophic areas is 2.8 times faster toward the retinal periphery than towards the fovea. In a study by Schmitz-Valckenberg et al. (2016), foveal GA progressed at 1.28mm2/year and extrafoveal at 2.05mm2/year ($P=0.001$). Indeed, another study showed that foveal sparing occurred until there was an area of GA greater in size than the optic disc (J. P. Sarks et al., 1988). Although visual acuity may be preserved for a period, perifoveal atrophy in itself is understood to affect general visual performance and scotopic vision in particular (Brown, Goldstein, Chan, Massof, & Ramulu, 2014). It has been hypothesised that

such trends indicate that RPs exhibit a higher vulnerability to GA induced cell death than CPs. Bhatt et al. (2010) and colleagues demonstrated that both RPs and CPs produce reactive oxygen species in response to stress of serum deprivation. Findings from other studies have suggested that RPs seem to be die preferentially to CPs in response to an oxidative stress stimulus (Komeima, Rogers, Lu, & Campochiaro, 2006). These data imply that RPs are more sensitive to increases in reactive oxygen species and therefore undergo cell death before CPs, resulting in further increases in oxidative stresses which eventually leads to CP death.

10.3 Choroidal Neovascularisation

The process of CNV defines nAMD, the second form of late stage AMD wherein there is a pathological growth of new and largely incompetent choroidal vasculature,extending from the choroidal complex into the sub-retinal or sub-RPE spaces (Kanski, 2003). Subsequent extravasation and haemorrhage create neovascular membranes, consisting of proteinaceous fluid, lipid and blood in the aforementioned anatomical spaces. Although less common than aAMD, comprising around 10% of late AMD cases, nAMD results in around 75-90% of AMD associated blindness(Morris et al., 2007; R. Klein, Klein, Jensen, & Meuer, 1997).

CNV lesions can be classified as classic or occult based on their fluorescein angiography appearance; occult lesions are poorly defined and haemorrhage less intensely whilst classic lesions are well defined and haemorrhage intensively (Kanski, 2003; Domalpally, A. and Danis, 2008). Occult neovascular membranes accumulate under the RPE (can also be termed type 1 lesions) whilst classic neovascular membranes lie above the RPE in the sub-retinal space (can also be termed type 2 lesions) (Lim, Mitchell, Seddon, Holz, & Wong, 2012). Occult lesions are the more common type; classic lesions tend to comprise around 20% of all neovascular membranes (Cohen et al., 2007; Olsen, Feng, Kasper, Rath, & Steuer, 2004).

There is much contention in the literature regarding anatomical changes to the choroidal complex during nAMD, but it seems this may be due to the different stages at which studies took place. Invernizzi et al. (2018) found that during the active disease phase of both CNV types, there was a significant increase in both sub-foveal and mean choroidal thicknesses compared to the control group ($p<0.0001$). Sub foveal choroidal thickness increased the most during active nAMD from 164m to 175m . Figure 6 is a collection of OCT scans depicting the findings of Invernizzi et al. (2018). There is an increase in the thickness of the choroidal layer in figure 6D (active CNV) compared to figure 6C (inactive CNV) over a follow-up period of 12 months. The arrow in figure 6B shows a large sub-retinal fluid accumulation, resulting from classic CNV extravasation.

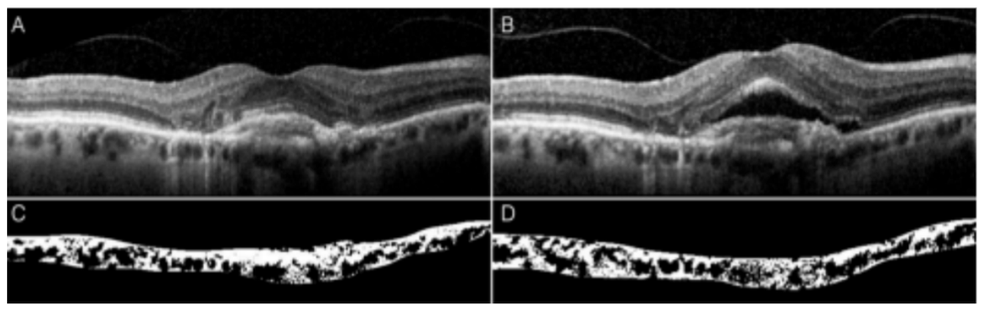

Figure 6. (From Invernizzi *et al.,* 2018). **CNV Imaged by OCT.** Figures 6A and 6B are transverse section OCT scans of the foveal region of a single patient. Figure 6A is a baseline image and figure 6B is taken after a 12-month period from which there is a progression from inactive CNV (6A) to active CNV (6B). Figures 6C and 6D are transverse section reconstructions of the choroidal complex represented in 6A and 6B, respectively. The arrow indicates a region of sub-retinal exudate accumulation resulting from classic CNV.

Since age and choroidal thickness are negatively correlated, the findings of Invernizzi et al. (2018) demonstrate involvement of anatomical changes in active nAMD that are not present in NA (Margolis & Spaide, 2009). Other authors have also investigated anatomical changes to the choroidal complex during nAMD (Manjunath, Goren, Fujimoto, & Duker, 2011; Govetto et al., 2017). Manjunath et al. (2011) carried out a retrospective review and discovered that the mean sub-foveal choroidal thickness in nAMD patients was 194.6m compared to a mean sub-foveal choroidal thickness of 213.4m in aAMD patients. Sub-foveal and temporal choroidal thicknesses were also found to be significantly lower in nAMD patients than in aAMD patients ($p=0.037$) by Govetto et al. (2017). In this study, participants were diagnosed with nAMD in one eye and aAMD in the other, providing a like-for-like comparison between the two disease types and reducing the inter-individual variability in choroidal measurements.

Both Manjunath et al. (2011) and Govetto et al. (2017) studied patients who were already diagnosed with nAMD based on clinical observations such as CNV, intraretinal or subretinal fluid. On the other hand, Invernizzi et al. (2018) enrolled participants as cases only if they had no signs of neovascular activity at the baseline appointment, and subsequently developed neovascular activity at the follow-up appointment 12 months later. It can be proposed based on these data that whilst there may be a transient increase in choroidal thickness during early active CNV in nAMD, the choroidal complex subsequently undergoes significant atrophy to become much thinner. Data from these studies also suggests that CNV and subsequent atrophy of the choroidal complex affects the foveal region of the macula to a greater degree than in GA (Invernizzi et al., 2018; Manjunath et al., 2011; Govetto et al., 2017).

A preferential vulnerability of the fovea to CNV compared to the rest of the macula may be explained in part by the particularly high density of foveal PRs which are likely to mean the metabolic demands of this region are higher than the rest of the macula (et al. Purves, 2001). Provis et al. (2005) argues that adaptations of the sub-foveal choriocapillaris such as wider lumens and a thinner elastic lamina, may in the long term make the region more vulnerable to senescent changes such as membranous debris accumulation. Indeed, membranous debris accumulation has a predilection

for the fovea and is especially linked to the manifestation of CNV (Curcio, 2018; J. Sarks et al., 2011; J. P. Sarks et al., 1994). The choriocapillaris also supplies little in excess of the metabolic demand under normal conditions and combined with its lobular morphology, is likely to both inadequately perfuse and insufficiently remove waste from the foveal region in particular (Provis, Penfold, Cornish, Sandercoe, & Madigan, 2005; Alten, Clemens, Heiduschka, & Eter, 2013).

Degeneration of the choriocapillaris may be the instigating mechanism behind CNV development. McLeod et al. (2009), examined the relationship between choriocapillaris and RPE changes in AMD by analysing post-mortem choroids. They found that there was a 50% reduction in viable choriocapillaris immediately adjacent to areas of CNV (i.e. in advance of CNV involvement). Interestingly, areas of significant choriocapillaris degeneration around sites of active CNV were associated with viable RPE. A limitation of this study is that findings were based on observations of 3 post-mortem specimens only. However, other studies substantiate the findings of McLeod et al. (2009). Moult et al. (2014) performed swept source OCTA in vivo imaging of 63 eyes of 32 healthy controls and 19 eyes of 15 nAMD patients. They discovered that in all 16 eyes with identifiable CNV on SS-OCT, severe choriocapillaris alteration (atrophy and/or flow impairment) was present under regions of CNV. Moreover, in 14 of the 16 eyes, they discovered that the CNV lesions were surrounded by a region of severe choriocapillaris alteration. These observations are strong evidence to suggest that the initial insult to the RR in nAMD is the loss of choroidal vasculature (Bhutto & Lutty, 2012).

Proliferation of new choroidal architecture during the CNV phase of nAMD is believed to be principally stimulated by RPE-mediated release of the pro-angiogenesis factor VEGF (Spilsbury, Garrett, Shen, Constable, & Rakoczy, 2000). Immunological involvement is inextricably linked with the production of VEGF (Thurman et al., 2009). The chronological order of the CNV process remains to be determined; some authors propose that diffuse membranous debris deposition induces hypoxia whilst others suggest pre-existing hypoxia may be the cause of debris deposition and subsequent CNV (Schlingemann, 2004; Feigl, Brown, Lovie-Kitchin, & Swann, 2007). It is likely both theories contribute to CNV development, but the latter may be more significant given that choriocapillaris degeneration appears to be the primary insult in CNV (J. P. Sarks et al., 1994; Bhutto & Lutty, 2012).

The differences between occult and classic neovascular membranes appear to have important implications on the resulting anatomical changes observed in the RR of nAMD eyes (Kanski, 2003; Lim et al., 2012). Schmidt-Erfurth (2007) explored the anatomical changes using fluorescein angiography and indocyanine green angiography in 158 patients. In fluorescein angiography of classic CNV, there was an irregular, steep elevation at the lesion site which was encircled by a prominent ring akin to a dark halo, as depicted in figure 7A below. The central prominence appears to result from the ventral displacement of the neural retina due to sub-retinal neovascular membrane, illustrated in figure 7B. Schmit-Erfurth (2007) deduced that the circumferential halo is a proliferating and actively leaking CNV zone, thus suggesting extravasation and haemorrhage occurs medially to this zone in classic CNV.

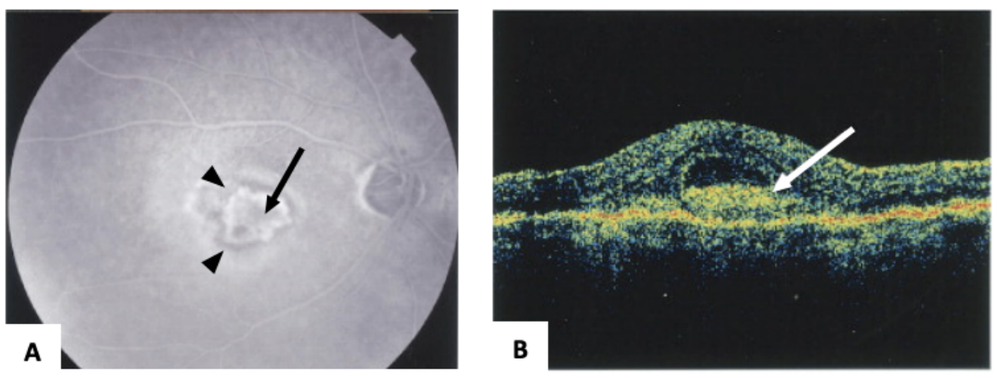

Figure 7. (From Hughes *et al.*, 2005). **Classic CNV in 91-year-old Female.**

Figure 7A: Fluorescein angiography image of well-defined classic CNV lesion (arrow) encircled by circular (halo) zone of choroidal proliferation (arrowheads). Figure 7B: Transverse section OCT image of discreet subretinal neovascular membrane with associated fluid (arrow) resulting from classic CNV.

Fluorescein angiography analysis of occult CNV demonstrated more numerous areas of confluence with a partially convex and flatter appearance (see figure 8B) (Schmidt-Erfurth, Kriechbaum, & Oldag, 2007). The flatter morphology of the occult lesion is suggested in literature to be resulting from neovascular membrane compression by a largely intact RPE layer (Grossniklaus & Green, 2004). Indocyanine green angiography observation revealed a relatively well-perfused central lesion; better perfusion of the RPE and neural retina than in classic CNV may explain the slower visual deterioration observed in occult CNV. Whilst histological analyses generally agree with fluorescein angiography and indocyanine green angiography findings, Lafaut et al., (2000), concludes that in classic CNV, there may also be smaller sub-RPE lesions. This data supports more wider theoretical opinion that nAMD may involve a greater degree of classic CNV than previously thought (J. D. Gass, Yannuzzi, Kramer, & Green, 1994; J. D. M. Gass, 1997). Indeed, figure 8B demonstrates an accumulation of subretinal fluid, in addition to the expected sub RPE fluid, in a patient with occult CNV.

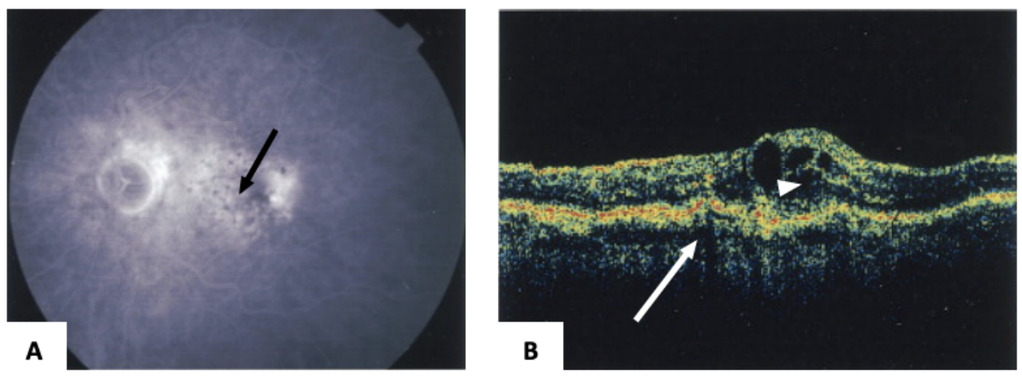

Figure 8. (From Hughes *et al.*, 2005). **Occult CNV in 82-year-old Female.**

Figure 8A: Fluorescein angiography image of poorly defined CNV consistent with the occult type (arrow). Figure 8B: Transverse section OCT image showing significant sub-RPE fluid (arrow) and resulting RPE disruption. Subretinal fluid is also present (arrowhead).

Unlike GA, CNV expansion rates are sparsely represented in medical literature, presumably due to a more rapid progression. Stevens et al. (1997) analysed the natural history of eyes in which occult or both occult and classic CNV was diagnosed over a period of 12 months. They found that 32% of the

lesions doubled or more in size and 38% increased by 4 or more MPS disc areas. 1 MPS disc area is equivalent to a retinal area of 2.54mm2 (Sunness et al., 2007). Functional deficits resulting from the anatomical changes during CNV are stark and more rapid than in GA. A systematic meta-analysis by Wong et al. (2008) showed patients with untreated nAMD can expect functional declines in visual acuity of 1 to 3 LogMAR lines over 3 months, and 3 to 4 lines over the course of a year. A loss of 3 lines represents a halving of the retina's resolving power. Severe vision loss of more than 6 lines was present in over 40% of untreated patients at 3 years. The more aggressive, classic CNV type is often associated with earlier and more substantial visual loss due to direct damage to the neural retina, whereas occult CNV is associated with a slower visual deterioration due to a greater preservation of the RPE layer (Bressler, Finklestein, Sunness, Maguire, & Yarian, 1990).

11 Discussion

This review explores and analyses the anatomical changes that take place in the RR during NA and in AMD, with explicit reference to how such changes relate to AMD pathophysiology. Based on anatomical changes, the senescent process appears to be strongly associated with, and has profound implications, on AMD pathophysiology.

Changes to BrM appear critical in the pathogenesis of all aspects of AMD. Such is the apparent role of BrM in NA and AMD modulation, Moore (2001) moots the idea of a BrM permeability threshold, below which NA changes occur and above which, AMD is more likely. Although likely to be a gross oversimplification, thickening of BrM such as via proteinaceous modification and accumulation of advanced glycation end products compounded by cross-linking as oxidative defence mechanisms deteriorate through senescence, is theorised to take place (Booij et al., 2010; Crabb et al., 2002). Such changes predispose to extracellular debris accumulations which appear to initiate early AMD. Extracellular deposits are anatomical changes common to the processes of both NA and AMD. In NA, drusen are characterised by small size (<63m), distinct borders and a hardened texture (S. H. Sarks et al., 1999). In contrast, amorphous (soft) drusen are a defining anatomical feature of AMD eyes contributing to clinical staging of the disease (Ferris et al., 2013).

Formation of hard drusen appears to be more strongly linked with BrM changes during NA than lipofuscin accumulation, creating a relatively diffuse distribution of hard drusen across the retina (S. H. Sarks et al., 1999). One way in which soft drusen may manifest is via coalescing BLinDs; this process is also linked to BrM thickening during senescence (J. P. Sarks et al., 1994; S. H. Sarks et al., 1999). Whilst appearing relatively innocuous in NA, hard drusen degradation has also been linked to AMD pathophysiology. Breakdown of hard drusen has been postulated to be the other main mechanism of soft drusen formation (J. P. Sarks et al., 1994). Whether the origin of soft drusen is from hard drusen or diffuse BLinD may therefore be important for the pathological course of AMD (Yehoshua et al., 2011; J. P. Sarks et al., 1994). A hard drusen origin appears to preferentially lead to GA in aAMD, whereas a BLinD origin appears to preferentially predispose to CNV in nAMD (J. P. Sarks et al., 1994). However, confounding factors complicate the process of deducing explicit conclusions as basal laminar deposits accumulation has also been linked to onset of GA; basal laminar deposits form across the RR like hard drusen but are not considered a key part of the NA process (S. Sarks et al., 2007; van der Schaft et al., 1993a). Soft drusen are almost universally confined to the macula, with membranous debris having a particular affiliation with the fovea (Curcio, 2018; J. Sarks et al., 2011; Ardeljan & Chan, 2013). The exact reason that hard drusen breakdown in AMD but remain relatively well preserved in NA remains to be discovered. However, this may be due to the lack of immunological involvement in NA compared to AMD (Crabb et al., 2002).

Static foveal RPE densities during NA have been reported, whereas RPE cell loss occurs elsewhere in the macula, thus creating a foveal sparing phenomenon (Gao & Hollyfield, 1992; Del Priore et al., 2002). Evidence suggests that foveal sparing is also demonstrated in GA of aAMD whilst there is cell loss in the parafoveal and perifoveal regions (Lindner et al., 2015; Schmidt-Erfurth et al., 2007; J. P. Sarks et al., 1988). The trends appear to be explained by increases in oxidative stress, although the mechanisms by which this occurs depends on the process. In NA, increased turnover of outer segments by RPs compared to CPs is thought to create a lipofuscin concentration profile that correlates well with RP density (Wing et al., 1978; Delori et al., 2001; Boulton et al., 1993; Ach et al., 2014). The main implication of lipofuscin appears to be photoinducible generation of reactive oxygen species and subsequent apoptosis of the RPE-PR complex, although some link it to hard drusen formation too (Davies et al., 2001; Mazzitello et al., 2009; Yasukawa et al., 2007). Literature tends to agree that RPE density shows greatest decline in regions of the retina where the density of RPs is high (Gao & Hollyfield, 1992; Del Priore et al., 2002). A close, but not parallel, correlation of RPE-PR loss with RP topography is understood to occur in NA owing to the lipofuscin pathway discussed previously.

