EMJ

March 2019 Vol 4.1 ISSN: 2397-6764

+ RESPIRATORY

Rocking Pneumonia and the Boogie Woogie Flu

+ DERMATOLOGY

Clinical Conditions that Masquerade as Urticaria

+ ALLERGY AND IMMUNOLOGY

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Marketing Authorisation Holder: Bayer AG, 51368 Leverkusen, Germany
Further information available from: *xarelto.medinfo@bayer.com*
Version: EU/8

Reference: 1. Xarelto (rivaroxaban). Summary of Product Characteristics, as approved by the European Commission.

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"Whether you are a fledgling physician looking for an update or a veteran scientist looking for inspiration for your next research project, this journal has something for everyone."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

Aims and Scope

The European Medical Journal (EMJ) is an online only, peer-reviewed, open access general journal, targeted towards readers in the medical sciences. We aim to make all our articles accessible to readers from any medical discipline.

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EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

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European Medical Journal is published four times a year. For subscription details please visit: www.europeanmedical-journal.com

EMJ 4.1

EMJ Innovations 3.1

View some of the latest advances and innovations from across the medical sphere and details their implications for treatment, education, and research.

VIEW ALL JOURNALS ←

Welcome

Spring is upon us, and with it comes a host of fantastic medical breakthroughs. *EMJ 4.1* is here to share with you some of the most exciting developments of recent months, across a host of therapeutic areas. Whether you are a fledgling physician looking for an update or a veteran scientist looking for inspiration for your next research project, this journal has something for everyone.

Selected by our venerable Editorial Board member, Prof Andy Bush, this edition's Editor's Pick is a fascinating article on experimental therapeutics for bronchopulmonary dysplasia. In this article, Silveyra et al. present a thorough review of the pathophysiology of the disease, exploring the latest therapeutic options that are being trialled in animal models and humans. With premature birth still such a prevalent issue in healthcare systems around the world, this paper provides an excellent framework for future study.

Respiratory diseases are well represented in this issue: Dal Negro et al. provide an insightful study examining the perceptions of the general public relating to persistent cough, and Rijkers et al. explore the relationship between pneumonia and influenza in the colourfully titled article 'Rocking Pneumonia and the Boogie Woogie Flu'. Do not worry, you do not have to be a music fan to enjoy this paper!

The immunologists among you also have much to look forward to in this edition. Hart et al. discuss the effects of obesity on host systems, particularly how the resulting inflammation can inhibit mobility, and Knibb et al. seek to enhance our understanding of self-diagnosed allergies. Increasing our understanding of biopsychological factors will be key to overcoming this growing issue, which creates an unnecessary burden on health systems every year.

With further papers on non-small cell lung cancer, urticaria, oocyte cryopreservation, and more, this edition showcases the highlights from all corners of the medical world. I would like to thank all of those who contributed to this excellent edition and I hope we have the opportunity to work with you all again in 2019. This coming year has a wealth of exciting research to share with us, but for now simply put your winter blues behind you and enjoy *EMJ 4.1*.

Spencer Gore

Chief Executive Officer, European Medical Group

Non-melanoma skin cancer (NMSC):

Getting to the root of the problem

NMSC is the most common cancer, and its incidence is rising[1]

Studies in Canada, the United States, Switzerland, and Australia have shown that the incidence of NMSC has been increasing at 2% to 8% per year since the 1970s.[2]

Advanced NMSC can be debilitating, with significant psychosocial and functional impacts on patients[3,4]

Typically, NMSC is curable with complete surgical excision. However, the cosmetic and functional results of treatment for advanced disease, such as scarring and disfigurement from surgery or radiation, can have a profound impact on patients.[1,3,4]