RPE atrophy is understood to be the defining insult to the RR in aAMD (Bhutto & Lutty, 2012). In GA pathophysiology, parafoveal and perifoveal loss of RPE similar to NA is observed, likely due to preferential macular BrM thickening and reduced permeability (Moore et al., 1995). Formation of hard drusen resulting from this and subsequent immunological involvement could lead to hard drusen breakdown which is known to induce GA (S. H. Sarks et al., 1999; Crabb et al., 2002; J. P. Sarks et al., 1994). The effect of such a decrease in BrM permeability (and subsequent hard drusen formation) is likely to be less marked in the fovea due to the significantly lower concentration of lipofuscin accumulated during NA (Delori et al., 2001). Additionally, it is understood that CPs have a higher resistance to increases in reactive oxygen species than RPs (Komeima et al., 2006). It is therefore proposed that enhanced lipofuscin accumulation and subsequent RPE cell loss during senescence, is more likely to result in aAMD when combined with decreases in macular BrM permeability and

subsequent hard drusen formation.

In NA, vascular and perfusion densities decrease; the reduction is most marked in the fovea and is probably due to reduced capillary diameters (Ramrattan et al., 1994; Sacconi et al., 2019). Similarly, CNV has been proven to have a predilection for the foveal region during nAMD (Invernizzi et al., 2018; Manjunath et al., 2011; Govetto et al., 2017). Although BLinD formation is linked to BrM thickening, it is probably more extensively modulated by choriocapillaris changes of the fovea (S. H. Sarks et al., 1999; Provis et al., 2005). Reduced perfusion during NA is likely to increase oxidative stress of the RR and decreasing RPE waste removal may aid production of membranous debris (Bhatt et al., 2010; Friedman et al., 1963; Alten et al., 2013; Curcio, 2018; J. Sarks et al., 2011). Choriocapillaris degeneration, unlike in aAMD, appears to be the primary insult to the RR in nAMD (Bhutto & Lutty, 2012; Mcleod et al., 2009; Moult et al., 2014). The effects of such an insult would have greater impact on the foveal region given the changes that take place in NA. It is therefore proposed that accelerated loss of the choriocapillaris during ageing is likely to preferentially predispose to BLinD accumulation and aggregation, leading to nAMD as a sequela. However, given the lack of knowledge regarding the manner in which this primary insult may occur, further research into the role of the choroidal complex in AMD pathophysiology is required.

Mechanisms leading to RPE cell death also appear to be noticeably different between NA and AMD; proportionally greater inflammatory and immunological involvement is likely to comprise AMD pathophysiology compared to the NA process. Increased oxidative stress from lipofuscin accumulation in particular, is theorised to result in RPE-PR apoptosis (Wihlmark et al., 1997; Bhattacharya, Chaum, Johnson, & Johnson, 2012; Del Priore et al., 2002). In AMD however, apoptosis appears to be supplementary to a predominantly necroptotic pathway (Hanus et al., 2015). The findings of Hanus (2015) agree with studies that have found extensive immunological involvement in soft drusen formation (Johnson, Ozaki, Staples, Erickson, & Anderson, 2000). It must be considered that Johnson et al. (2000) also found some degree of immunological involvement in hard drusen formation, thus suggesting that cellular population changes in NA are not entirely apoptotic.

There is a noticeable disparity between the age of research investigating NA of the RR, compared to AMD. With few exceptions, literature on NA changes predates current AMD morphometric research by decades, indicating a general acceptance within the scientific community that sufficient analysis of this area has been undertaken. By comparison, there is an unprecedented intensity of AMD research at present and this is unsurprising given the prevalence and clinical impact of the disease. However, this study has uncovered noticeable contention in NA findings. Given the level of technological advancement over the past 20 years and the inextricably close relationship between NA and AMD, research into NA changes to the human RR has never been more important.

A limitation of this study is the explicit focus on late stages of AMD. Whilst investigation into the development of AMD from the early stages was undertaken, it was necessary to preferentially analyse late AMD given the overt anatomical changes that occur during these stages. It is acknowledged that changes observed in early AMD may be more closely aligned with NA changes. A further limitation is the absence of discussion regarding other structures of the neurosensory retina. Further research into anatomical changes in regions of the retina not investigated in this review may aid understanding of issues, such as the decline in visual acuity in NA with relative absence of foveal RPE-PR anatomical changes, highlighted by this paper.

12 Conclusion

The senescent process is strongly associated with AMD and has profound implications for its pathophysiology. In addition to predisposing to the pathological state, NA appears to play a key role in determining the pathophysiological course of the disease.

Reductions in BrM permeability through proteinaceous modification, collagen cross linking and advanced glycation endproducts in senescence, appears to be critical in the general pathophysiology of AMD. Production of hard drusen and BLinDs are associated with the structural changes to BrM during ageing. These extracellular deposits appear to significantly influence AMD pathophysiology; breakdown of hard drusen is more associated with aAMD whilst coalescing of BLinDs seems to be more linked with nAMD development. Oxidative stress is a common theme in NA and AMD and extensively contributes to AMD pathophysiology. This review has also highlighted various areas of ambiguity in AMD pathophysiology, and the importance of ongoing research into AMD pathophysiology to simultaneously consider the retinal ageing process.

It is proposed that enhanced lipofuscin accumulation and subsequent RPE cell loss during senescence, is more likely to result in aAMD when combined with decreases in macular BrM permeability and subsequent hard drusen formation. RPE atrophy is understood to be the defining insult to the RR in aAMD. Patterns of RPE cell loss are similar in NA (principally modulated by lipofuscin) and aAMD, both demonstrating foveal sparing. Macular BrM thickening facilitates hard drusen accumulation, which can breakdown, leading to aAMD. The effect of such a decrease in BrM permeability (and subsequent hard drusen formation) is likely to be less marked in the fovea due to the significantly lower concentration of lipofuscin accumulated during NA. It is also posited that accelerated loss of the choriocapillaris during ageing is likely to preferentially predispose to BLinD accumulation and aggregation, leading to nAMD as a sequela.

Choriocapillaris degeneration appears to be the primary insult to the RR in nAMD. CNV has a predilection for the fovea during nAMD, and the fovea also appears to be disproportionately affected in NA. Although BLinD formation is linked to BrM thickening, it is probably more extensively modulated by choriocapillaris changes of the fovea. The effects of this

primary choriocapillaris insult would therefore be greater in the fovea given the changes that take place in NA, thus nAMD is likely to ensue. However, there is a lack of understanding regarding the exact mechanism by which the primary choriocapillaris insult occurs. Indeed, further research is required to elucidate the exact role of the choroidal complex in AMD pathogenesis.

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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.

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Neurodegeneration in epilepsy and the relationship between epilepsy and Alzheimer's disease

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Abstract

Alongside memory loss and cognitive impairment, seizures are reported in patients living with Alzheimer's disease, typically in the later stages. Similarly, epilepsy is not commonly referred to as a neurodegenerative disease despite repeated global and focal seizures having the potential to cause loss of function, sclerosis and neurodegeneration in patients. A diagnosis of epilepsy carries and increased risk of developing Alzheimer's disease and vice versa, a diagnosis of Alzheimer's disease makes the risk of seizure higher than the average population. The extent to which these diseases are related is under reported and not well understood. This review of the current research aims to identify the connections between Alzheimer's disease and epilepsy at the level of physical presentation, pathophysiology and the extent to which one may be responsible for the incidence of the other.

1 Introduction

Epilepsy is diagnosed based on the presence of more than one generalized, or focal seizure and can be present from childhood, or develop in later life. Seizures are defined by inappropriate synchronous activity of neurons due to an imbalance of inhibitor and excitatory activity (Staley, 2015), induced by heterogeneous cellular and molecular alterations (Pitkänen & Lukasiuk, 2009).

Epilepsy is not typically referred to as neurodegenerative. As a lifelong syndrome, cumulative seizures could feasibly cause neurodegeneration by consistent alteration of cellular mechanisms (Thom, 2014). Temporal lobe epilepsy (TLE) is the most common and widely studied epilepsy syndrome, predominantly characterized by hippocampal sclerosis (HS). Epileptic neurodegeneration is best described here, hence this essay will focus largely on TLE, exploring possible mechanisms for neurodegeneration such as seizures, protein aggregation and inflammation

Some of these mechanisms are shared with Alzheimer's Disease. This is unlikely a 33 coincidence. Increased risk of epilepsy within AD (and vice versa) has been known for some time. Seizures occur in AD patients up to 6-10 times more often than in healthy controls (Cretin et al., 2016), classically later in the disease course. However, this essay presents evidence for seizures occurring early or even preceding an AD diagnosis. Seizures in TLE originate in the temporal lobes, where AD pathology is highly prevalent, suggesting a disease spectrum may be present here.

Precisely how these diseases are associated remains to be elucidated. The relationship between AD and epilepsy is discussed with respect to potential interconnecting pathways, converging at the temporal lobes.

2 Methods

Articles were collected from PubMed, Medline and Google scholar. Search syntax included: epilepsy; atrophy; neurode-

generation; progressive; neuronal loss; Alzheimer's disease; mechanisms and seizures. Relevance was evaluated based on titles, abstracts and using the inclusion criteria: peer reviewed; published from 2007 onwards; clinical diagnosis if epilepsy, AD or MCI where appropriate; primary research data and reviews. Exclusion criteria included dissertations, abstracts, editorials and unpublished comment.

3 Results and Discussion

3.1 Epilepsy as a neurodegenerative disease

3.1.1 Evidence for neurodegeneration in epilepsy

Diagnostic criteria of HS in TLE is noteworthy in assessing neurodegeneration. Criteria requires neuronal loss in hippocampal pyramidal cells, principally in CA1, 3 and 4 (Pitkänen & Lukasiuk, 2009) (Thom, 2014), suggesting neuron reduction can indeed give 58 rise to an epileptic disease course. Additionally, kainic acid induced rat models of 59 epilepsy exhibit neuronal loss (Bertoglio et al., 2017). Conversely, the term 'neurodegeneration' implies ongoing loss, for which data is conflicting. Thom, 2014 report a general absence of necrotic or apoptotic neurons in surgically removed tissue in TLE treatment, suggesting lack of ongoing loss and indeed neurodegeneration is not necessary for epileptogenesis (Pitkänen & Lukasiuk, 2009).

Contrastingly, longitudinal analysis by Bernhardt et al., 2009 demonstrated progressive cortical atrophy in TLE using serial MRI, increasing with disease duration outside normal ageing. This study removed bias from post-surgery samples where patients have severe epilepsy, and shows the effects of focal seizures on the entire brain. It also provides high impact longitudinal MRI evidence, other studies largely using retrospective or post-surgery analysis. Exclusion criteria for randomized patient selection was absent, therefore comorbidities particularly in old age, where neurodegenerative disease is highly prevalent aren't accounted for.

3.2 Possible mechanisms of neurodegeneration

3.2.1 Seizures

Neurodegeneration often involves transient protein aggregation, associated with cellular dysfunction. Where this is absent in epilepsy, synchronous activity of seizures could induce neurodegeneration through cumulative excitotoxic insults. Lin et al., 2007 demonstrated through MRI, a reduction in cortical thickness ipsilateral to the site of seizure propagation in TLE (30 patients). One cannot definitively conclude seizures directly cause neurodegeneration from this, as patients were seizure free for 2 years. Alternatively, this could demonstrate long-term effects of seizures such as changes in cellular function and protein transcription via miRNA control (Thom, 2014). In support, pilocarpine-induced mouse models of TLE showed a negative correlation between seizure number and hippocampal neurons (Lopim et al., 2016). Here, seizure number peaked at 180 days of life before decreasing, whereas neuronal loss continued. Animal models enable the study of TLE where human post operation/post mortem samples are limited, although do not mimic human disease precisely; The effect of pilocarpine in this instance induced status epilepticus for 24 hours, unrepresentative of human epilepsy, although this may 'speed up' parallel events of human disease.

3.2.2 Inflammation

Inflammation could provide novel explanations for seizure induced neurodegeneration. Translocator protein (TPSO), is expressed on activation of glia and inflammation, and is upregulated in TLE. Amhaoul et al., 2015 demonstrated TPSO increase correlates with a decrease in hippocampal neuronal markers in kainic acid induced rat models of TLE, progressing with seizure number and time. This supports a progressive disease course.

However, measurements were only taken up to 3 months in a small number of rats, therefore applicability to lifelong human disease is modest.

3.2.3 Tauopathy

Hyperphosphorylated tau protein is implicated in several neurodegenerative diseases including AD and is now recognised in epilepsy. Tau may induce neurodegeneration via the 'protein aggregation' model. Cerebral tau deposition is measured using Braak stages 1-6. A study by Thom et al., 2011, evidenced a correlation between higher Braak stage and epilepsy duration, and higher Braak stages were associated with a younger age at death. Although there was no association between Braak stage and seizure number or type, suggesting tau may act independently from seizures. Analysis here was limited to Braak staging, hence tau present outside Braak areas will not be included.

The incidence of tau in epilepsy suggests a possible interconnecting mechanisms with. Tau, and other pathways have been explored, uncovering why a clinical relationship exists, at a molecular level.

3.3 Relationship between epilepsy and AD

3.3.1 Tauopathy

Tai et al., 2016, analysed the distribution of tau relative to that seen in AD using immunohistochemistry on surgically removed tissue from TLE patients. 10 of 24 cases showed 'Braak-like' distributions, although overall tau deposition did not correlate with AD distribution and markedly, most cases were categorized to lower Braak stages. Exact mechanisms of tau tangle formation may differ in epilepsy resulting in differential patterns through selective vulnerability depending seizure type (focal or general). Pooler et al., 2013 suggest tau is released by synaptic activity, clearly altered in epilepsy and differing seizure types.

Despite bias to severe disease through surgical samples, studying this raises an interesting hypothesis: If severe TLE is more likely to be associated with AD and hyperphosphorylated tau, could this represent a subcategory of TLE?

3.3.2 ApoE4

The ApoE4 allele is a well recognised risk factor for AD. Aboud et al., 2013 found ApoE4 carriers with either epilepsy or AD exhibited a reduction in neuron size, increase in caspase

3 levels, a reduction in S100 (neuron promoting cytokine) and an increase in oxidative stress. These differences were not observed in neurologically normal controls despite ApoE4 genotype. These mechanisms could link the 2 diseases and support the notion of epilepsy as neurodegenerative and the increase in oxidative stress could demonstrate an increase in inflammation, supporting its neurodegenerative role. The authors suggest a 'self-perpetuating' pathway whereby glutamate released in a seizure promotes ApoE4 expression, increasing APP, sAPP, IL-1, and subsequently glutamate.

3.3.3 Amyloid beta (a)

In the same study, ApoE4 carriers with epilepsy showed an increase in a plaque, associated with AD, compared with ApoE2/3 carriers. a accumulation could be explained by the self-propagating mechanism previously described. The study does not compare epileptic ApoE4 carriers against control ApoE4 carriers, which would show whether an epilepsy diagnosis amplifies ApoE4 effects and increases AD risk with respect to the population. However, the notion that ApoE4 can cause plaques to mature rapidly, as the authors demonstrate in a patient 10 years old, could highlight an early onset AD predisposition in epilepsy patients.

In support, in animal models, a plaque triggers epileptogenesis (Cretin, Di Bitonto, Blanc, & Magnin, 2015).a could be responsible for network synchronization and increased seizure risk in AD patients (Palop & Mucke, 2009).

3.3.4 Seizures

Seizures are often reported at late stage AD. Conflictingly, Cretin et al., (2015), Cretin et al., (2016) and Vossel et al., (2013) showed seizures preceded amnesic mild cognitive impairment (aMCI) by up to 7 years and was correlated with cognitive decline. In case control studies by Cretin et al., 2016, patients had existing aMCI and were retrospectively studied for comorbid epilepsy. Retrospective detail was given by patients/carers. Unresponsive/non-convulsive spells (now known to be common in early AD (Vossel et al., 2013), may be forgotten or missed, underestimating seizure frequency.

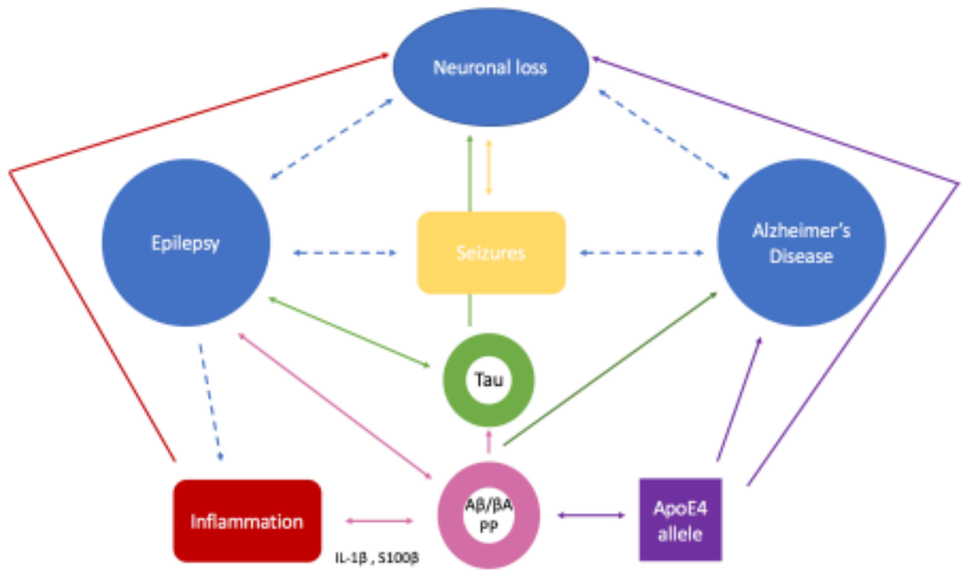
AD patients experiencing seizure disorders have greater cognitive impairment than AD alone, and clinical symptoms correlate with location of seizure onset. Vossel et al., (2013) found patients who developed epilepsy had a quicker onset to AD from MCI than those who did not by 6.8 years. Epileptiform activity was also largely apparent in the temporal lobes, consistent with AD pathology. This evidence shows epilepsy not only predisposes patients to AD, but also perpetuates symptoms.

4 Conclusions

Although evidence for epilepsy as a neurodegenerative disease is conflicting, strong evidence is given by serial MRI in humans correlating with disease duration. Global seizures are widely understudied and could represent another mechanism of neuronal loss separate from TLE. The data suggests rather than being a disease mechanism, neuronal loss could manifest as a result of epilepsy over time. Future studies should consider biomarkers for inflammation/plaque accumulation supporting current studies for mechanisms of neuronal loss.

Results demonstrate a huge potential connection between seizures, a and tau deposition summarized in Figure 1. Particularly at the level of tau tangle formation and implications for AD risk. Further studies should indicate at what level tau is correlated with a in epilepsy and by comparison with AD. From the presently discussed data, it seems feasible there could be a spectrum of the two diseases or even, an epileptic variant of AD.

Figure 1.



Summary of possible interconnecting mechanisms between Alzheimer's disease and TLE and modes of neurodegeneration discussed. The figure demonstrates how complex this interaction could be with many factors being implicated. Potentially the most important connection highlighted here could be the correlation between seizures and neuronal loss, leading to cognitive dysfunction seen at MCI, and secondly, the implication of tau and amyloid in epilepsy, which could give rise to a direct link to AD.

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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.

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Ketogenesis and Alzheimer's Disease: Literature Review

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Abstract

Context Whilst Alzheimer's Disease (AD) aetiology is unclear, diet has been implicated as a modifiable risk factor. Despite this, current AD dietary advice is vague. Recent research has suggested ketone bodies can be used to slow or even improve the rate of AD deterioration.

Aim This literature review evaluates the role of ketones in AD amelioration through the Ketogenic Diet (KD) and Medium-chain triglyceride (MCT) therapy. This review also analyses potential mechanisms by which ketone bodies improve AD pathophysiology.

Method Three searches were carried out on PubMed database covering: KD effectiveness in animal models of AD and AD patients, and MCT effectiveness in AD patients. Animal studies focused on both physiological and behavioural/cognitive functioning outcomes whilst human studies solely focused on the latter.

Findings Studies in this review generally support the link between KD and AD cognitive improvement, through the induction of ketogenesis in the body. Similar results have been achieved for MCT therapy. However, effects of both seem to be largely limited to mild, APOE4- AD patients.

Conclusions Whilst the ketosis effects of the ketogenic diet seem promising, more research needs to investigate why cognitive improvement is lacking in APOE4 positive patients. MCT emulsion therapies could be a welcome alternative to achieve ketosis, considering strict adherence needed in KD for similar effects.

1 Introduction

Alzheimer's Disease (AD) is a neurodegenerative disorder characterised by amyloid-beta $(A\beta)$ plaque deposition and neurofibrillary tau tangles within neurons, leading to cortical degeneration. Over time, this presents with cognitive and behavioural impairments, starting with mild memory loss (Weller & Budson, 2018).

AD pathophysiology implicates many biological processes e.g. glucose hypometabolism, free-radical damage, and gut dysbiosis leading to neuroinflammation (Seneff, Wainwright, & Mascitelli, 2011).

Whilst AD aetiology is unclear, diet has been implicated as a modifiable risk factor. Despite discrepancies in which macronutrients exacerbate AD risk, a low fat and high carbohydrate diet has been cited. (Seneff et al., 2011) suggested that this diet leads to cholesterol deficiency which causes increased oxidative damage and ultimately, neuronal apoptosis. Cholesterol enables the cell membrane to form a closer configuration. This protects membrane fatty acids from oxidative damage caused by excess glucose exposure from high carbohydrate intake. Insufficient fatty acid supply, through

low fat intake, reduces neuronal ability to repair damaged cell membranes. This suggests that AD progression is exacerbated by diet as people with AD have a greater preference for foods high in carbohydrates (Mungas et al., 1990).

Current AD dietary advice is vague (Yerstein & Mendez, 2020), however, if low fat and high carbohydrate intake is suggested to worsen AD outcomes, the inverse (low carbohydrate and high fat (LCHF)) could slow or even improve the rate of AD deterioration.

In this literature review, I will focus on research regarding the ketogenic diet, an LCHF diet, as a potential way of improving AD symptoms. There has been increased interest in this area, using this diet as an adjunct to current Alzheimer's Disease treatment. Previous reviews have focused on its role on improving cognitive symptoms, but few have further explored the practicalities of implementing the ketogenic diet (KD) in AD patients. Therefore, I will also focus on potential objections to KD implementation and future steps research could take, such as the use of medium-chain triglyceride therapy as an adjunct to Alzheimer's Disease treatment.

2 Background

2.1 Ketogenic diet

The Ketogenic Diet (KD) was a term coined by Dr Wilder in 1921. It is an LCHF diet which involves substantial carbohydrate reduction. This minimises glucose stores and replaces them with dietary fat, inducing ketogenesis. A standard ketogenic diet (SKD) involves a macronutrient divide of 55-60% fat (mainly saturated fat), 30-35% 63 protein and 5-10% carbohydrates (20-50g/day) (Dhamija, Eckert, & Wirrell, 2013).

Carbohydrates are the main energy source in the body. They are metabolised via glycolysis, the TCA cycle and oxidative phosphorylation to produce ATP. When carbohydrate intake is reduced to $\leq 50g$, insulin secretion is reduced, and glucagon is used to access glycogen stores to provide energy. Once glycogen stores are depleted, gluconeogenesis and ketogenesis are initiated. During ketogenesis, ketones become the main energy source for both peripheral tissue and the brain. They are produced by the liver from excess fatty acid oxidation. This leads to elevated circulating levels of ketones: β-hydroxybutyrate (BHB) and acetoacetate, as seen in Figure 1.

2.2 KD and AD Pathophysiology

2.2.1 Fuel Exchange

In AD, global cerebral glucose metabolism is reduced by 20-25%, in turn, reducing ATP output. Presumably, this starts from the hippocampus and precedes clinical symptom onset, resulting in reduced synaptic function and atrophy (Cunnane et al., 2016). This leads to a further reduction in energy demand, forming a vicious cycle of hypometabolism and neuropathology. As amyloid precursor protein (APP) cleavage is also ATP-dependent, a lack of ATP could lead to increased Aβ plaque accumulation, a hallmark of AD (Maloney, Minamide, Kinley, Boyle, & Bamburg, 2005). As brain ketone uptake remains the same in AD patients compared to cognitively healthy aged-matched controls (Hartman et al., 2007), it can be used to supplement neural ATP demand.

2.2.2 Anti-oxidant properties

There is evidence of free-radical oxidative damage in AD, caused by reactive oxygen species (ROS). The brain is especially vulnerable to this due to its high content of readily oxidised fatty acids, high oxygen consumption and low antioxidant levels. Aβ_{1-42} plaques and their high copper concentration are thought to mediate ROS generation and lead to mitochondrial damage and lipid peroxidation, a prominent feature of AD degeneration (Huang, Zhang, & Chen, 2016). Both BHB and acetoacetate have been shown to have an antioxidant effect against ROS, preventing neuronal ATP decline (McPherson & McEneny, 2012).

2.2.3 Gut Microbiota

The microbiota-gut-brain axis describes the interaction between commensal microbiota and brain function via multiple pathways. Research suggests age-related changes to the gut contribute to neuroinflammation, which is amplified by AD pathology. Over time, there is a decrease in 'beneficial' gut bacteria e.g. *Bifidobacteria* and *Lactobacillus*, which maintain gut barrier integrity and contribute to anti-inflammation. Low *Bifidobacterium* levels have been correlated with elevated plasma LPS concentration, a component of gram-negative bacteria (Ling, Linglong, Weixia, & Hong, 2016). LPS production is shown to increase IL-6 and TNF-α levels – pro-inflammatory cytokines. (Zhang et al., 2009) found LPS concentrations 3 times higher in AD patients than healthy, age-matched controls.

Ageing is also linked to low-grade chronic inflammation termed 'inflammaging', which can contribute to increased gut permeability and blood-brain barrier compromise (Jiang, Li, Huang, Liu, & Zhao, 2017). This would allow proinflammatory cytokines more access to the brain, causing neuroinflammation.

Studies in healthy mice have found KD enhances neurovascular functions after 16 weeks, by increasing relative abundance of *Lactobacillus* and decreasing pro-inflammatory taxa (Ma et al., 2018). Theoretically, this could reduce neuroinflammation. However, conflicting evidence in young epilepsy patients shows that KD reduces the relative abundance of beneficial bacteria and increases pro-inflammatory *Escherichia coli* abundance after 3 months (Lindefeldt et al., 2019). This would suggest that KD promotes inflammation, worsening AD pathology. It could be argued that AD gut microbiota changes may not be seen in younger patients so they cannot be used as a valid comparison however, more research needs to be done on KD-induced microbiota changes.

2.2.4 Upregulation of Protein Channels directly involved in Amyloid Clearance

More recently, Versele et al. (2020), using human in vitro blood-brain barrier (BBB) models and brain-like endothelial cells (BLECs), have found that the combined use of ketone bodies (acetoacetate and BHB) promotes Aβ efflux through

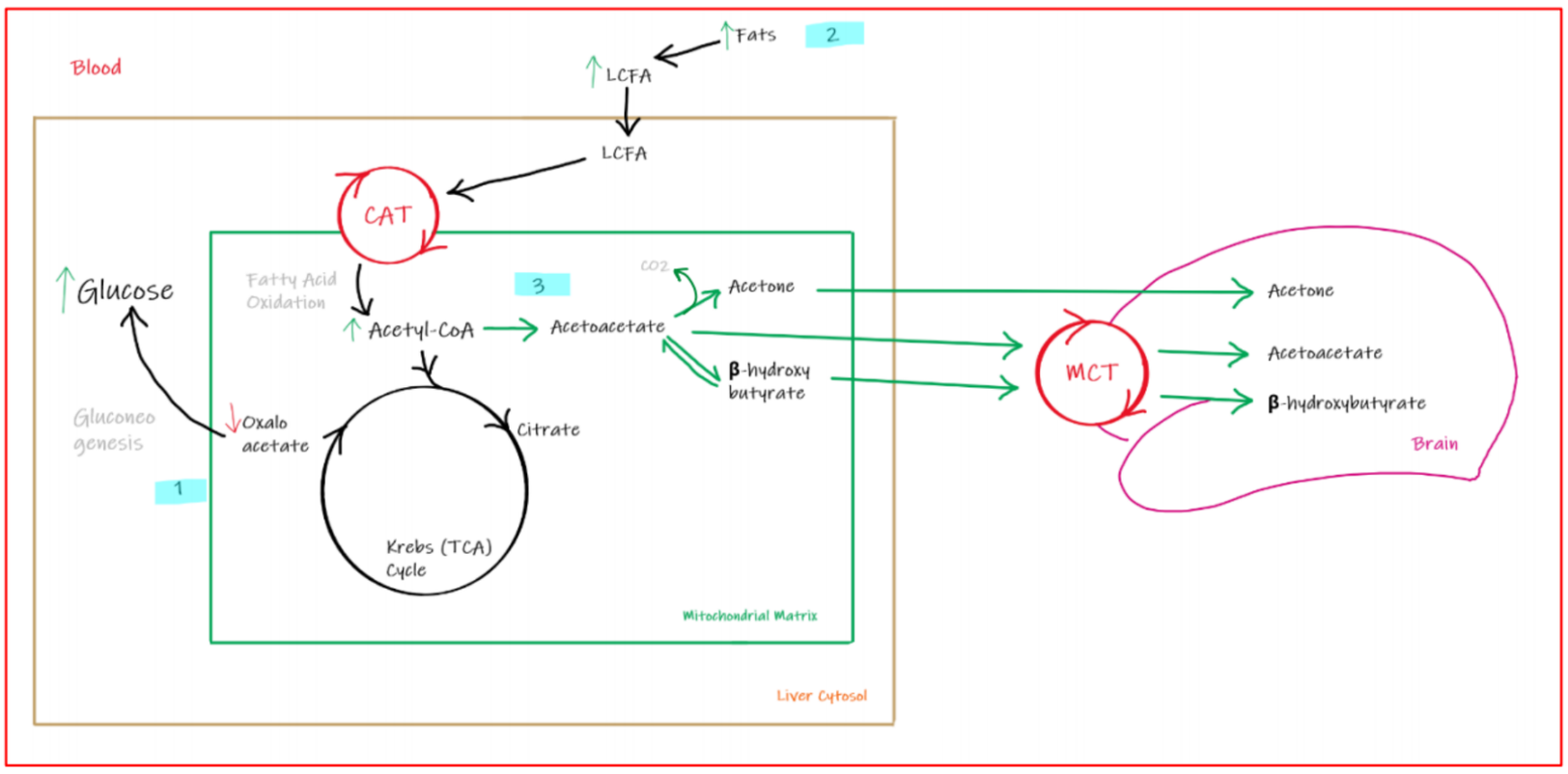

Figure 1: Ketone effect on TCA (Krebs) Cycle (Hartman et al., 2007). (1) Low blood glucose causes oxaloacetate (Krebs Cycle) conversion to glucose in the liver via gluconeogenesis. (2) Increased long chain fatty acid (LCFA) intake (from high saturated fat diet e.g. KD) increases acetyl CoA concentrations. Due to low oxaloacetate levels caused by gluconeogenesis, it cannot be combined with excess acetyl CoA to make citrate needed in the TCA cycle. (3) As a result, excess acetyl CoA is converted to acetoacetate, a ketone; this spontaneously degrades to acetone. It can also be reversibly converted to β-hydroxybutyrate, another ketone, via β-hydroxybutyrate dehydrogenase. Ketones can readily cross the Blood-Brain Barrier (BBB) either via simple diffusion (acetone) or via Monocarboxylic Acid Transporters (MCT) (β-hydroxybutyrate and acetoacetate) to be used for energy metabolism; MCT expression in the brain responds rapidly to hyperketonaemia. CAT: Carnitine-acetylcarnitine translocase

upregulation of protein channels involved in amyloid clearance, such as low-density lipoprotein receptor-related protein 1 (LRP1). This suggests that KBs can have a direct effect on the blood-brain barrier which has not been previously demonstrated. More research into the direct effect of KBs on protein channels would be appreciated to corroborate results shown in this study.

3 Methods

3.1 Data Sources and Search Methods

Two separate PubMed searches were conducted in October 2018 and March 2020 to find relevant, up-to-date papers. Previous literature reviews were used to form the basis of the initial search, using backwards citation to identify useful papers. This search investigated the following topic areas:

1. KD effectiveness in animal models of Alzheimer Disease
2. KD effectiveness in Alzheimer patients
3. Medium-chain triglyceride (MCT) therapy effectiveness in Alzheimer's patients

The primary literature search utilised studies published from January 2004 to March 2020. This sixteen-year span was used as a starting point for the topics mentioned above due to a general lack of studies in these areas.

3.2 Inclusion and Exclusion Criteria

English-language only papers (print or electronic) were included in this review. Randomised controlled trials and clinical trials were looked on more favourably, as these are stronger levels of evidence. However, case reports were also included, if relevant, due to a general scarcity of evidence in the topic areas listed below. Reviews were excluded from reporting as they are not primary research.

Both MeSH heading and free text searches were combined to find papers. All three searches included *'alzheimer*'* as a search term, to encompass all iterations of Alzheimer's Disease spellings. The screening process was refined for each topic area following the initial search:

3.2.1 KD effectiveness in animal models of Alzheimer Disease

The inclusion criteria were as follows:

1. English-language papers (print or electronic) published between 1 January 2004 - 1 March 2020. This span was selected due to the paucity of studies in this area.
2. Papers including ('ketogenic diet') AND ('alzheimer*') AND ('animal studies OR animal models') in either title or abstract.
3. Papers assessing physiological or behavioural/cognitive functioning outcomes in animal models of Alzheimer's Disease.

Studies were excluded if:

1. Animals were given ketones or additional supplements to induce ketosis
2. Animals were given vitamin supplementation

3.2.2 KD effectiveness in Alzheimer patients

The inclusion criteria were as follows:

1. English-language papers (print or electronic) published between 1 January 2012 - 1 March 2020. The initial sixteen-year search span detailed above was later refined to seven years due to increased research interest in this area in the recent decade.

2. Papers including ('ketogenic diet') AND ('alzheimer*') AND ('human studies') in either title or abstract.

3. Studies with populations of AD and/or mild cognitive impairment (MCI), as MCI patients are at increased risk of AD.

4. Papers assessing cognitive or behavioural changes in patients with AD.

Studies were excluded if:

1. A non-human study population was investigated

2. Lack of primary or secondary behavioural/cognitive functioning outcomes investigated

3. Use of MCTs without patient achieving ketosis

4. Combined use of MCT therapy with adjuncts such as vitamin/protein supplementation seen

4 Results

See Appendix A for full table summary of findings.

4.1 KD Effectiveness in Animal Studies

van der Auwera et al. (2005) found that KD can reduce amyloid deposition by 25% in transgenic mice brains over 43 days, compared to controls on a standard diet (high carbohydrate/low fat). This suggests that KD could reverse AD pathology. However, it may not reverse clinical symptoms as, despite ketone and Aβ level changes, no cognitive improvements were seen.

Brownlow et al. (2013) found KD-fed transgenic mice performed better on motor tasks than controls. Yet, tissue measures of amyloid and tau deposition showed no difference between mice fed on KD or control diet over 3 months. Similar results were corroborated by (Beckett, Studzinski, Keller, Paul Murphy, & Niedowicz, 2013). This again shows that symptom improvement and pathology improvement can be separate phenomena.

Going forward, if these are separate, perhaps non-human studies should focus on human trial endpoints i.e. symptom improvement, as it is more practical to measure patient outcome.

4.2 KD Effectiveness in Human Studies

Krikorian et al. (2012) found that mild AD/MCI patients randomly assigned to a very low carbohydrate diet significantly improved verbal memory after 6 weeks, compared to controls on a high carbohydrate diet. High blood ketone levels positively correlated with memory performance. However, depressive symptoms were not affected suggesting that KD might have better efficacy in cognitive impairment.

One problem found in KD studies is that efficacy is largely tested on mild AD patients. In a 3-month medium chain triglyceride (MCT)-supplemented KD, patients had significant cognitive improvement, which reverted to their baseline post-1-month washout (Taylor, Sullivan, Mahnken, Burns, & Swerdlow, 2018). Despite positive results, all diagnosed with moderate AD (Clinical Dementia Rating (CDR) = 2.0) withdrew due to caregiver burden. This highlights that KD may not be practical for moderate to severe AD patients, as strict adherence is needed to achieve ketogenesis. Further, neither study was able to demonstrate similar cognitive improvements in APOE4 positive patients, suggesting a genetic difference in treatment effectiveness. The APOE4 genetic variation is the strongest genetic risk factor for AD and is associated with increased levels of amyloid deposition in both early and late stage AD (M. Di Battista, M. Heinsinger, & William Rebeck, 2016).

However, a recent case study has shown that a 10-week carbohydrate restricted,high-fat KD has been linked to cognitive improvement in a heterozygous APOE4 positive, mild AD patient (Morrill & Gibas, 2019). Whilst it is unclear how much the KD contributed to cognitive improvement, as the patient had also been prescribed time-restricted eating and low-impact physical/cognitive exercise, which have been shown to improve cognition in mild AD (Du et al., 2018); (Jia, Liang, Xu, & Wang, 2019), this still suggests that KD could have a role in cognitive improvement in APOE4 positive patients.

4.3 MCT Therapy

Medium-chain triglyceride (MCT) therapy involves the consumption of medium chain triglycerides (6-12 carbons long), a form of saturated fat, to induce ketogenesis. This is administered via capsules or liquid emulsions.

Medium-chain triglyceride therapy is an alternative to strict KD as AD patients can achieve ketogenesis with limited dietary modification. In contrast to LCFAs in KD, MCTs are oxidised readily in the liver regardless of nutrient consumption. Therefore, macronutrient restrictions are unnecessary as described in Figure 2 (Cunnane et al., 2016).