References: 1. Fahradyan A, Howell AC, Wolfswinkel EM, Tsuha M, Sheth P, Wong AK. Updates on the management of non-melanoma skin cancer (NMSC) [published online November 1, 2017]. *Healthcare (Basel).* 2017;5(4):82. doi:10.3390/healthcare5040082 **2.** Brougham NDL, Dennett ER, Tan ST. Non-melanoma skin cancers in New Zealand—a neglected problem. *N Z Med J.* 2010;123(1325):59-65. **3.** Rhee JS, Matthews BA, Neuburg M, Logan BR, Burzynski M, Nattinger AB. Validation of a quality-of-life instrument for patients with nonmelanoma skin cancer [published online for public access]. *Arch Facial Plast Surg.* 2006;8(5):314-318. doi:10.1001/archfaci.8.5.314 **4.** Philipp-Dormston WG, Müller K, Novak B, Strömer K, Termeer C, Hammann U, Glutsch JW, Krähn-Senftleben G, Lübbert H, Koller M, Szeimies RM, the NMSC-QoL Study Group. Patient-reported health outcomes in patients with non-melanoma skin cancer and actinic keratosis: results from a large-scale observational study analysing effects of diagnoses and disease progression. *J Eur Acad Dermatol Venereol.* 2018;32(7):1138-1146.

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Sanofi Genzyme and Regeneron are committed to providing resources to better understand the incidence, identification, and treatment of NMSC and to research the unmet needs of patients with this disease.

Foreword

Dear colleagues,

It is a pleasure and an honour to write the Foreword to *EMJ 4.1.* It includes great papers, and I herewith present a personal view of what took my fancy.

Is there a bigger heartsink than a children's party at which everyone seems to have a different food allergy? Or the social bore droning on interminably about their allergies? The number of people with food allergy documented following double-blind challenge is far exceeded by the number of those who self-diagnose allergy. Rebecca Knibb tries to understand why; she was unable to come to a conclusion but performed a useful service in pointing out the flaws of current research, and especially the need to understand the phenomenon of pseudo-food allergy. Also, on the allergy theme, all that blisters is not urticaria, and Kimchi and Bernstein review the differential diagnoses of these rashes.

Has there ever been a better title than 'Rocking Pneumonia and the Boogie Woogie Flu'?! The musical introduction from Rijkers et al. gives way to a serious discussion on the interactions between the two diseases, including the possible pathophysiology. Anyone doubting whether influenza immunisation is a good thing should read this article; they will then soon be off for their 'flu shot.

When times are tight, we still waste money. Both in resource-rich and, depressingly, in resource-poor settings, expensive-coloured placebos claiming to cure cough are purchased by the bucket-load. This is explored by Dal Negro et al., who show that the prevalence of cough is rising in Italy. Coughers were prepared to pay to try to get relief, showing there is a big unmet need. The authors are properly cautious about their methods, but their data suggest that the market for cough linctus shows no signs of decreasing.

There are also manuscripts covering such diverse topics as coronary atherosclerosis, bronchopulmonary dysplasia, obesity and diabetes, small cell lung cancer, and oocyte cryopreservation, so there is plenty here for all interests. Congratulations to the authors, the peer-reviewers, and the whole journal team!

Best wishes,

Prof Andrew Bush
Royal Brompton Hospital, UK

Discover the Potential: Exploring New Frontiers of IL-23 Inhibitors

This symposium took place on 14th December 2018, as part of the 3rd Inflammatory Skin Disease Summit (ISDS) in Vienna, Austria

Chairperson: Antonio Costanzo[1]

Speakers: Antonio Costanzo,[1] Kilian Eyerich[2]

1. Humanitas University, Milan, Italy
2. Technical University of Munich, Munich, Germany

Disclosure: Prof Costanzo has provided research support to Celgene, Janssen, LEO Pharma, and Novartis; he has received honoraria from, and has been a scientific advisory board member for, AbbVie, Celgene, Janssen, Novartis, Pfizer, and Sanofi. Prof Eyerich has declared no conflicts of interest.

Acknowledgements: Medical writing assistance was provided by Lorena Tonarelli.