MCTs have been shown to increase serum ketone levels in both APOE4+ and APOE4- AD patients (Reger et al., 2004). It also significantly improved cognition and paragraph recall in APOE4- AD subjects, compared to placebo. The lack of cognitive improvement in APOE4+ patients despite increased ketone levels could be investigated, because this indicates that ketones might be used differently to the proposed methods above. Other MCT-based ketogenic formulas have shown to improve verbal memory and processing speed in mild to moderate AD patients after 12 weeks of consistent administration along with their usual diet (Ota et al., 2019). If MCT supplements can be taken alongside patient's normal diets, this can improve adherence as it is not a substantial disruption to their normal routine, compared to the challenges the KD might pose.

Studies have also investigated the effects of Caprylidene

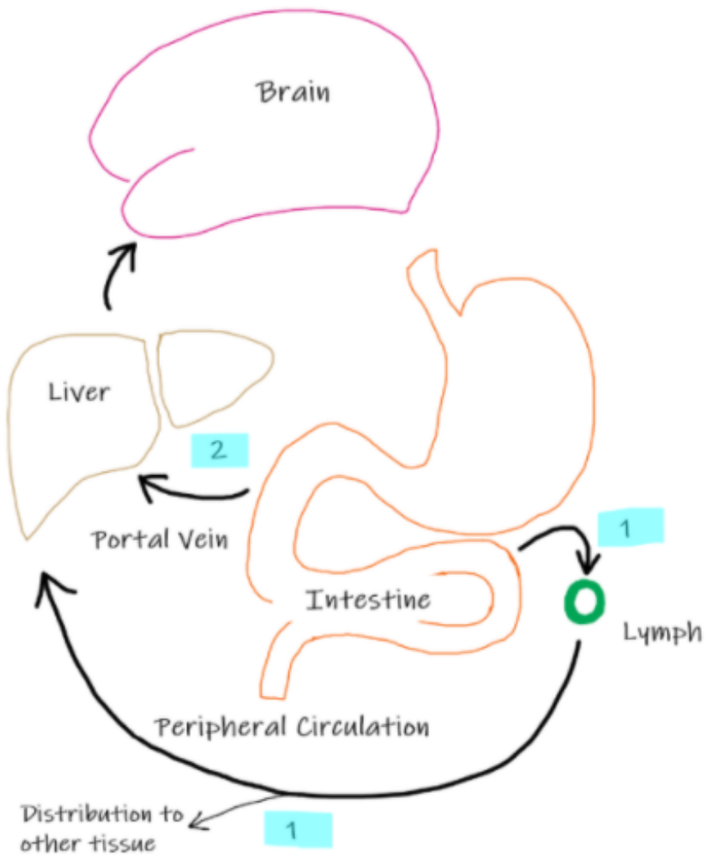

Figure 2: LCFA metabolism is different to MCFA metabolism (Cunnane et al., 2016). Long chain fatty acids (LCFAs), used mainly in KD, are absorbed into the intestinal villi (1) where they are packaged into chylomicrons. These are transported via the lymphatic (lacteal) system into peripheral circulation where they are either distributed to adipose tissue or other organs (excluding the brain), for storage. They are utilised once glucose stores are depleted. Medium chain fatty acids (MCFAs), used in MCT emulsion therapy, directly pass into the portal vein (2) via diffusion across the intestinal villi, leading to the liver. Here, MCFAs undergo complete fatty acid oxidation. Excess acetyl CoA from thisreaction is converted into ketones which can be used as an energy substrate for the brain.

(Axona®), an American Food and Drug Administration (FDA)-approved, MCT-rich medical food for AD treatment. Henderson et al., (2009) found significant cognitive improvement from baseline in ADAS-cog (Alzheimer's Disease Assessment Scale-Cognitive Subscale) scores over 90 days in compliant mild APOE4- AD subjects, compared to placebo, highlighting Caprylidene's effectiveness. In a similar trial, Kimoto et al., (2017) also found cognitive improvements in mild APOE4- AD subjects through Caprylidene. Kimoto et al. also found that patients with more severe cognitive decline (Mini-mental State Exam (MMSE) scores $<$15) did not show cognitive improvement. These studies suggest that MCTs are most impactful in early AD stages. The next step in implementing MCT therapies may be to investigate how early in AD the therapy is most effective.

Reported side effects of MCT therapies such as Caprylidene are gastrointestinal limited: diarrhoea, flatulence and dyspepsia – especially if not taken with meals. Diarrhoea, especially, can lead to long term problems in frailer patients with AD. Whilst results are mixed, studies have suggested these side effects can be mitigated through putting MCTs into an emulsification (e.g. in a smoothie) rather than a straight oral dose (Courchesne-Loyer et al., 2017). Caprylidene is further contraindicated in type 1 diabetes mellitus due to ketoacidosis risk, and in those with renal dysfunction(Roman, 2010). Moreover, the effects of MCT supplementation, on long-term lipid levels and CVD risk factors need to be established before recommendations can be made.

Overall, as MCT therapy produces similar effects to KD, it might help patients who may be less compliant with KD, to obtain similar results. Furthermore, it may be more convenient than KD as the patient can avoid diet changes which reduces caregiver burden.

5 Potential Objections to KD

Despite much research, it is still unclear why a standard KD works as studies have also found correlations between high saturated fat intake and AD risk (Ruan, Tang, Guo, Li, & Li, 2018). Mice studies have found that high saturated fat diets can increase amyloid levels in microvasculature and exacerbate cognitive deficits (Thériault, ElAli, & Rivest, 2016). Diets high in saturated fat are also shown to increase plasma LPS levels 2-3-fold, contributing to neuroinflammation, according to the gut microbiota hypothesis (Jiang et al., 2017).

It could be argued that saturated fat intake associated to AD risk, is related to the Western Diet where it is coupled with high levels of simple carbohydrates (Thériault et al., 2016). This could mean that it is not saturated fat alone which increases risk but its links to other poor eating habits. This also suggests that KD success is due to carbohydrate-fat (LCHF) interaction.

Furthermore, as high saturated fat is linked to high cholesterol, it could theoretically increase cardiovascular disease (CVD) risk. However, studies show KD is associated with significant reductions in variables associated with high CVD risk, such as: total cholesterol, triglycerides and LDL cholesterol in both obese and normal weight participants (Kosinski & Jornayvaz, 2017). This could be explained theoretically as fat is used as a main energy source in KD so it will be utilised immediately, rather than stored. Longitudinal studies would be ideal to assess long term CVD risk in KD.

6 Future Research

Overall, research generally supports the link between KD, a form of high fat diet, and AD cognitive improvement. How-

ever, effects seem to be limited to mild, APOE4- AD patients.

This poses two further research interests:

1. **Stratification of mild AD patients to assess KD effectiveness.** There is currently a lack of standardisation when assessing AD severity. The studies above either used CDR or MMSE to stratify patients. If this can be standardised, more studies could investigate KD effectiveness at these stratified levels (based on score) to discover which AD stage the diet is most effective.

2. **Differences in APOE4+ cognitive outcomes despite ketone elevation.** More research needs to investigate why cognitive improvement is lacking in these patients despite treatment producing the same ketone level increases seen in APOE4- participants. In the meantime, if this form of treatment were to be implemented, genetic screening should be done to determine the patient's APOE4 status as this may influence treatment outcome.

As strict KD adherence is needed to achieve ketogenesis, it may reduce patient compliance and thus the effectiveness of the treatment presented in findings. MCT emulsion therapies could be a solution as one's usual diet can be maintained. There is yet to be a direct comparison between MCT therapy and the KD, regarding clinical outcomes – this could be investigated via clinical trial, comparing MMSE cognitive scores. Additionally, longitudinal trials are impact to investigate both the impact of MCT-induced ketogenesis on AD improvement and any long-term cardiovascular risks.

Generally, many KD efficacy studies only look for cognitive improvement whilst AD is a multi-faceted disease, including behavioural components e.g. apathy. Research could expand to investigate the KD effect on other symptoms apart from cognitive impairment.

Lastly, as it is unclear which mechanism KD uses to improve AD symptoms, more research needs to be done to understand this. That way, more insight can be shed into the pathophysiology of AD, which could lead to further treatment discovery.

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.

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7 Appendix A

Table 1: Summary findings of studies assessing effectiveness of KD and MCT therapies

**CDR - Clinical Dementia Rating; **ADAS-cog - Alzheimer's Disease Assessment Scale-Cognitive Subscale; ***MMSE - Mini-Mental State Examination*

KD Effectiveness in Animal Studies			
Study Details	Participants	Diet Composition and Study Length	Key Findings
Van der Auwera et al., 2005 Randomised Controlled trial	Sixteen, 3 months old, transgenic (London APP mutation) female mice	KD chow: 1. 79% fat (29% of this is saturated fat) 2. 8% protein 3. 0.76% carbohydrate 4. 12% water, fibre and ash Compared with standard chow: 1. 4.5% fat 2. 21% protein 3. 35% carbohydrate 4. 39.5% water, fibre and ash Fed for 43 days ad libitum	Compared to controls, KD-fed mice found: 1. 25% reduction in total brain Aβ levels 2. No changes in behaviour
Brownlow et al., 2013 Randomised Controlled trial	Five months old APP (model of amyloid deposition) and Tg4510 (model of tau deposition) mice.	KD chow: 1. 77% fat (especially MCT-rich) 2. 22% protein 3. 1% carbohydrate Compared with NIH-31 control diet: KD chow: 1. 14% fat 2. 24% protein 3. 62% carbohydrate Fed for 3 months. Food replaced 3 times a day.	Compared to controls, KD-fed mice found: 1. Improved motor performance independent of genotype. 2. No difference in amyloid or tau deposition on tissue examination.
Beckett et al., 2013 Randomised Controlled trial	1–2 months old APP/PS1 knock-in mice	LCHF, KD diet: 1. 79% fat 2. 8% protein 3. 1% carbohydrate Compared with control diet: KD chow: 5. 5% fat (29% of this is saturated fat) 6. 20% protein 7. 62% carbohydrate Fed for 1-month ad libitum.	Compared to controls, KD-fed mice found: 3. Improved motor performance on rotarod apparatus. 4. No difference in amyloid deposition or APP levels in the brain.

KD Effectiveness in Human Studies			
Study Details	Participants	Diet Composition and Study Length	Key Findings
Krikorian et al., 2012 Randomised control trial	23 older adults with mild cognitive impairment and mild AD (CDR*) = 0.5 or 1). Mean age = 70.1 years.	Very low carbohydrate (VLC) diet: 1. 5-10% of total calorie intake 2. Fat and protein intake allowed to vary 3. No total calorie intake restrictions Compared with high carbohydrate diet (50% of total calorie intake). Diet lasted 6 weeks.	Compared with controls, VLC group found: 1. 2.8-mean point increase in V-PAL score (verbal memory test) 2. No difference in depressive symptoms
Taylor et al., 2018 Randomised, single-arm controlled trial	15 AD participants with CDR ratings: 1. 0.5 (very mild AD) 2. 1 (mild AD) 3. 2 (moderate AD). Mean age 73.1 years. 5 participants withdrew due to caregiver burden	Very high-fat KD diet: 1. 73.4% fat (MCT-rich) 2. 17.6% protein 3. 9% carbohydrates Diet lasted 3 months. Later followed by 1-month washout (reverting back to normal diet).	Participants found, compared to preintervention: 1. 4.1-mean point reduction in ADAS-cog** scores 2. 1.1-mean MMSE*** score increase. Improvements reverted to baseline post-washout. Note: Point reduction in ADAS-cog assessment is seen as cognitive improvement.
Morrill & Gibas, 2019 Case report	Morbidly obese 71-year-old female, APOE4 positive. Family history of AD. Diagnosis: Mild AD and metabolic syndrome.	LCHF ketogenic diet (composition unspecified), time-restricted eating and physical/cognitive exercise. Diet lasted 10 weeks.	After 10 weeks: 1. Triglycerides and VLDL: 50% reduction 2. HbA1c reduction from 5.7& to 4.9%. 3. 7-point increase in Montreal Cognitive Assessment score

MCT Effectiveness in Human Studies			
Study Details	Participants	Diet Composition and Study Length	Key Findings
Reger et al., 2004 Double-blinded randomised control trial	20 subjects with mild AD or mild cognitive impairment. Mean age = 74.7 years old 50% participants were APOE4+	Emulsified MCT drink: 1. 40ml MCT oil 2. 152ml heavy whipping cream Compared with placebo: 232ml of heavy whipping cream Study conducted under isocaloric conditions (690 calories) over 2 visits.	MCT-fed participants found, compared with controls: 1. Improved paragraph recall and 1.5-mean point reduction of ADAS-cog score in APOE4- participants 2. 1-point increase in ADAS-cog scores in APOE4+ participants
Henderson et al., 2009 Double-blinded randomised controlled trial	152 subjects with mild to moderate AD. Mean age = 76.8 years old. 52 withdrew before end of study. 55.6% participants were APOE4+.	Oral ketogenic compound AC-1202 (Axona®) – 30g powder sachet mixed with liquid (e.g. water, milk, juice): 1. 33% AC-1202: Glycerine and caprylic acid 2. 64% gum acacia Compared to placebo: 1. 51% gum acacia 2. 37% dextrose 3. 10% safflower oil Given daily for 90 days.	Dosage compliant, AC1202 (Axona®) participants found, compared to controls: 1. 2.6-mean point reduction in ADAS-cog scores in APOE4- participants from day 45 2. No significant ADAS-cog changes in E4+ participants throughout study.
Kimoto et al., 2017 Randomised clinical trial	24 patients with sporadic mild to severe AD. Mean age = 63.9 years old. MMSE scores pre-treatment range = 10 – 25 2 patients withdrew from study 7 patients were APOE4+.	Axona® (40g of powder containing 20g of caprylic triglycerides) administered to all patients. Given daily for 3 months.	Study found during treatment: 1. No significant difference between APOE4- and E4+ patients. 2. Participants with MMSE >15: Improved mean orientation and memory in ADAS-cog test (0.5-point reduction) 3. Participants with MMSE <15: Decreased orientation (0.6-point increase in ADAS-cog)
Ota et al., 2019 Randomised clinical trial	20 patients with mild-to-moderate AD Mean age = 73.4 years old 4 withdrew from study due to side effects (diarrhoea)	Patients given 50g Ketoformula® (contains 20g MCTs) or isocaloric placebo formula (370 calories each), as a control. Taken daily for 12 weeks.	Compared with controls, Ketoformula® participants found: 1. At 8 weeks: Mean 4-point in immediate and delayed logical memory tests 2. At 12 weeks: Mean 7-point increase in digit-symbol coding test and mean 4- point increase in immediate logical memory test

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Aphasia after left hemisphere stroke in users of British Sign Language

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Abstract

Aphasia after stroke in users of British Sign Language is an understudied area with patients often missing a diagnosis hence unable to access appropriate rehabilitation services for aphasia post-stroke. There is a lack of understanding of British Sign Language in stroke units and the need for further assessment of these patients with respect to aphasia is often not recognised. Aphasias in British Sign Language are complicated by the physical element of speech using bilateral hand use which is often not possible post-stroke, as well as the lack of staff understanding of the language and the difference between sign language and gesture. Here, the processing of British Sign Language is outlined with similarities and differences between post-stroke aphasias in spoken language compared to British Sign Language. Current research highlights the necessity for appropriate and timely assessment of these patients for improved outcomes.

1 Introduction

Approximately 100,000 people in the UK suffer from a stroke each year with one third resulting in aphasia Stroke Association (2015). Aphasia is assessed typically within the first week post-stroke by professional speech and language therapists (SALT). Where a patient's first language is a non-spoken language such as British Sign Language (BSL), assessment is challenging and often, aphasia in deaf patients is not acknowledged. Approximately 200 people from the deaf community in the UK experience strokes (or other aphasia causing injuries), every year. Indeed, aphasia does occur in deaf users of sign language depending on lesion location. However, few of these patients receive language therapy compared to the hearing population Marshall, Atkinson, Thacker, and Woll (2003). This is due to several reasons: Clinicians are inexperienced with BSL, with no reference to normal signing; patients and clinicians are unable to communicate and deaf signers use gesture well, potentially misinterpreted as fluent BSL.

Aphasia has profound outcomes for patients including quality of life, depression, and has been associated with a worse prognosis. Moreover, subtypes of aphasia have different outcomes 1-year post-stroke Pedersen, Vinter, and Olsen (2004). Therefore, proper assessment is important to access appropriate therapy and ensures healthcare is equitable in the population. Left-sided lesions most commonly result in aphasia, where language centers are dominant for most people. Common post-stroke aphasias are global, Broca's and Wernicke's aphasia Pedersen et al. (2004), presenting with phonological, semantic, agrammatic and anomic errors amongst others. Whether aphasias are similar in signed languages depends on the way it is processed. Evidence is presented showing sign language has similar unilateral processing as spoken languages, and common errors in aphasia are compared between English and BSL for parallel presentations.

2 Methods

Articles were acquired through PubMed, Google Scholar and Mendeley. Search syntax included: Stroke, Sign Language, BSL, deaf patients, aphasia and subtypes, in combination. Relevance was evaluated based on the inclusion criteria: English language; published from year 2000 onwards; peer reviewed; patients fluency in British Sign Language where applicable; primary research data and reviews, and the exclusion criteria: Dissertations; abstract only; editorials; unpublished comments; articles published prior to the year 2000. Aphasia in deaf patients is an understudied topic with few papers addressing this problem directly. A wide publishing range (up to the year 2000) was chosen to accumulate sufficient data. Articles published before 2000 were omitted to present up to date evidence. Reviews were used in support of primary literature only where highly applicable.

3 Results and Discussion

3.1 Is sign language processed similarly to spoken language?

In most right-handed hearing individuals, language is processed in the front-left cerebral hemisphere. Broca's area is activated in speech production, with Wernicke's area posterior, including the temporal lobes, for comprehension Campbell, MacSweeney, and Waters (2008). It was previously thought sign language was processed bilaterally due to the visual elements of the language, which would require right hemisphere activation. Hickok et al., (2002) showed deaf signers with left hemisphere damage (LHD) from stroke, have reduced linguistic ability compared with right hemispheric damage (RHD), despite visuospatial impairment. Lesions in the left frontal regions were associated with poor speech production, and perception difficulties were associated with damage to the temporal lobe. The study found LHD with temporal lobe involvement, induced more severe impairment than LHD without temporal lobe involvement, concordant with speaking languages. This would suggest sign language is more lateralized than previously thought.

Most studies analysing sign language processing report post lesion dysfunction. Lesions may have indirect effects on proximal regions not directly involved in language. Imaging techniques showing activity during comprehension/production such as fMRI would remove this. fMRI has shown healthy non-deaf English speakers reading English activate the same left-sided regions as deaf signers watching sign language Campbell et al. (2008), supporting previous studies. The right hemisphere is more engaged in sign language than spoken language, particularly the visual cortex Campbell et al. (2008). Patients with RHD still perform under average in aphasia testing with some extra-grammatical errors after RHD (Atkinson, Campbell, Marshall, Thacker, & Woll, 2004; Hickok, Love-Geffen, & Klima, 2002). Nonetheless, the evidence overwhelmingly favors the left temporal and peri-sylvian involvement in deaf signers and visuospatial problems do not necessarily result in aphasia.