Support: The symposium and the publication of this article were funded by Janssen via an educational grant. The views presented here are those of the speakers and not necessarily those of Janssen.

Citation: EMJ. 2019;4[1]:12-19.

Meeting Summary

Affecting up to 11.4% of the population worldwide,[1] psoriasis is one of the most common chronic autoinflammatory diseases. It is associated with multiple comorbidities and can have profound negative effects on physical and emotional wellbeing and overall quality of life, making it a serious public health concern. A primary objective of this symposium was to explain the pathogenesis of psoriasis and its relation to the development of novel targeted immune therapies. Psoriasis is characterised by skin and systemic damage consequent to pathogenic cytokine production under the influence of both environmental and genetic factors. Differentiation of Th17 cells from naïve T cells is central to the development of psoriasis, and recently pathogenic models have identified IL-23 as the pathogenic cytokine responsible for promoting Th17 cell proliferation and IL-17 production. Therefore, selective blockade of IL-23 may be instrumental in controlling Th17-mediated inflammation in psoriasis. Another key objective of the symposium was to evaluate key learnings from the latest available clinical trial data on agents targeting the IL-23/Th17 signalling pathway and how these learnings can be harnessed to improve the management of patients with psoriasis. Both IL-17 inhibitors (e.g., ixekizumab and secukinumab) and IL-23 inhibitors (e.g., guselkumab and risankizumab) have demonstrated high efficacy and a good safety profile. Anti-IL-17 agents have faster onset of action and allow the achievement of good response rates very rapidly. Efficacy is better maintained over time with anti-IL-23 agents, including in patients who have stopped and those that then restarted anti-IL-23 therapy after a withdrawal period. Despite the availability of effective treatments, undertreatment in psoriasis is common. This can be attributed to factors such as the heterogeneous nature of psoriasis and relatively large prevalence of addictive behaviours in patients with the condition. When making treatment decisions, it is important to consider these factors as well as patient preferences and expectations, so that treatment can be

individualised as much as possible. The symposium concluded with an interactive session, which offered the audience the opportunity to ask questions and discuss relevant issues of interest.

Advancing the Science of Psoriasis: The Underlying Pathology and the Role of IL-23

Professor Antonio Costanzo

Both genetic and environmental factors contribute to the development of psoriasis. From a genetic point of view, multiple loci are involved in harbouring psoriasis susceptibility genes, including human leukocyte antigen (*HLA*)-*Cw6*, *IL-23R* and *IL-12B*, and the two members of the late cornified envelope (LCE)-3 group, *LCE3B* and *LCE3C*.[2-6] Sixteen new susceptibility loci have been identified within a large-scale meta-analysis of genome-wide association studies (GWAS), bringing the total number of known psoriasis-associated loci to 63.[5] The majority of these loci are related to genes involved in lymphocyte differentiation and regulation, response to stimuli, and type 1 IFN/pattern recognition pathway.[5] The highest degree of genetic predisposition has been reported for *HLA-Cw6*.[7] This has been indicated as the psoriasis susceptibility 1 (*PSORS1*) risk allele conferring a predisposition specifically to psoriasis, particularly the early-onset (type 1) form of the disease.[7] *PSORS1* is considered the major genetic determinant of psoriasis, accounting for up to 50% of the heritability of the disease.[8] Conversely, psoriasis susceptibility genes, such as *IL-12B* and *IL-23R*, are shared with other chronic autoimmune or inflammatory diseases, including psoriatic arthritis, Crohn's disease, multiple sclerosis, asthma, and diabetes.[9]

From a medical viewpoint, psoriasis can be defined as an exaggerated immune response to bacteria, as evidenced by elevated skin levels of certain antimicrobial peptides, such as β-defensin, the encoding genes of which are highly expressed in the genome of individuals with the condition. Notably, overexpression of β-defensin has been found in keratinocytes from psoriatic skin but not in those from atopic dermatitis lesions, despite the latter being remarkably similar to psoriatic lesions.[10]

Cathelicidin hCAP18/LL37, another antimicrobial peptide frequently found in humans, is also overexpressed in psoriasis.[11] Both cathelicidin hCAP18/LL37 and β-defensin are thought to play a prominent role in psoriatic plaque development.[8] Stress, bacteria, and trauma can trigger LL37 overproduction and, in turn, the formation of LL37-DNA complexes that bind to plasmacytoid dendritic cells, thus activating them.[8] Activated dendritic cells are then internalised by lymph nodes where they induce naïve T cells to differentiate into effector cells, such as Th1 and Th17 cells.[8] Effector cells migrate into skin tissue followed by putative presentation of autoantigens to T cells and the release of IL-23.[8] These and other processes eventually lead to the activation and proliferation of keratinocytes with consequent overexpression of β-defensin.[8]

Importantly, Lande et al.[12] demonstrated that LL37 is the key mediator of plasmacytoid dendritic cell activation in psoriasis. Specifically, the binding of LL37 to DNA induces plasmacytoid dendritic cells to produce IFN-α via activation of toll-like receptor (TLR)9. This pathway could potentially be a driver of autoimmunity in psoriasis.[12]

Also of note is that, following internalisation in DNA complexes, LL37 is processed within the cell and presented to lymphocytes in the context of *HLA-Cw6*.[13] The fact that *HLA-Cw6* can present an autoantigen, such as LL37, to T cells would explain the observed high frequency of this particular type of HLA among patients with psoriasis, and this is supported by the higher peptide-binding selectivity of HLA-C, compared with HLA-A and HLA-B.[14]

Autoantigens other than LL37 have recently been identified, most notably the melanocyte-expressed molecule ADAMTS-like protein 5 (ADAMTSL5), which can be presented to lymphocytes in the context of *HLA-Cw6*.[15] Initially identified in the melanocytes of patients with psoriasis, ADAMTSL5 was discovered by Fuentes-Duculan et al.[16] to be widely distributed in keratinocytes. Subsequent immunohistochemistry analyses by the same authors demonstrated that LL37 and ADAMTSL5 are significantly upregulated in lesional, versus non-lesional, skin biopsies from patients with

moderate-to-severe psoriasis ($p<0.05$).[16] In both lesional and non-lesional skin, LL37 and ADAMTSL5 are coexpressed by keratocytes, dendritic cells, and other leukocytes.[16]

The key event leading to psoriatic plaque formation is the differentiation of Th17 cells from naïve T cells following presentation to the latter of autoantigens by plasmacytoid dendritic cells. Several cytokines are necessary for Th17 cell differentiation and IL-17 production in humans, including TGF-β, IL-β, IL-6, IL-23, and IL-21.[17] A crucial role is played by IL-21. In a human psoriasis xenograft mouse model, Caruso et al.[18] discovered that IL-21 blockade via an IL-21-specific antibody reduced keratinocyte proliferation and transcript levels of both IFN-α and IL-17A, effectively inducing psoriatic plaque regression.[18] IL-21 is directly involved in the expression of IL-23 receptors (IL-23R).[17] Thus, the findings by Caruso et al.[18] suggest that IL-21 plays a prominent role in T cell differentiation and the epidermal hyperplasia of psoriasis, probably via the activation of IL-23 receptors.

Binding of IL-23 to IL-23R on mature Th17 cells has been shown to maintain IL-17 production *in vivo* and to promote Th17 cell survival and pathogenic activity.[19,20] It can, therefore, be assumed that IL-23, rather than IL-17, plays a central role in Th17-related autoimmunity and is a driver of inflammation mediated by innate lymphoid cells.[20] Clinical data from other diseases support this view. For example, in animal models of inflammation, blockading IL-23 has been associated with a protective effect against immune colitis, whereas blocking IL-17 has been found to have no protective effect, or a worsening effect, on the condition.[21]