3.2 Symptoms of spoken aphasias after stroke

The most common spoken aphasias post-stroke are global (30-40%), Wernicke's (15%) and Broca's aphasia (12%), Pedersen et al. (2004). Anomic and transcortical aphasias also manifest Hoffmann and Chen (2013). Common aphasic errors are outlined in table 1.

3.3 Clinical presentation of aphasia in deaf users of sign language: Similarities with spoken language

Studies have shown these errors occur in sign language aphasia. Unfortunately, as this topic is understudied, most studies rely on single patient analysis, however the results are profound and demonstrate that rather than aphasia presenting differently to spoken language, the current lack of knowledge of BSL may be the limiting factor in establishing a diagnosis. Table 2 outlines the presentation of aphasias between spoken language and users of BSL.

3.4 Anomia

A case study by Marshall et al., (2003) found several errors in a patient after a left 106 sided stroke. In a picture-naming task, the patient was presented with 3 pictures (1 target and 2 semantic distractors) and asked to point to one in response to a sign. The patient scored well in this test and avoided semantic errors. In another test, the patient was asked to sign the word for pictures presented to him. Here, the patient was unable to retrieve many signs, often using finger spelling or gesture instead. These results indicated understanding was intact, but sign finding/word retrieval was impaired, indicating anomia. Visuospatial tasks were performed normally, hence language problems cannot be attributed to this. As in spoken anomia, the patient performed better on high frequency words than lower frequency words. Hemiplegia was accounted for, and all targets used were known to be in the patient's vocabulary. This should be considered by SALTs, as deaf signers have different cultural references to English speakers.

3.5 Phonology

BSL is lexical, with signs produced in specific places, motions and hand shapes. Phonological errors are produced when one component is incorrect. In the same study errors such as the correct hand motion and location but with the incorrect hand shape were made Marshall, Atkinson, Smulovitch, Thacker, and Woll (2004). Phonological errors may not have meaning, however errors can be made between meaningful signs also (which may not be phonologically related in English). The authors gave phonological cues when the patient struggled to find a sign, which was helpful, and could be valuable in developing therapy.

The authors should consider including MRI or CT scans to further evidence similarities between spoken and sign aphasia to give a diagnosis akin to that of spoken language, for example for the patient, either Broca's or anomic aphasia Alexander and Hillis (2008). A firm diagnosis may give better access to appropriate treatment.

Table 1: Common errors made in aphasias of spoken language

Error type	**Presentation in spoken language**
Phonological	Incorrect syllable used/understood in a word. For example, mistaking DOOR for FLOOR, or BOOK for LOOK
Semantic	Mistaking a word for another noun in the same category. For example, mistaking DOG for CAT or TOMATO for CARROT
Anarthria	Inability to articulate remembered words
Agrammatism	Producing sentences with content but not meaning/lack of grammar
Anomia	Trouble remembering words
Repetition and jargon	Repeating words often in the same sentence and producing words with no meaning

Table 2: Parallel aphasic presentations between spoken and signed languages

Error type	**Presentation in spoken language**	**Presentation in signed language**
Phonological	Incorrect syllable used/understood in a word	One of the 3 components of a sign is incorrect: hand shape, motion, position in space
Semantic	Mistaking a word for another noun in the same category	Identical to spoken language
Anarthria	Inability to articulate remembered words	May attempt to finger spell or make use of gesture
Agrammatism	Producing sentences with content but not meaning/lack of grammar	Identical to spoken language, lack of head, torso movement
Anomia	Trouble remembering words	May see 'groping' for signs and hesitant language
Repetition and jargon	Repeating words often in the same sentence and producing words with no meaning	Identical to spoken language

3.6 Anarthria

Saito et al., (2007), described the substitution of meaningless finger patterns when asked to name a line drawing, and related this to anarthria. The authors do not confirm that the patient recognized the word they were trying to sign by performing the inverse test (picture pointing after seeing a sign), therefore anomia cannot be ruled out here. Notably the patient had occipital lobe lesions. The authors suggest problems with sign execution cannot be explained solely with temporal lobe lesions. The patient had trouble reading and writing English words. The occipital lobe is activated in visual processing, therefore these problems could arise from lesions here and indeed sign production and finger spelling could be affected by this also. The authors did not confirm the patient's fluency with English hence cultural differences could account for this. Involvement of multiple regions has huge implications for sign language aphasia as the language relies heavily on a visual element, whereas this may not be so in English.

3.7 Semantic errors

A single case study of a deaf signer with left hemisphere stroke, presented with semantic errors comparable to spoken languages for example mistaking DOG for CAT Marshall, Atkinson, Woll, and Thacker (2005). The patients was shown a sign and asked to point to the corresponding picture from 5 options (the target or a semantic, phonological, visual or unrelated distractor) Atkinson et al. (2004). A challenge in BSL aphasia assessment is iconicity, for example the sign for CIGARETTE mimics how one would use it. Therefore, use of gesture may be misinterpreted as comprehension. The test removes this possibility by using 20 iconic and 20 non-iconic signs, thus identifying if a patient truly understands a sign. The patients showed no difference between iconic or non-iconic signs but made primarily semantic errors scoring only 25/40 (control average: 39.15). The patient was bilingual in BSL and English. Similar tests in English would ascertain whether the patient's aphasia is similar between both languages, supporting the hypothesis. Indeed, the patient scored 23/40 in a similar English test, with largely semantic errors.

3.8 Key differences between aphasia between BSL and spoken language

Anterior left hemisphere damage induces agrammatism in users of BSL Marshall et al. (2003). BSL relies on space for grammatical structures and uses multiple body parts, for example moving the torso forward and backward to indicate tenses Marshall et al. (2005). Apraxia post-stroke due to lesions of the motor cortex may prevent proper sign formation and grammar production. Apraxia (outside orofacial/bulbar muscles) would not affect spoken language. Although deaf signers can switch dominance and communicate with one hand, hemiplegia may affect communication, particularly as speaking whilst performing tasks will become affected.

Another difference is the potential for visual errors in sign language. For example, the sign for DOG may be mistaken for a knife and fork gesture, as the sign resembles the action of using these items (first two fingers of both hands pointing downwards, palms facing towards the speaker) Marshall et al. (2005). This error could indicate a patient is relying on iconicity or gesture to communicate rather than truly comprehending signs.

4 Conclusions

Despite only a small number of existing studies compromising a small patient population, there is clear evidence sign language aphasia presents similarly to spoken languages and could be given parallel diagnosis of aphasia subtypes. Recognition of this alone will ensure better access to therapy. Moreover, risks for aphasia post-stroke are well recognized such as age, previous stroke and urinary problems Plowman, Hentz, and Ellis (2012). A clinician should be made aware aphasia is a possibility in a deaf patient presenting with these risk factors and be monitored closely, particularly where communication between patient and clinician is limited. Future studies should aim to elucidate how lesions of the motor cortex and visual cortex influence aphasia and any other potential differences to administer appropriate, high quality therapy.

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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.

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

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The use of non-steroidal anti-inflammatory drugs in chronic kidney disease: a primary care audit

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Abstract

The incidence of chronic kidney disease (CKD) is increasing alongside the use of non-steroidal anti-inflammatory drugs (NSAIDs). The use of these medications in patients with CKD is associated with an increased risk of renal impairment and disease progression. As a result, the latest guidance by the National Institution of Care Excellence cautions against the prescription of NSAIDs in these patients. With this in mind, we conducted a large primary care audit to identify cases of patients with CKD being prescribed an NSAID. We identified 53 such cases out of 1490 patients with CKD (3.6%). The factors contributing to this result are discussed and 10 proposals are given to build upon these findings in future.

1 Introduction

Chronic kidney disease (CKD) is a common condition with an estimated global prevalence of 11-13% (Hill et al., 2016). According to Kidney Care UK, 1 in 8 people will go on to develop it at some point in their lives (Kidney Care UK, n.d.).

Unfortunately, CKD is also a significant cause of morbidity and mortality. In 2015 alone, 1.2 million people died from renal failure; an increase of 32% since 2005 (H. Wang et al., 2016). As a result, clinicians must be careful when managing these patients. This is especially true for the elderly who make up the largest proportion of CKD patients, and are at the highest risk of kidney injury (Public Health England, 2014; X. Wang, Bonventre, & Parrish, 2014).

Non-steroidal inflammatory drugs (NSAIDs) are also in very common use, with over 15 million prescriptions dispensed in England in 2014 (Davis & Robson, 2016). This is excluding private prescriptions and over-the-counter forms which are readily available in most pharmacies across the world. However, NSAID use is potentially dangerous (Davis & Robson, 2016).

NSAIDs have been shown to rapidly decrease glomerular filtration rate (GFR) and long-term use is associated with the progression of renal disease (Hörl, 2010). It has been reported that in patients aged over 65 years, NSAIDs more than doubled the risk of acute kidney injury in the next 30 days (Schneider, Lévesque, Zhang, Hutchinson, & Brophy, 2006). In spite of this, 9% of patients aged over 70 were found to receive a prescription for NSAIDs for more than 3 months in 2016 (Hörl, 2010).

Similar findings have been described by other authors internationally (Guirguis-Blake et al., 2018; Plantinga et al., 2011).

Given the frequency of both CKD and NSAID use, even a small percentage overlap of the two could lead to a large cumulation of patient harm. With this in mind, we performed a primary care audit of the number of patients with a diagnosis of CKD that were being prescribed NSAIDs. This took place at a large medical centre in England.

2 Criteria

The criteria for this study are based on the latest guidelines as set out by the National Institute for Care Excellence (NICE) in 2014 (NICE, 2014). Notably, these recommendations are reiterated in the 2019 revision of 'NSAIDs – prescribing issues', a part of the NICE Clinical Knowledge Summaries (CKS) (NICE, n.d.).

In addition, several other independent organisations have produced similar advice on NSAID use in CKD. This includes the UK's National Health Service (NHS) and international groups such as Kidney Disease: Improving Global Outcomes (Levin et al., 2013; NHS, n.d.). The guidance on this area therefore appears to be robust.

Specifically, we will be comparing our data set against the following two criteria (NICE, 2014):

1. 'NSAIDs should not be prescribed in people who have an eGFR of less than 30 mL/minute/1.73 m^2 i.e. severe renal impairment.'

2. 'For those with any renal impairment or even those at risk of developing renal impairment, prescribing NSAIDs should be avoided (if possible).'

3 Evidence

There is a plethora of evidence which supports the claim that NSAIDs, especially at a high dosage, may increase the risk of CKD and/or worsen kidney function (reviewed in (Nderitu, Doos, Jones, Davies, & Kadam, 2013; Yaxley, 2016)). In support of this, a literature search of the PubMed database in December 2019 for the terms 'non-steroidal anti-inflammatory drug' and 'chronic kidney disease' returned 7502 results. A small selection of these are described in Table 1. Interestingly, different NSAID sub-types are associated with different risk values.

4 Standards

After consultation with members of the primary care team, we decided on a standard of 90% against which we have compared our findings (Table 3). In other words, we expected to see that 90% of patients with CKD were not prescribed an NSAID. Whilst we would have liked this figure to be as high as possible, we realised this standard must consider a number of influencing factors.

Firstly, CKD comes in different stages Table 2 (Levey et al., 2003). Whilst all CKD patients should generally avoid NSAIDs, only stages 4-5 are absolute contraindications (Levin et al., 2013; NICE, 2014). Practitioners may therefore feel that in some cases, the benefits of NSAID use outweigh the risk. This is commonly the case with low-dose aspirin for cardiovascular risk control which has been reported to have no significant effect on kidney failure (Su et al., 2019).

Other GPs may be unconvinced by the latest guidelines which seem to lump all NSAIDs and doses together. It is understandable that they may wish to form their own opinions based on the literature. More simply, but rarely, some doctors may even be unaware of the most recent guidelines. It is also possible that patients may be intolerant or allergic to alternative analgesia such as opioids, or simply prefer NSAIDs due to other factors such as fear of addiction.

5 Methods

A search of the GP database (SystemOne) was performed to identify patients with CKD who had visited the practice in the last 12 months (from 01/01/2019 to 01/01/2020). The resultant patient list was then filtered to identify those which were also prescribed an NSAID at any point during this period. Patients were stratified by the stage of their CKD and the data was analysed as described below.

6 Results

At the time of this study, 1490 patients were found to be on the CKD register. Of these, 53 had been prescribed a NSAID on repeat prescription (3.6%). Nine were found to be CKD stage 3 but fortunately, no patients were stages 4 or 5. Out of these 9 patients with stage 3 disease, 5 were receiving aspirin for cardiovascular risk reduction. The distribution of NSAIDs prescribed in this sub-population are given in Figure 1.

Considering these results, each patient was marked for review and the decision was made not to implement a second cycle at this time.

7 Discussion

As shown in Table 3, 96.4% of patients with CKD were not prescribed a NSAID in this GP practice. This exceeded our standard of 90%. Moreover, no patients with CKD 4 or 5 were given an NSAID. This follows the latest evidence-based guidelines (NICE, 2014). Observation of standard practice revealed a number of factors that contributed to this result.

Firstly, being a large practice, patients would often see a variety of clinicians of different grades over time (as well as medical students). This introduced a fresh perspective with every other consultation and enabled treatment plans to be double or triple checked.

Secondly, the computer system (SystemOne) automatically

Table 1: Studies focusing on the effects of NSAIDs in CKD

Study	Methods	Results
Nderitu et al 2013	Systematic review and meta-analysis of NSAIDs and CKD progression.	High-dose NSAID use significantly increased the risk of accelerated CKD progression (OR: 1.26; 95% CI: 1.06– 1.50).
Hsu et al 2015	Cohort study of 10184 subjects over 2.75 years using multiple regression analyses.	High-dose NSAID users experienced a 26% increased risk (OR: 1.26, 95% CI: 1.04-1.53). No risk differential was seen for selective and nonselective NSAID users.
Sandler et al 1989	Multi-centre, retrospective case control study of 554 patients with newly diagnosed kidney disease.	Daily paracetamol ingestion was associated with higher risk of CKD compared with non-consumers (OR: 3.2; 95% CI: 1.05 to 9.80).
Ingrasciotta et al 2015	Nested case-control study of 1989 CKD cases using multivariate models for NSAIDs.	Statistically significant increase in CKD risk for current users of oxicams (OR: 1.68; 95% CI: 1.15-2.44).
Fored et al 2001	Case-control study of 926 CKD patients that regularly or sporadically used aspirin.	An average intake of 500g or more of aspirin per year during periods of regular use resulted in an increased risk of chronic renal failure (OR: 3.3; 95% CI: 1.4-8.0)

OR = odds ratio. CI = confidence interval.

Table 2: Simplified staging system of CKD

Stage	Description	GFR (ml/min/1.73m^2)
-	At increased risk	≥60
1	Kidney damage with normal or increased GFR	≥90
2	Kidney damage with mild decreased GFR	60-89
3	Moderately decreased GFR	30-59
4	Severely decreased GFR	15-29
5	Kidney failure	<15 (or dialysis)

Table 3: Results on NSAID use in CKD

	CKD + no NSAIDs	CKD + NSAIDs	Total
Number of patients	1437	53	1490
Patient percentage (%)	96.4	3.6	100
Pre-set standard (%)	90	10	100

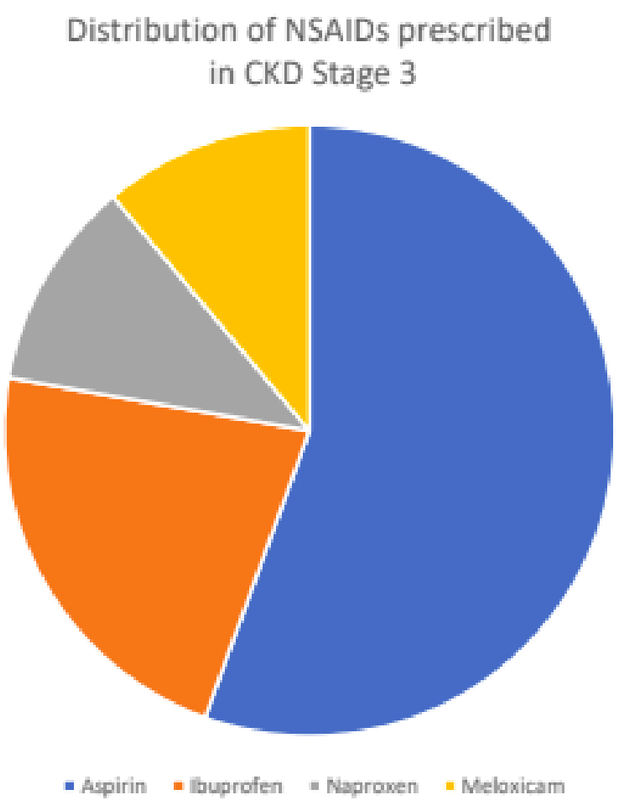

Figure 1: Pie chart of NSAID use in CKD stage 3 (n=9)

generates a 'pop-up' to warn against NSAID use in renal impairment. This creates the question as to why NSAIDs were given in 53 patients with CKD. One possible answer is that GPs might have been over-riding these prompts. This was observed on a number of occasions and one doctor explained to us that having so many different pop-ups for so many different things, actually made them all less effective. Another reason could be due to the perceived risk to benefit ratio of prescribing NSAIDs, as mentioned above.

Thirdly, the staff at the medical centre regularly meet to discuss topics such as prescribing issues and audit results. Focusing on the latter, there is a dedicated audit manager and an excess of keen medical students who are all constantly evaluating the service provided.

However, this audit is not without its limitations. We realise that the results above are a gross underestimate of NSAID use in CKD. To better investigate this topic, one should also consider over-the-counter medication by conducting patient interviews. This is important as NSAIDs are commonly over-used in both dose and indication (Kaufman et al., 2018; Wilcox, Cryer, & Triadafilopoulos, 2005). During these interviews, one could also assess what patients with CKD know about NSAID use and whether they received any advice on diagnosis (e.g. verbally, as a leaflet, or from a website). Similarly, GPs could be interviewed and/or tested on NSAID prescribing issues. This could help establish why there were 53 cases of NSAID use in CKD identified.

8 Conclusions

This medical centre is exceeding standards with regards to NSAID use in CKD. There are multiple factors that have contributed to this and we advise that similar measures are employed by other practices. However, there is still room for improvement and as such, each case of NSAID use in CKD was followed up individually.

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No conflicts of interest have been declared by any authors.

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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.

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Can stem cells revolutionise therapy for osteoarthritis?

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Abstract

Osteoarthritis is a debilitating joint condition which primarily causes damage to the articular cartilage, but also affects surrounding joint tissues such as the subchondral bone and overlying synovium. The condition presents itself through impaired joint function, reduced movement, increased pain and stiffness. There are various molecular mechanisms underlying osteoarthritis from abnormal activation of proteolytic enzymes to pathological inflammatory pathways; the end result is gradual cartilage (and ultimately other joint tissue) damage. Current therapy involves symptomatic management, with most patients undergoing total joint replacement eventually. New research into the functional mechanisms of stem cells introduces exciting opportunities for osteoarthritis therapy. Mesenchymal stem cells make an ideal candidate for the role. These cells possess the ability of self-renewal and are also able to produce a variety of anti-inflammatory molecules, which are hugely beneficial for the osteoarthritic joint. Numerous clinical studies have been analysed in this study to determine the efficacy of mesenchymal stem cells. This review aims to outline mechanisms underlying osteoarthritic pathology and potential therapeutic options for the future.