Interestingly, Th17 cells are characterised by a high degree of plasticity, which is strongly dependent on the microenvironment.[19] The phenotype of Th17 cells can change from one in which cells produce pathogenic cytokines to one in which cells produce anti-inflammatory cytokines, and is influenced by epigenetic mechanisms that affect whether relevant genes are expressed or silenced.[19] This is illustrated in an *in vitro* study in which Aschenbrenner et al.[22] were able to characterise two divergent activated subsets of Th17 cells. Of these subsets, one maintained proinflammatory activity; the other, however, acquired anti-inflammatory activity.[22] A distinctive feature of the latter subset was the ability to produce IL-10, a potent anti-inflammatory cytokine.[22] Expression of *c-MAF* was found to be upregulated in IL-10-positive Th17 cells, leading to the conclusion that it may be important in driving divergent fates of human Th17 cells.[22]

Tissue-resident memory (TRM) T cells are thought to contribute to psoriasis development. This is substantiated by the finding that epidermal TRM cells are retained in resolved psoriatic lesions and can produce IL-17A and IL-22.[23] Given the pivotal role played by these cytokines in the pathogenesis of psoriasis, TRM cells in resolved lesions have been indicated as potential drivers of recurrent disease.[23] Defective Foxp3 regulatory T cells (Tregs) have been identified in psoriasis, which could also contribute to the development of the disease; Sanchez et al.[24] found proliferating defective memory Tregs that produce IL-17 in human psoriatic skin. In other research, blood-derived Tregs from patients with severe psoriasis were more likely to differentiate into IL-17-producing cells, compared with Tregs from healthy subjects; on *ex vivo* stimulation, the increase in the percentage of intracellular IL-17A-producing cells (mean±standard error of the mean) was 9.3±2.4% (n=7) for patients with psoriasis and 3.2±0.7% (n=8) for healthy controls ($p=0.0236$).[25] Crucially, supplementation of IL-23 increased the percentage of IL-17-producing Tregs, and the effect was more pronounced in patients with psoriasis than in controls (17.0±2.6%, n=6 versus 6.3±1.6%, n=8; $p=0.0006$).[25] In another study, IL-23 was found to be central to the conversion of functional Foxp3 Tregs into IL-17-producing Foxp3 Tregs with pathogenic phenotype.[26]

Importantly, Th17 cells are not the only lymphoid cells regulated by IL-23. Other targets include γδT cells and Type 3 innate lymphoid cells, both of which can produce pathogenic cytokines such as IL-17 and IL-22 in response to IL-23 stimulation.[27] Neutrophils and mast cells are also influenced by IL-23, and have been indicated as the major producers of IL-17 in human psoriatic skin.[28-30]

Updated pathogenic models of psoriasis attribute IL-23 with a central role in all stages of skin lesion formation; specifically, IL-23 is responsible for initiating Th17 cell activation and IL-17 production in pre-psoriatic skin, promoting

pathogenic Th17 cell expansion and survival in early psoriatic lesions, and maintaining IL-17 production in chronic psoriatic plaques.[31]

It is evident that the IL-23/Th17 signalling pathway is of critical importance in the pathogenesis of psoriasis and that selective blockade of IL-23 may represent an effective, targeted approach to psoriasis treatment.[31] In light of this, several anti-IL-23 agents have been approved in recent years (e.g., guselkumab, tildrakizumab, and ustekinumab) and others are being developed (e.g., mirikizumab and risankizumab). Anti-IL-23 agents typically target a specific subunit of IL-23, usually p19 or p40. Since these subunits are not exclusive to IL-23, but are shared with other cytokines, off-target activities must be considered.[32]

It can be concluded that psoriasis is an autoimmune disease characterised by skin and systemic damage that occurs as a result of the secretion of cytokines, and that IL-23 is a key pathogenic cytokine in psoriasis development.

New Horizons in Managing Psoriasis: Individualising Care One Patient at the Time

Professor Kilian Eyerich

Psoriasis has been recognised by the World Health Organization (WHO) as a serious health problem of global importance that has a significant impact on quality of life.[1] Over recent decades, our understanding of the pathogenic pathways of psoriasis has improved substantially and new, effective, targeted therapies have become available. However, this does not seem to have been paralleled by an improvement in the management of real-world patients in clinical practice. In a large population-based survey conducted in Europe and North America, 41% of patients with psoriasis with up to three palm lesions (n=2,112) reported they were not receiving any treatment.[33] No treatment was also reported by 37% of patients with >10 palm lesions (n=166) and 32% of those with 4–10 palm lesions (n=393).[33] In all three groups, only 1% of patients reported being on oral plus biologic treatment.[33]

A key contributing factor may be the heterogeneity of psoriasis,[34] which makes it important to provide treatment based on disease phenotype as well as patient expectations and characteristics. Regarding the last point, an important but often-overlooked issue is the high prevalence of addictive behaviour among patients with psoriasis.[35] In a study by Zink et al.,[35] 57 of 102 patients were assessed over a period of 4 months exhibited addictive behaviour. Of these, 41% were regular smokers, 23.8% were high-risk drinkers, 19% were compulsive gamblers, 11% were at risk of drug abuse, and 4.1% were at risk of food dependency. Providing effective therapies may not be enough for these patients, and this factor should be considered when making treatment decisions.

From an efficacy point of view, agents that can selectively block IL-17 (e.g., ixekizumab and secukinumab) or IL-23 (e.g., guselkumab and risankizumab) have demonstrated important benefits in patients with psoriasis.[36-39]

In the UNCOVER-3 study, 80.5% of patients treated with the high-affinity monoclonal antibody ixekizumab achieved at least 75% improvement from baseline in Psoriasis Area Severity Index (PASI) at Week 156.[36] Sixty-six percent of patients achieved PASI 90 and 45.1% achieved PASI 100.[36] Ixekizumab achieved and sustained high responses of action and the observed skin improvements were maintained over the 3-year period, with no new safety signals identified.[36] Notably, the number of nonresponders was limited despite the elevated heterogeneity of psoriasis. Similar results have been reported with secukinumab in a 5-year randomised (1:1), double-blind, noninferiority clinical trial (SCULPTURE) of adults with moderate-to-severe psoriasis.[37] The study compared fixed-interval (FI) secukinumab with retreatment as needed (RAN) secukinumab. Both regimens demonstrated good efficacy, with superiority of FI 300 mg secukinumab. With this regimen, PASI 75, 90, and 100 response rates (as observed) were 88.9%, 68.5%, and 43.8%, respectively, at Year 1, and were maintained through Year 5 (88.5%, 66.4%, and 41.0% for PASI 75, 90, and 100, respectively).[37]

Among IL-23 inhibitors, risankizumab has demonstrated superior sustained efficacy compared with ustekinumab in two Phase III

randomised, double-blind, placebo-controlled, and active comparator-controlled trials (UltIMMa-1 and UltIMMa-2).[38] Significantly more patients with moderate-to-severe psoriasis treated with risankizumab achieved PASI 90 at Week 16 compared with patients treated with ustekinumab or placebo (75.3% versus 42.0% and 4.9% in UltIMMa-1; and 74.8% versus 47.5% and 2.0% in UltIMMa-2; p<0.0001 versus placebo and ustekinumab for both studies).[38]