1 Introduction

Osteoarthritis (OA) is a chronic progressive functional and structural degenerative joint disease (Hunter & Feldon, 2006). It is estimated that approximately 8.5 million individuals in the UK have clinical OA, as defined by signs and symptoms such as joint pain, joint stiffness and functional limitations (Al-Najar M, Khalil H, Al-Ajlouni J, Al-Antary E, Hamdan M, Rahmeh R, Alhattab D, Samara O, Yasin M, Al Abdullah A, 2017). Prevalence increases with age, 13.9% of adults over the age of 25 experience OA in at least one joint while for individuals over the age of 65 that statistic jumps up to 33.6% (Peach CA, Carr AJ, 2005). By the end of 2020, OA is set to become the fourth most disabling disease globally. Its negative consequences don't just end there; OA was also found to have evident links with other conditions such as depression, sleep disorders and neuropathic pain (Makris EA, Gomoll AH, Malizos KN, Hu JC, 2015a; Malchau H, Herberts P, 1993). Unfortunately, left to its own resources the dysfunctional osteoarthritic joint has little hope of convalescence as it does not possess healing properties unlike most other tissues in the body (Klatt E, 2015). The aim of this review is to critically analyse the current prognosis and treatment of OA and evaluate the potentially different patient outcomes through the introduction of stem cell therapy.

2 Osteoarthritis

OA is a degenerative disease resulting in gradual joint dysfunction. Although all joints can be affected the knee,

hip and hand joints are the most common (Makris EA, Gomoll AH, Malizos KN, Hu JC, 2015b). The resulting pain is usually significant enough to hinder other physiological processes evidenced by knee OA being associated with a 1.55-fold increased risk for all-cause mortality (Tchetverikov I, Lohmander LS, Verzijl N, Huizinga TW, TeKoppele JM, Hanemaaijer R, 2005). All joint tissues undergo pathology in OA; from bone and menisci to ligaments and synovium. However it's the hyaline articular cartilage (AC) which is most affected; the eventual destruction of which prompts the need for surgical intervention. From a macroscopic prospective the AC goes through three phases of destruction; fibrillation, erosion and cracking. Eventually inflammatory alterations to the synovium and subchondral bone results in chondrocyte necrosis, extensive loss of cartilage and marked subchondral bone changes. The whole process is termed 'eburnation'. On a cellular level the AC undergoes an increase in water content and a reduction in proteoglycan and collagen. In maintaining homeostasis chondrocytes can synthesise new extra cellular matrix (ECM) to replace the degraded matrix although this process is incredibly timely; proteoglycan turnover can take upwards of 2 decades (Endres M, Andreas K, Kalwitz G, Freymann U, Neumann K, Ringe J, Sittinger M, Häupl T, 2010). In OA the rate of ECM destruction is higher than the rate of synthesis leading to an overall net reduction (Goldman L, 2011). These changes limit the AC's compressible properties and increase the permeability to tissue breakdown products.

AC is composed of sparse specialised chondrocytes and (mainly) ECM. ECM consists of type II collagen proteins which form a structural skeleton, elastin micro fibrils and aggrecan (aggregated proteoglycans) all of which are synthesised by AC chondrocytes (Malchau H, Herberts P, 1993). The swelling pressure of aggrecan and the tension of the collagen matrix is equal and opposing, giving AC the property of resilience under compression. Through the course of OA it is these molecules which undergo proteolysis. Enzymes of the 'A disintegrin Metalloproteinase with Thrombospondin motif' (ADAMTS) family are responsible for the damage that occurs to the aggrecan, especially ADAMTS 4/5. Whereas it's the 'collagenolytic matrix metalloproteinases' (MMPs) which provoke collagen breakdown. MMPs are secreted from chondrocytes and synovial lining cells undergoing physiological stress. These enzymes hydrolyse the collagen network effectively softening it, leading to the initiation of the superficial layer of AC into the fibrillation phase. This process of cartilage softening is called 'chondromalacia'. It was found that chondrocyte mRNA for various MMPs was significantly higher in OA versus non-OA patients (Cross M, Smith E, Hoy D, Nolte S, Ackerman I, Fransen M, Bridgett L, Williams S, Guillemin F, Hill CL, 2014).

Ultimately, the entire joint suffers. As the AC erodes it exposes the underlying subchondral bone. The unprotected bone is susceptible to wear and tear which results in the formation of micro fractures on the bony trabeculae. This induces osteoblast activity to regenerate the bone on a microscopic scale. Osteophyte formation and focal pressure necrosis is quick to follow leading to the formation of cysts on the subchondral surface. As a result of these alterations, subchondral bone is less able to dissipate energy upon excursion, thus increasing the force exerted on the joint which further enhancing cartilage damage (B Marcu K, Otero M, Olivotto E, Maria Borzi R, 2010; Scanzello CR, Umoh E, Pessler F, Diaz-Torne C, Miles T, Dicarlo E, Potter HG, Mandl L, Marx R, Rodeo S, 2009). Bone marrow oedema can build up in the subchondral bone along with vascular engorgement and reduced blood flow to the bone marrow, all of which contribute to the clinical features of pain in OA. Prevailing therapeutic options are aimed at targeting these features.

3 Current Treatment

Current therapeutic options for OA are limited. Often treatment is targeted towards alleviating the signs and symptoms of OA, rather than targeting the underlying pathology (Klatt E, 2015). Obesity is a fundamental risk factor for OA, and consequently weight loss is effective in non-pharmacological management. Clinical trials from 2005 showed that patients with knee OA experienced a 28% improvement in knee function (stiffness and joint pain) following a 10% reduction in body weight (B, 2006). Contrastingly pharmacological interventions focus mainly on pain management, ranging from NSAIDs (Non-steroidal anti-inflammatory drugs) to opioid use. NSAIDs and paracetamol are widely prescribed (Cryer B, 1998; Towheed T, Maxwell L, Judd M, Catton M, Hochberg MC, 2006) however the effects, while statically significant, are minimal as found by a Cochrane review in 2006 (Chalmers JP, West MJ, Wing LM, Bune AJ, 1984). Nonetheless chronic use of NSAIDs and paracetamol pose their own risks including cardiovascular, respiratory and GI side effects. More invasive therapeutic options include intra-articular corticosteroid injections and total joint replacement (TJR). TJR application introduces its own complications. While (for knee joints) 82% of TJRs last for 25 years (on average); TJRs are not completely effective and have numerous complications both during and after surgery (Sophia Fox AJ, Bedi A, 2009). Ranging from risk of infection and technical error during surgery to instability, misalignment, recurrent dislocation and osteolysis thereafter (Goldring MB, 2004; Moore K, 1998). Surgery for many is still not the preferred option for management. Finding an alternative therapeutic cure for OA is therefore a necessity, especially one which targets the elemental pathology in OA. Upcoming research into the regenerative abilities of stem cells introduces exciting new prospects for OA treatment.

4 Stem cells

Stem cells are defined as cells which possess the capability of self-renewal for a prolonged period, along with the ability to differentiate to produce at least one type of mature cell. These cells have the capacity to become all (totipotent) or most (pluripotent/multipotent) tissue types within the body. Subpopulations of stem cells are classified as embryonic stem cells (ESCs), adult stem cells (ASC) and cancer stem cells (CSCs). ESCs are pluripotent, giving rise to all cell types. CSCs are the basis for cancer development and have more sinister implications such as escaping physiological apoptosis

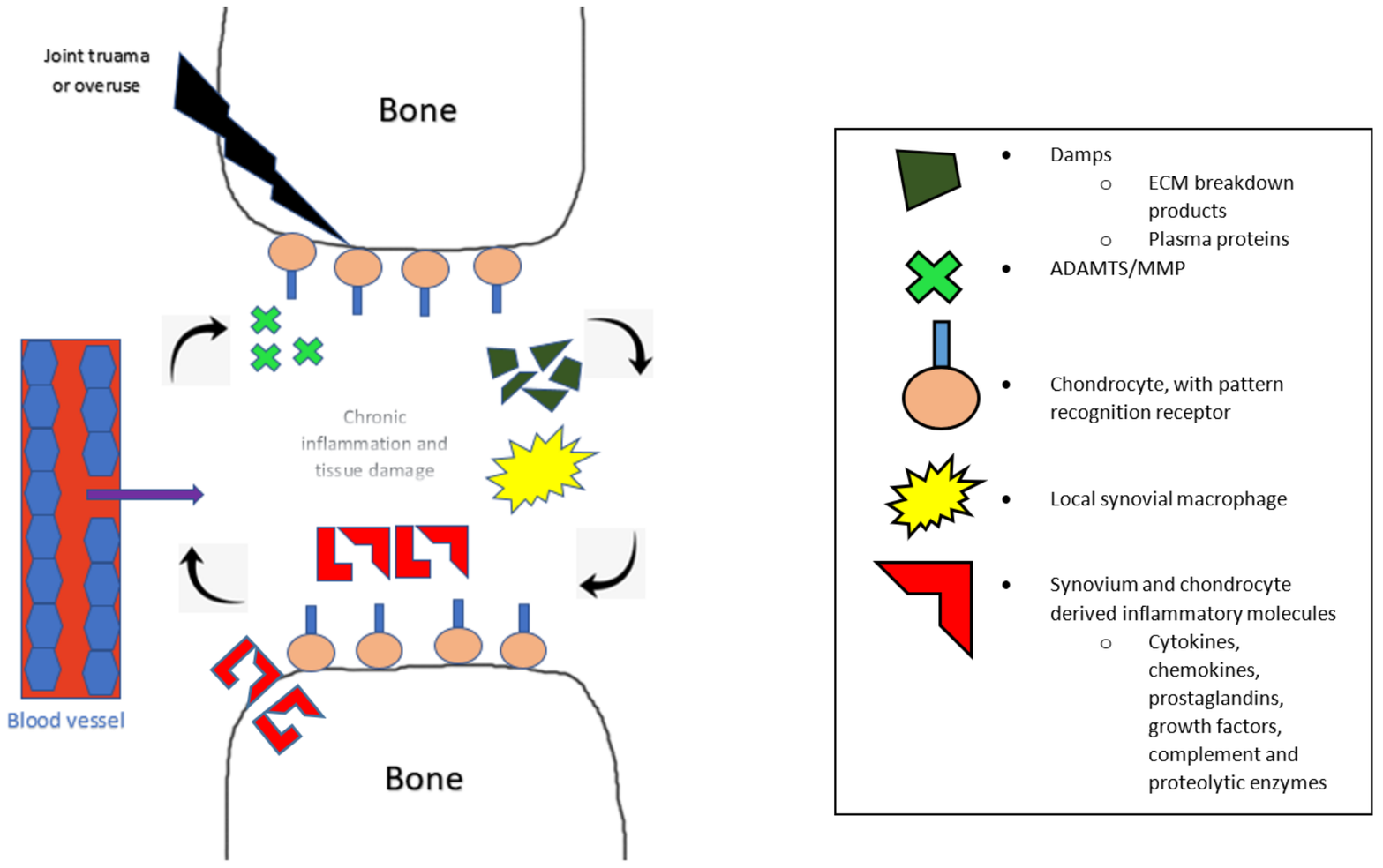

Figure 1: Schematic representation of chronic inflammation and pathological occurrences in an OA joint (Sokolove J, 2013)

(Goldman L, 2011). Induced pluripotent stem cells (IPSCs) are another prospective area of study. These cells exhibit many similarities to ESCs but are derived from somatic cells. These cells can be genetically re-engineered in vitro to regain proliferative properties and the potential to differentiate into various cell types (Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, 2007). The most important stem cells for OA therapy however are ASCs. ASC's main function is to replenish cells which undergo stress or death due to physiological wear and tear or pathology (Goldman L, 2011). Examples of such cells are mesenchymal stem cells (MSCs).

4.1 Mesenchymal stem cells

MSCs can differentiate into osteoblasts, adipocytes, myocytes and chondrocytes and (in adults) are derived from two main sources; adipocytes and bone marrow (Caplan AI, 2007; Somoza RA, Correa D, Labat I, Sternberg H, Forrest ME, Khalil AM, West MD, Tesar P, 2018). MSCs possess immunomodulatory and trophic properties through the release of growth factors and cytokines (Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, 2002). Cytokines including TGF-β, HGF (hepatocyte growth factor) and PGE2 are all released. These function to reduce immune cell function, mainly by upregulating T-cell function (Dyer DP, Thomson JM, Hermant A, Jowitt TA, Handel TM, Proudfoot AE, Day AJ, 2014; Engela AU, Hoogduijn MJ, Boer K, Litjens NH, Betjes MG, Weimar W, 2013). TSG-6 (TNF-alpha stimulated gene/protein 6) is also expressed by MSCs and is a key component in the regulation of inflammation by inhibiting neutrophil chemotaxis while also possessing chondroprotective properties (Vonk LA, De Windt TS, Slaper-Cortenbach IC, 2015). Other T cells (CD4+ and CD8+) are also affected by MSCs. By releasing inflammatory soluble factors MSCs induce G0 arrest of the cell cycle of these cells or even cause apoptosis, exhibiting inhibition of both innate and adaptive immune pathways (Pers YM, Ruiz M, Noël D, 2015; Vonk LA, De Windt TS, Slaper-Cortenbach IC, 2015). MSCs also disrupt the local inflammatory response by suppressing B cell activation and antibody secretion which effectively eradicates the risk of tissue rejection for prospective stem cell transplantation.

5 Clinical Application

Before application to the patient stem cells often undergo in vitro 'expansion' (induced mitotic divisions) (Lai RC, Yeo RW, 2015). Although it is difficult to prevent phenotypic instability during this process there are numerous studies which show that factors such as TGF-β, Proline, Insulin, BMPs and FGF all enhance chondrogenesis and cartilage formation (Lefebvre V, 2015). In clinical application the MSCs are generally obtained from healthy donors, often from the posterior superior iliac spine region and expanded to large number before transplant. Minimal amounts of BM aspirate are required (between 2-4ml) which is mixed with heparin to prevent blood clotting. Clinical trial studies of MSC application are critical to analyse, in order to determine the efficacy and potential success of stem cell therapy.

6 Clinical Data

From all the studies that have been discussed intra-articular injections containing autogenic or allogenic MSCs showed positive clinical manifestations with no graft-related death, tumorigenesis or infection (Vega A, Martín-Ferrero MA, Del Canto F, Alberca M, García V, Munar A, Orozco L, Soler R, Fuertes JJ, Huguet M, 2015). Since the method of transfer of stem cells is minimally invasive and does not require surgery this significantly reduces risks of many surgical side effects unlike TJR.

Key:

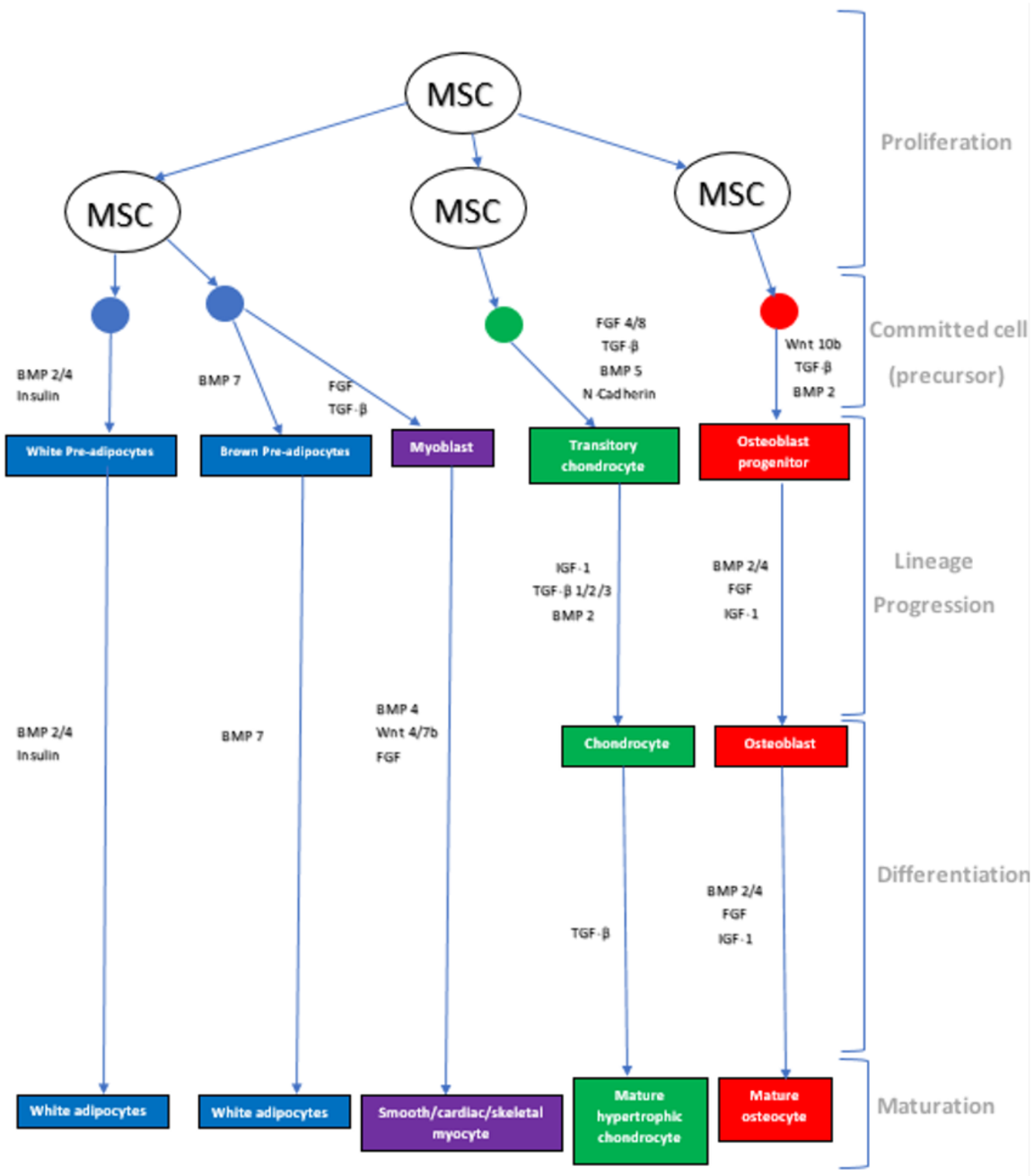

Figure 2: Schematic showing multiple differentiation pathways of MSCs. A diverse range of growth factors, receptors, intracellular signalling molecules and transcription factors are involved in aiding differentiation. Chondrogenic differentiation is displayed as the green lineage (Kolf CM, Cho E, 2007).