It is apparent from the evidence presented above that both IL-17 and IL-23 inhibitors are associated with considerable and sustained skin improvements, with the former class of drugs providing a more rapid response but slightly less sustainability than the latter class. Another factor to consider in treatment decision-making is the extent to which the benefits of a therapy can persist beyond withdrawal. This has been investigated in psoriasis by Reich et al.[39] in a Phase III, multicentre, randomised, double-blind, placebo and adalimumab comparator-controlled study (VOYAGE 2). Guselkumab-treated patients achieving ≥90% improvement from baseline in PASI score were randomised at Week 28 to either guselkumab (maintenance group) or placebo (withdrawal group). At Week 48, although PASI 75, 90, and 100 response rates were significantly higher (p<0.001) in the maintenance group (96.0%, 88.6%, and 59.0%, respectively), a large proportion of patients in the withdrawal group maintained PASI response (62.0%, 36.8%, and 20.0% for PASI 75, 90, and 100, respectively) despite the fact that they were no longer receiving treatment.[39] Upon retreatment with guselkumab, the majority of patients rapidly regained PASI 90 response; within 6 months of restarting guselkumab treatment, the PASI 90 response rate for this group of patients was 87.6%.[40] This last point raises the question of how to determine which patients will be long-term responders without being on the drug and which, instead, will need continuous treatment. In this regard, parameters predicting PASI 90 maintenance following guselkumab withdrawal have recently been identified by Liu et al.[41] They include lower IL-17A at baseline, shorter disease duration, lower BMI, and complete skin clearance and higher guselkumab levels at Week 28. Regression models using these and other parameters are currently being evaluated for response prediction.

Recently, guselkumab has been compared with secukinumab in the ECLIPSE study.[42] Patients with moderate-to-severe plaque psoriasis were randomised to guselkumab 100 mg (administered by subcutaneous injection at Weeks 0, 4, 12, and then every 8 weeks thereafter through to Week 44; n=534) or secukinumab 300 mg (administered by subcutaneous injection at Weeks 0, 1, 2, 3, 4, and then every 4 weeks thereafter through to Week 44; n=514).[42] In total, patients treated with guselkumab received 7 active injections versus 30 active injections in patients treated with secukinumab.[42] The primary endpoint was PASI 90 response rate at Week 48.[42] Prof Eyerich proceeded to explain that baseline demographics, disease characteristics, and exposure to previous psoriasis treatment were similar across study arms. Guselkumab demonstrated superior long-term efficacy compared with secukinumab; at Week 48, PASI 90 response rate was 84.5% for guselkumab-treated patients and 70% for secukinumab-treated patients (treatment difference: 14.2%; 95% confidence interval [CI]: 9.6–18.8; p<0.001).[42] Secukinumab showed faster onset of action, consistent with what has been reported in other studies of anti-IL-17 agents.[42] Both drugs were highly effective, with PASI 90 reaching 80% at Week 15–20; however, the elevated response rate was better maintained by selectively targeting IL-23 with guselkumab.[42] In terms of safety, observations of both guselkumab and secukinumab demonstrated a profile that was consistent with previous reports, with three events of Crohn's disease reported in the secukinumab group compared to none in the guselkumab group.[42]

Clearly, multiple factors affect the treatment decision in psoriasis, which also needs to be individualised by addressing patient preferences, needs, and expectations. Options to consider might include topical therapy (PASI <10), conventional systemics (chronic stable disease with PASI >10), or first-line biologics (inflammatory unstable disease with PASI >10 or 20). The choice of conventional systemic depends on several factors. For example, methotrexate might be suitable for patients with psoriatic arthritis who have no liver damage, whereas dimethyl fumarate might be a more appropriate choice for patients with no psoriatic arthritis and lymphopenia who want flexibility and prefer

oral administration. Other options might include apremilast (as a second-line conventional in subjects with affected nails and enthesitis), retinoids (for cases of pustular palmoplantar psoriasis), and cyclosporine (for short-term use, if no renal dysfunction is present). For biologics, the choice can be influenced by the presence of certain comorbidities; for example, TNF/IL-12/IL-23 for patients with IBD or candida; IL-17/17R/12/23 if tuberculosis is present, and TNF/IL-12 for women with psoriasis who are pregnant. If convenience is a factor, IL-12/23 may be preferred due to the need for less frequent injections, whereas IL-17/17R may be more suitable if a fast response is required.

Interactive Question and Answer Session

The symposium concluded with an interactive question and answer session chaired by Prof Costanzo.