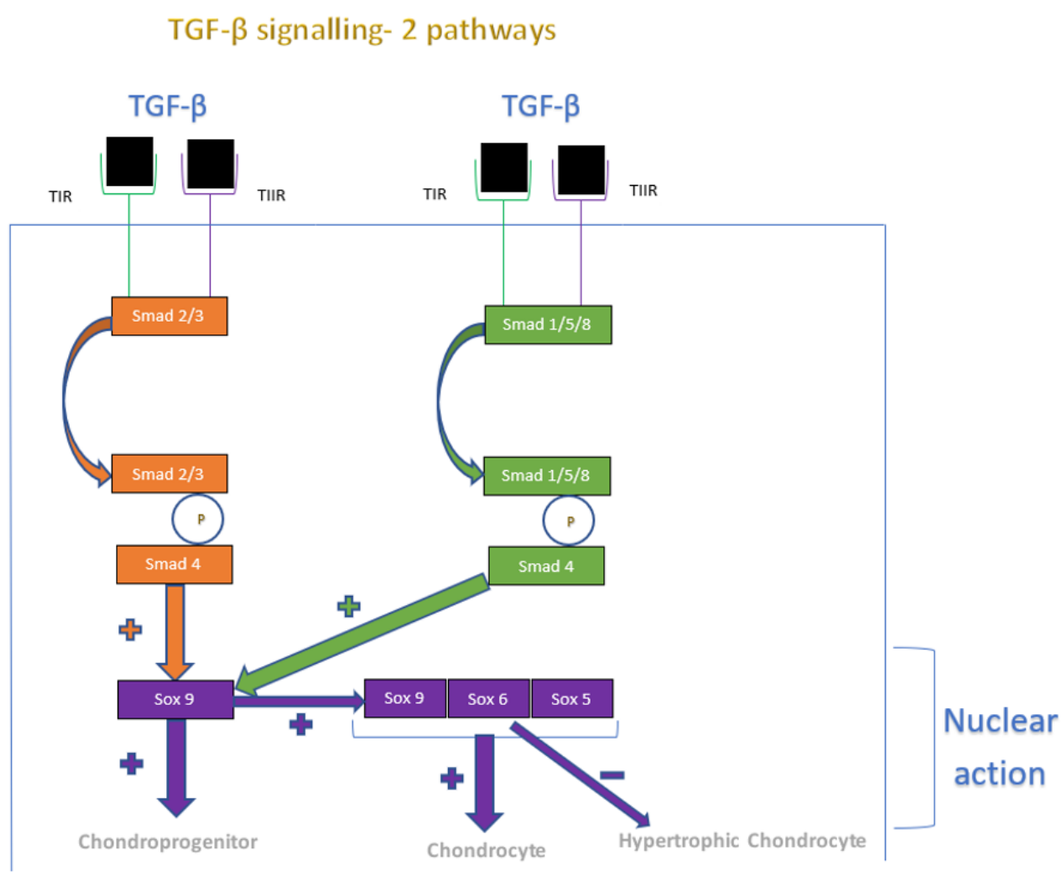

Figure 3: Schematic diagram displays two signalling pathways of TGF-β. Signals are transmitted via a pair of transmembrane serine/threonine kinases (TIR/TIIR). Multiple intracellular molecules are subsequently phosphorylated, resulting in an increase in activity of various transcription factors (Sox molecules). These molecules aid differentiation of the chondroprogenitor, while preventing excessive hypertrophy (chiefly through the action of Sox6) (Szychlinska MA, Stoddart MJ, D'Amora U, Ambrosio L, Alini M, 2017; Tang X, Fan L, Pei M, Zeng L, 2015).

1. KOOS: Knee injury osteoarthritis outcome score (measures 5 different categories, pain, other symptoms, ADL, function in sport and recreation and knee related QOL).

2. WOMAC: Western Ontario and McMaster universities osteoarthritis index (questionnaire with 3 categories measures, joint pain, stiffness, and functional limitations). Used in conjunction with HOOS (Hip injury osteoarthritis outcome score).

3. VAS: Visual analogue scale. Patients specify their level of agreement to particular statements; a more subjective scale.

4. Phase I/II clinical trials: Trials which determine the safety, side effects and best dosage of a new treatment and monitors disease progression. Usually different doses of drug are given in phase I and phase II of the trial.

5. Tegner activity scale: Sports based scoring system, on a scale of 0 to 10. 0 is disability, 10 represents international level sporting ability.

6. OARSI: Osteoarthritis research society international. Scoring system for the progression of OA.

7. PRP: Platelet- rich plasma.

All studies had expanded their initial harvest of cells. As can be seen from the numerous studies in the table there is overwhelming evidence to support the use of MSCs for OA treatment from both BM and AD sources, both allogenic and autologous, with many of the clinical trials reporting no tissue rejection. Testing categories ranged from safety to clinical outcomes and numerous studies have found MSCs to be effective in improving function and inducing repair in osteoarthritic joints. It is interesting to note however that certain studies, such as the one done by Pers et al in 2016 suggests the lowest dosage of cells given gave the most successful results. Hence investigating the optimal cell dosage is something which still requires further research. Potential use of a scaffold, most appropriate timing of intervention and method of MSC delivery are all among the components of methodology which require further investigation (Montoya F, Martínez F, García-Robles M, Balmaceda-Aguilera C, Koch X, Rodríguez F, Silva-Álvarez C, Salazar K, Ulloa V, 2013). The minimally invasive mechanism of intra-articular injections minimises risk of infection and significantly lowers risk of technical error during the procedure. While MSCs are an incredibly effective treatment option, costs of application to the NHS need to be considered.

7 Economic Implications

OA itself represents an enormous economic burden on the NHS. In 2000 there were 3 million GP consultations and 115,000 hospital admissions due to OA. Patients with OA often have multiple comorbidities, further adding to the complexity of care required. £250 million is spent annually on community and social services (as of 2010) and the total cost to the gross national product is estimated to be around 1%. In 2000 the economy suffered £3.2 billion in 'lost production', this brings into prospective the impact this condition has on our wider community. Surgical treatment introduces its own costs. There were 116,000 hip and knee TJR surgeries in 2010 which ultimately cost the NHS around £890 million (Pers YM, Rackwitz L, Ferreira R, Pullig O, Delfour C, Barry F, Sensebe L, Casteilla L, Fleury S, Bourin P, 2016). Demand for TJR has increased significantly with more than 160,000 TJR surgeries taking place in 2019 (Clinic, 2020a). Data on the costs of stem cells is limited although it is estimated that stem cell regeneration for one total knee OA would cost £6,400 (Clinic, 2020b). Affordability may not be the only hindrance; several eligibility criteria also prevent individuals from receiving stem cell treatment (such as needing to have a BMI of less than 35) (Clinic, 2020b).

8 Conclusion

There is overwhelming evidence to support the efficacy and safety of MSC application for OA therapy. After analysing multiple clinical studies I have found numerous positive outcomes from MSC therapy; not just to alleviate patient symptoms (as current OA therapy does) but to target and cure the fundamental underlying pathology. Nonetheless certain aspects of treatment require further research such as the correct dosage of cells and appropriate timing of intervention. Thus the need for a gold standard is necessary, to ensure maximum safety for patients. The nature of stem cells themselves however pose some ethical dilemmas which need to be addressed. Concerns regarding intentions for stem cell use, especially in the domain of ESCs, are often raised. Some argue this could violate the respect for nascent human life. Often in IPS/ASC research donors are not explicitly informed of which research procedures their donated cells will be part of, and who will be performing the study. As these databases are often accessed by large numbers of people, those who oppose this kind of research would argue that potential breach of data would be an invasion of privacy and confidentiality. These concerns can be addressed with more robust legislation, although the ethical stigma would still likely be associated. It is also critical to consider the expense of MSC therapy which as it stands would generate further economic strain on an already financially struggling NHS. However, with technological advancements and improvements in culturing and application methods, MSC therapy holds a very promising stance in the future of OA therapy.

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.

Type of study	Type of MSC /scaffold used	Number of participants/length of study	Cell dosage	Measurement technique used	Findings/results	Reference /year of study
Phase I/II study.	Autologous AD-MSCs with saline.	18 participants, with knee OA, 6 months follow up.	Three doses of cells administered: 1*10^7, 5*10^7, 1*10^8.	WOMAC.	Cartilage defect was reduced, while volume increased. Most significantly in higher dosage. Hyaline cartilage regeneration detected with clinical improvement in pain and function.	36. Year of study 2017 Jo Ch et al.
Phase I/II study.	Autologous BM-MSCs.	13 participants with knee OA, 24 months follow up.	One dose of cells administered: 30.5*10^6.	MRI and KOOS.	Significant KOOS improvements, and knee cartilage thickness had improved detected by MRI.	2. Year of study 2017 Mahasen Al Najar et al.
Phase I/II study.	Autologous BM-MSCs.	30 participants with knee OA, 12 months follow up.	Two doses of cells administered: 10*10^6 and 10*10^7.	VAS, WOMAC, X-ray and MRI.	Injection of the highest dose showed most clinical/functional improvement. Significant improvement in joint pain.	37. Year of study 2016 Lamo-Espinosa JM et al.
Phase I study.	Autologous BM-MSCs.	4 participants with knee OA, 60 months follow up.	One dose of cells administered: 8-9*10^6.	Walking time, X-ray and VAS.	Significant improvement in clinical knee function. Earlier transplantation had better results.	38. Year of study 2016 Soler R et al.
Phase I/II study.	Autologous AD-MSCs.	18 participants with knee OA, 24 months follow up.	Three doses of cells administered: 10*10^6, 50*10^6, 100*10^6.	VAS, WOMAC and MRI.	The highest dosage of cells were most effective in improving clinical joint function/mobility.	39. Year of study 2017 Jo CH et al.
Case series study.	Autologous AD-MSCs with PRP.	30 participants with knee OA, 24 months follow up.	One dose of cells administered 1.9*10^6.	VAS, Tegner activity scale.	Significant reduction in pain and improved joint function after 24 months of follow up.	40. Year of study 2012 Koh YG et al.
Case series, Phase I study.	Autologous AD-MSCs.	18 participants with knee OA, 20 months follow up	Three doses of cells administered, 2*10^6, 10*10^6, 50*10^6.	WOMAC, VAS, KOOS and OARSI.	Lowest dosage group showed best results. Improvements in joint pain/mobility.	41. Year of study: 2016, Pers et al.
Case series study.	Allogenic AD-MSCs.	30 participants with knee OA, 12 months of follow up.	One dose of cells administered: 40*10^6.	VAS, WOMAC and MRI.	Improvement in joint cartilage quality and reduction in pain.	42. Year of study, 2015, Vega et al.
Case series study.	Allogenic BM-MSCs.	30 participants with knee OA, 12 months of follow up.	One dose of cells administered: 40*106. Control group given 60mg of hyaluronic acid (15 participants in each group).	VAS and MRI (Figure 1).	Improvements in functional/structural properties of joint. Increases in cartilage size and quality.	35. Year of study, 2015, Vega Aurelio et al.

Table 1: In reference to Section 6 (Clinical Data)

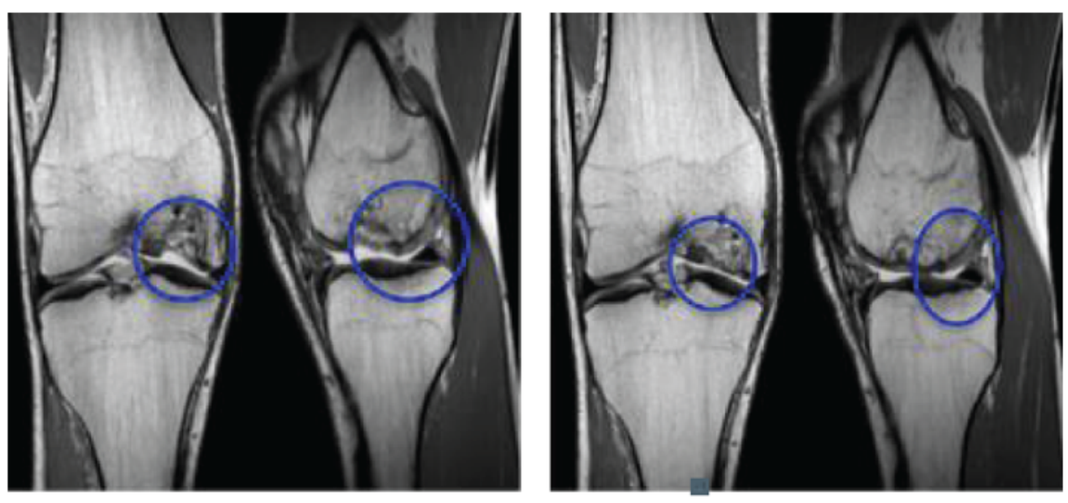

Figure 4: Schematic of MSC bases therapies in OA. MSC self-renewal properties and methods of application (left) and their molecular mechanisms in maintaining joint homeostasis (right) are all shown. Notably the immunomodulatory and anti-inflammatory effects via alterations to anti/pro-inflammatory molecules (Zhang R, Ma J, Han J, Zhang W, 2019).

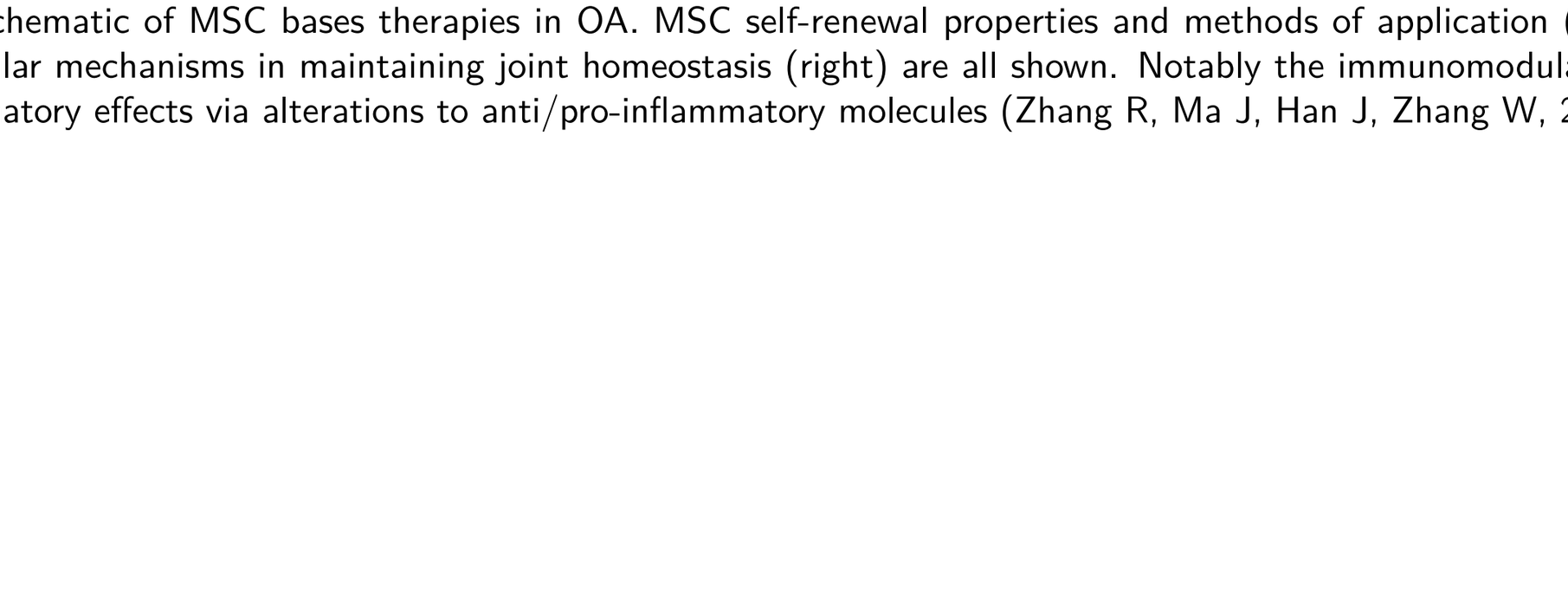

Figure 5: (Freitag J, Shah K, Wickham J, Boyd R, 2017) MRI of knee joint. Coronal (left) and sagittal (right) T2 weighted MRI images shown. A is pre-treatment, B is post treatment 12 months after initiating treatment. B shows significant regeneration of the articular cartilage.

Ethics statement

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

Editorial and peer review statement

The review process for this manuscript was double blind, where authors and peer reviewers were blinded to each others identity and institution.

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Reliability of Fracture Neck of Femur Classification Systems – A Retrospective Study

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Abstract

Introduction Fractured neck of femur (NOF) is a common injury, and the majority occur in the elderly frail population. Classification systems are useful in helping guide management and determine prognosis, therefore having a reliable system is of clinical benefit. In the literature, two main classification systems exist for NOF, Garden and Pauwel's, but there is debate over which is more reliable. The aim of this study was to compare the reliability of Garden and Pauwel's classification systems for intra-capsular NOF.
Methods Two observers (A and B), both fourth year medical students, independently evaluated anteroposterior pelvis X-rays of patients with an intra-capsular NOF on two separate occasions, three weeks apart. Each X-ray was graded using both Garden and Pauwel's classification systems. The data was statistically analysed to determine the inter- and intra-observer reliability of each system through kappa (k) values and intra-class correlation coefficients.
Results Forty-five x-rays in total were analysed. Pauwel's classification showed greater interobserver reliability on both the first (k=0.56, p<0.001) and the second assessment (k=0.46, p<0.001) when compared to Garden (k=0.22, p=0.027 and k=0.33, p=0.002) respectively. Pauwel's demonstrated higher levels of intra-observer reliability for observer A (k=0.81, p<0.001) when compared to Garden (k=0.71, p<0.001). However, there was a negligible difference in intra-observer reliability for observer B, with values for Pauwel's and Gardens of k=0.47 (p=0.002) and k=0.46 (p<0.001) respectively.
Conclusions Pauwel's classification had higher inter-observer reliability and higher intra-observer reliability for observer A but there was negligible intra-observer difference between the systems for observer B. Pauwel's is a more reproducible system for use with intra-capsular NOF than Garden's classification.

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

The review process for this manuscript was double blind, where authors and peer reviewers were blinded to each others identity and institution.

Open access and distribution statement

Reliability of Fracture Neck of Femur Classification Systems – A Retrospective Study

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1. Background

Neck of femur fractures (#NOF) are common (66,313 new cases in the UK during 2018)[1] and mainly occur in the elderly and frail . Classification systems are useful to help determine prognosis and management. The aim of the study was to compare the reliability of Garden[2] (Table 1) and Pauwel's[3] (Figure 1, 2 and Table 2) classification systems.

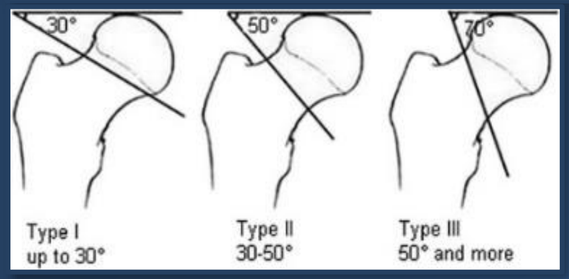

Figure 1 (Left): Pauwel's Classification Types[3]
Figure 2 (Below): Measuring Pauwel's Angle[3]

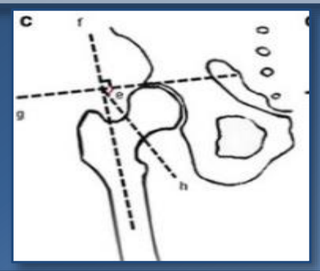

Table 1: Garden Criteria [2]

Type	Fracture	Displacement
I	Valgus Impacted	None
II	Complete	None
III	Complete	Partially
IV	Complete	Complete

Table 2: Pauwel's Criteria[3]

Type	Angle
I	0° - 30°
II	30°-50°
III	>50°

2. Methods

Two observers (A and B) both 4th year medical students, independently studied antero-posterior pelvis X-rays of patients (n=45) with intra-capsular #NOF and classified them according to both Garden and Pauwel's. This was done on two separate occasions, three weeks apart (Time 1 & 2). The reliability was assessed, producing kappa values, intra-class correlation coefficients and confidence intervals (k, ICC and CI respectively).

3. Results

11 Male | 34 Female

25 Left Femur | 20 Right Femur

Mean Age 82 (55-96)

Table 3: Inter-observer Reliability

	Time 1	Time 2
Garden Classification	k=0.22 p=0.027	k=0.33 p=0.002
Pauwel's Classification	k=0.56 p<0.001	k=0.46 p=0.001
Pauwel's Angle (ICC, 95% CI, p)	0.91 0.85 to 0.95 p<0.001	0.77 0.55 to 0.88 p<0.001

Table 4: Intra-observer Reliability

	Observer A	Observer B
Garden Classification	k=0.71 p<0.001	k=0.46 p<0.001
Pauwel's Classification	k=0.81 p<0.001	k=0.47 p=0.002
Pauwel's Angle (ICC, 95% CI, p)	0.91 0.82 to 0.95 p<0.001	0.79 0.62 to 0.89 p<0.001

4. Discussion

- Using validated grading systems[4,5], inter-observer agreement for Garden was rated *fair*, whilst Pauwel's was *moderate* on both occasions.
- Pauwel's uses measured angles, making it more *objective* than the *subjectivity of* determining if *partial* or *full* displacement has occurred. In theory less variable and thus more consistent, even with little observer experience, shown by *excellent* ICC values at Time 1.
- Intra-observer agreement was *substantial* and *almost perfect* for Observer A using Garden and Pauwel's respectively, whilst *moderate* for Observer B for both.
- Explanations are, A is more consistent, and thus has more agreeable results, or that B improved on the second attempt, whilst A did not, thus, less agreeable.

5. Limitations

This is a small study making the clinical applicability of any findings limited. Neither observer had a high level of orthopaedic knowledge at Time 1. Therefore, there was an additional variable of their learning curve between Time 1 and Time 2 which is independent of the classification systems. At Time 2, our results were comparable to those of Povilas et al. (k=0.33)[6]. A greater number of females reflects the nature of the injury and the prevalence in this particular patient demographic.

6. Conclusion

Pauwel's classification had greater inter-observer reliability than Garden and also greater intra-observer reliability for Observer A. Further studies are needed with a greater number of patients and observers to confirm the findings and appreciable clinical implications.

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How does gender influence behavioural traits in children with conduct disorder?

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Keywords: Poster Presentation, Behavioural Traits, Conduct Disorder

Abstract

Introduction: Conduct disorder (CD) is a persistent pattern of antisocial behaviour, which violates the rights of others and basic age-appropriate social norms. In 2015, CD had a prevalence of 5.6% amongst 5-16 year olds in England and was more common in boys. Our study aimed to identify the differences in behavioural traits in boys and girls diagnosed with CD. This could help tailor more specific psychosocial interventions in the management of young people with CD.
Methods: Data was taken from the 2004 British Child and Adolescent Mental Health Survey (BCAMHS). The Development and Wellbeing Assessment (DAWBA) was used to assess presence of CD according to DSM-V and ICD-10 criteria. We compared the prevalence of eight parent-reported variables for girls and boys with CD. Chi-squared test was used for analysis of categorical variables. Continuous samples T Test was used for analysis of continuous variables. Multivariable logistic regression was adjusted for confounding factors.
Results: A population sample of 5-16 year olds was utilised. Children who met diagnostic criteria for CD (N=420) were included- 140 females, 280 males. Smoking, self-harm and truancy from school is more common in females, compared to males, with CD. Exclusion from school and told lies in the past year is less common in females, compared to males, with CD. There is no significant difference between genders in the rates of stealing, bullying people and age ($p>0.05$).
Discussion: This study was retrospective, therefore we faced challenges with incomplete data. Analysis included parent-reported variables, as most of the data for teacher-reported variables was missing. In the future, the analysis of the recorded teacher-reported variables to answer the hypothesis can be completed and compared to parent-reported variables.

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

The review process for this manuscript was double blind, where authors and peer reviewers were blinded to each others identity and institution.

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How does gender influence behavioural traits in children with Conduct Disorder?

Daniyal Raja, Tamsin Newlove-Delgado, University of Exeter Medical School

Introduction

- Conduct Disorder (CD) is a persistent pattern of antisocial behaviour, which violates the rights of others and basic age-appropriate social norms (1)
- In 2015, CD had a prevalence of 5.6% amongst 5-16 year olds in England and was more common in boys (2)
- Our study aimed to identify the differences in behavioural traits in boys and girls diagnosed with CD
- This could help tailor more specific psychosocial interventions in the management of young people with CD

Method

Data collection

- Data was taken from the 2004 British Child and Adolescent Mental Health Survey (BCAMHS) (3)
- The Development and Wellbeing Assessment (DAWBA) was used to assess presence of CD according to DSM-V and ICD-10 criteria (4)
- A population sample of 5-16 year olds was utilised
- Children who met diagnostic criteria for CD (N=420) were included- 140 females, 280 males

Statistical analysis

- We compared the prevalence of eight parent-reported variables for girls and boys with CD
- Chi-squared test was used for analysis of categorical variables
- Continuous samples T Test was used for analysis of continuous variables
- Multivariable logistic regression was adjusted for confounding factors

Results

Graph 1

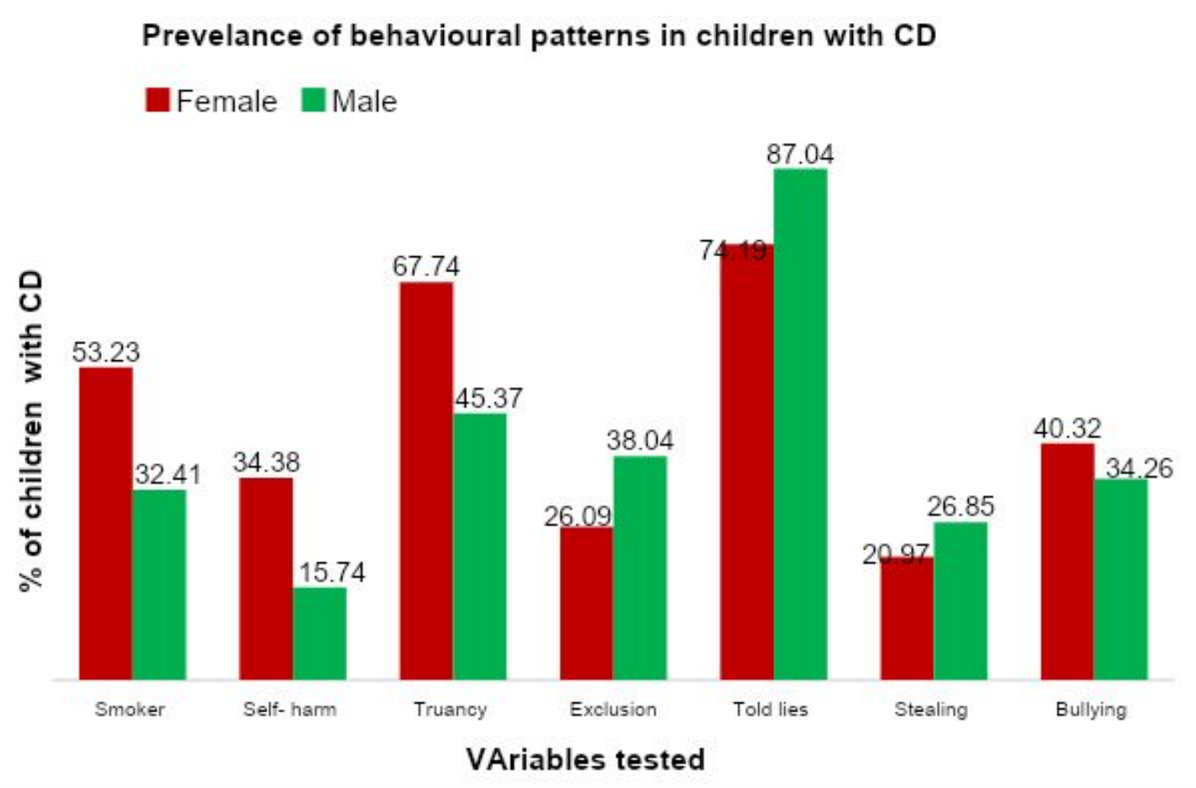

Table 1- Regression model data

Variables	Adjusted OR	95% CI	Sig. (p-value)
Smoker	**2.79**	**1.20-6.47**	**<0.05**
Self-harm	**2.61**	**1.10-6.15**	**<0.05**
Truant from school	**2.29**	**1.12-4.66**	**<0.05**
Exclusion from school	**0.49**	**0.25-0.98**	**<0.05**
Told lies in past year	**0.42**	**0.18-0.98**	**<0.05**
Stolen valuables	0.67	0.30-1.52	>0.05
Bullied people	1.17	0.58-2.36	>0.05
Average age	1.14	0.90-1.44	>0.05

Adjusted odds ratios, 95% confidence intervals and significance (p-value) data from regression models are presented in Table 1. The significant results are highlighted in bold and red.

Discussion

- Smoking, self-harm and truancy from school is more common in females, compared to males, with CD
- Exclusion from school and told lies in the past year is less common in females, compared to males, with CD
- There is no significant difference between genders in the rates of stealing, bullying people and age ($p>0.05$)
- This study was retrospective, therefore we faced challenges with incomplete data
- Analysis included parent-reported variables, as most of the data for teacher-reported variables was missing
- In the future, the analysis of the recorded teacher-reported variables to answer the hypothesis can be completed and compared to parent-reported variables

References: **1.** ICD-10. Conduct Disorders. 2016. [Available from https://icd.who.int/browse10/2016/en#/F91]. (Accessed 14/04/19)
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Could early identification of Changes in Olfactory Function be an Indicator of Preclinical Neurodegenerative Disease? A Systematic Review

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Abstract

Introduction: Alzheimer's disease (AD) is a debilitating neurodegenerative disease that currently affects 850,000 individuals in the UK with estimates continuing to rise. Diagnosis is only available in the presence of significant neuronal pathology and apparent cognitive decline, meaning that treatment avenues are often limited and carry little to no effect on prognosis. Olfactory function has been shown to have a direct correlation with cognitive function and therefore may serve as a potential diagnostic tool for the detection of preclinical disease. Despite this, olfactory testing is not a clinical tool used routinely, which may represent a missed opportunity. The aim of this review is to critically appraise relevant literature to establish whether olfactory testing provides a suitably accurate preclinical biomarker of Alzheimer's Disease for clinical use, and if so, to make recommendations for future research to increase its accuracy.
Methods: A systematic review was performed using the search terms and Boolean operators 'Dementia OR Alzheimer's AND olfaction AND cognitive impairment' yielding 111 results. Articles were assessed via the inclusion/exclusion criteria alongside a PICO strategy.
Results: Despite different study designs, all studies included in this review found a correlation between OI and cognitive decline. This aligns with previous evidence. However, this review highlights novel limitations that may strengthen future work and result in the ability to use olfactory testing with greater accuracy in the future.
Discussion: The findings of this review align with current literature in demonstrating the correlation between olfactory and cognitive function. However, the strength of this review lies in the highlighting of multiple limitations that, if addressed in future work, may increase the accuracy of olfactory resting, and therefore its utilisation in clinical practise.
Conclusion: This systematic review aligns with the current literature; there is a connection between olfaction and cognition. However, the strength of this paper is in identifying limitations that may be preventing increasingly accurate conclusions to be found, which may facilitate regular clinical use, and the possibility of designing new therapeutic targets.

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

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Open access and distribution statement

Could Early Identification of Changes in Olfactory Function Be an Indicator of Preclinical Neurodegenerative Disease? A Systematic Review

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Introduction

Alzheimer's disease (AD) is a debilitating neurodegenerative disease that currently affects 850,000 individuals in the UK with estimates continuing to rise. Diagnosis is only available in the presence of significant neuronal pathology and apparent cognitive decline, meaning that treatment avenues are often limited and carry little to no effect on prognosis. Olfactory function has been shown to have a direct correlation with cognitive function and therefore may serve as a potential diagnostic tool for the detection of preclinical disease. Despite this, olfactory testing is not a clinical tool used routinely, which may represent a missed opportunity.

The aim of this review is to critically appraise relevant literature to establish whether olfactory testing provides a suitably accurate preclinical biomarker of Alzheimer's disease for clinical use, and if so, to make recommendations for future research to increase its accuracy.

Methods

A systematic review was performed using the search terms and Boolean operators 'Dementia OR Alzheimer's AND olfaction AND cognitive impairment' yielding 111 results. Articles were assessed via the inclusion/exclusion criteria alongside a PICO strategy (shown below).

Acronym	Definition	Determinants
P	Patient/population	Patient's with Alzheimer's Disease
I	Intervention	Preclinical detection of olfactory disturbance by the olfactory assessment tools: University of Pennsylvania Smell Identification Test (UPSIT) and Sniffin' Sticks
C	Control/comparison	Adult participants without AD or mild cognitive impairment
O	Outcome	How preclinical olfactory changes correlate with diagnosis/progression of cognitive decline

Table 1: PICO Framework used to guide research question
AD was selected due to being the most prevalent neurodegenerative disease and therefore an increased understanding of pathogenesis has the potential to benefit the greatest number of patients. UPSIT and Sniffin' Sticks were selected due to being the most commonly used olfactory tests in the literature and providing the widest amount of data for critical appraisal.

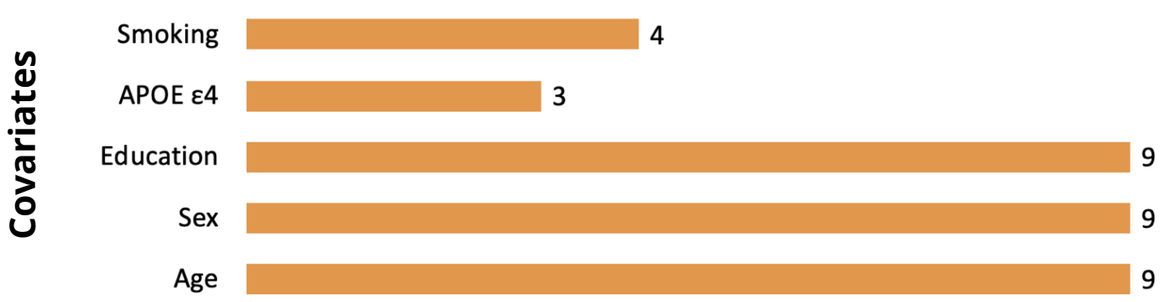
Articles were excluded based on age (>5 years old) (n = 37), review articles (n = 13), if the predominant neurodegenerative disease being investigated was not AD (n = 8), if participants had co-morbidity (n = 1) if not performed on humans (n = 1), or were unable to be readily attained (n = 4). 47 full text articles were assessed for eligibility, and further excluded based on ineligible study design or no comparable group (n = 25) incorrect olfactory assessment tool being used (n = 13). 9 studies met this criteria with a total of 14,760 participants for inclusion in this systematic review. Therse articles were then critically appraised using the AXIS tool for cross-sectional studies and the CASP tool for longitudinal studies.

Results

Despite different study designs, all studies included in this review found a correlation between OI and cognitive decline. This aligns with previous evidence. However, this review highlights novel limitations that may strengthen future work and result in the ability to use olfactory testing with greater accuracy in future.

Medication use 4

Only considered in 3 of 9 articles, and stated as a limitation in another, despite its proven effect on olfactory function.



- Age, sex and education considered in all studies
- APOE E4 only considered in 3/9 studies
- Smoking only considered in 4/9 studies

- **Cultural:** the use of an olfactory test on a population it was not designed for (5/9 studies)
- **Sampling:** not considering factors such as head trauma, sinonasal disease and infection (4/9 studies)
- **Selection:** recruitment from a specialist service (5/9 studies)

Discussion

The findings of this review align with current literature in demonstrating the correlation between olfactory and cognitive function. However, the strength of this review lies in the highlighting of multiple limitations that, if addressed in future work, may increase the accuracy of olfactory testing, and therefore its utilisation in clinical practise.

Recommendations for future work include:

- Modifying olfactory tests for individual cultures. This review highlights studies in which a test designed for one culture is used on another. This creates an inherent bias as each culture has an increased prevalence of certain odours, and lack of identification may be due to lack of recognition, not due to dysfunction.

- Including a consistent set of inclusion/exclusion criteria which covers factors that can influence olfaction outside of neurodegenerative disease, such as head trauma, infection and sinonasal disease.

- Considering a consistent set of covariates such as smoking and APOE E4 to increase the accuracy of association.

If these recommendations are utilised in future work, there is the potential to use olfactory testing as a regular clinical tool with the aim of providing earlier diagnosis and prompting therapeutic intervention with may slow or halt disease progression.

Conclusion

This systematic review aligns with the current literature; there is a connection between ofaction and cognition. However, the strength of this paper is in identifying limitations that may be preventing increasingly accurate conclusions to be found, which may facilitate regular clinicial use, and the possibility of designing new therapeutic targets.

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Comparison of the Effectiveness of Endovascular versus Open Repair of Abdominal Aortic Aneurysms: An Evidence Review?

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Abstract

Introduction:
Abdominal Aortic Aneurysms (AAAs) are defined as enlargements of the abdominal aorta in which the maximum diameter exceeds 3cm or is 50% greater than normal. If the diameter exceeds 5.5cm, AAAs require surgical repair. Endovascular Aneurysm Repair (EVAR) or open repair are the two main methods employed. The aims of this review are to explore the relative merits of both and to determine whether either method offers significant advantages over the other in terms of short-term mortality, long-term mortality and post-operative complications.

Methods:
A NICE Evidence search was conducted and it identified two sets of NICE guidelines, which were then appraised using the AGREE II framework. Secondly, Cochrane and Pubmed databases as well as NICE evidence were searched for systematic reviews. After an abstract and full text screen, five systematic reviews were selected which were then appraised using the CASP systematic review framework. Finally, Pubmed and Embase databases were searched for primary studies: two randomised controlled trials (RCTs) were identified for appraisal using the CASP RCT framework.

Results:
Five of the five systematic reviews found that EVAR had a significantly lower 30-day all22 cause mortality than open surgical repair, ranging from a 64-67%. However, none of the five found a statistically significant improvement in long-term mortality. In terms of post-operative complications, the only significant finding was a 494% increase in the risk of aneurysm rupture in EVAR as compared to open repair.

Conclusion:
Our review suggests that EVAR benefits patients in the short-term, although, despite many trials and systematic reviews, it remains unclear whether this benefit in all-cause mortality persists in the longer term.

Author Statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

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

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