The use of *HLA-CW6* status as a biomarker of response to biologics was discussed. It was noted that a study by Gulliver et al.[43] has demonstrated that positivity for the susceptibility gene is associated with improved efficacy of biologic therapies, such as ustekinumab, in patients with moderate-to-severe psoriasis. Therefore, early screening for *HLA-CW6* might be a useful approach to individualising therapy.

The idea of psoriasis being a primary autoimmune condition was challenged and it was suggested that this might be characteristic of a subgroup of patients, rather than all subjects with the condition. Autoimmunity was said to be important in psoriasis, but it was acknowledged that the fact that certain patients have permanent clearance of psoriasis may indicate that the autoimmune phenotype is stronger in some individuals.

Regarding the impact of disease duration on TRM cells, it was highlighted that the number of T cells increases during the course of the disease. This may be important to consider in treatment decision-making. Early treatment initiation, as opposed to a 'wait and see' approach, was said to be preferred in psoriasis in that it may help prevent complications.

It was asked whether patients on ustekinumab should be switched to guselkumab, given the overlap of the mechanisms of action of the two drugs. There is evidence that this approach may be appropriate in some cases. In the NAVIGATE clinical trial,[44] patients with psoriasis who did not fully respond to ustekinumab achieved a better response after switching to guselkumab. However, switching may not be appropriate for patients who are stable on ustekinumab. It was noted that there may also be risks in switching rapidly from one therapy to another, and that the reason for patients not responding to treatment should be investigated. Most patients respond to most biologics; lack of response is often due to noncompliance and other factors unrelated to the efficacy of the therapy.

The occurrence of Crohn's disease in patients treated with the IL-17 inhibitor secukinumab in the study by Langley et al.[42] presented earlier in the symposium was discussed, with some members of the audience arguing that the condition might have been pre-existent and therefore unrelated to the use of secukinumab. Abdominal complaints have been reported by patients on secukinumab, and some clinicians are increasingly referring these patients for endoscopy.

The importance of determining which patients will be long-term responders without being on drug was reiterated. It was noted that although predictors have been identified, as described by Prof Eyerich in his presentation, accurate predictions at the level of individual patients are not yet possible.

It was explained that young people with type 1 psoriasis tend to respond better to treatment. This may be due to the presence in this patient population of *HLA-CW6* but also to the fact that treatment is initiated early in the life course.

The skin of patients with psoriasis was said to be an important indicator of the general activity of psoriasis; skin and extracutaneous manifestations of psoriasis may occur in parallel.

Conclusion

Differentiation of Th17 cells and production of IL-17 following stimulation of naïve T cells play a central role in psoriasis development and are

thought to be activated and maintained by the pathogenic cytokine IL-23. Clinical trial data indicate that selective inhibitors of IL-23 are associated with significant and sustained skin improvements, even when patients have stopped receiving treatment or are retreated after a period of withdrawal. Anti-IL-17 agents have also demonstrated good efficacy in psoriasis and have faster onset of action but less sustainability of response compared with anti-IL-23 agents. Despite the availability of these and other treatments, a large proportion of patients with psoriasis remain untreated. The heterogeneity of the condition and the presence of addictive behaviours may play a role in this, indicating that an individualised approach to psoriasis management is crucial to ensuring more patients receive the treatment they need.

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Bronchopulmonary Dysplasia: An Update on Experimental Therapeutics

EDITOR'S PICK

This review starts with an excellent description of the pathophysiology of bronchopulmonary dysplasia, which should be informative to physicians who will be seeing adult survivors of neonatal intensive care in their clinics. The authors provide an informative overview of human therapeutic trials and what is currently being tested in animal models. Until we crack the conundrum of the prevention of premature birth, bronchopulmonary dysplasia will continue to be a lifetime respiratory disease that will mandate energetic and innovative therapeutic interventions.

Andrew Bush

Imperial College and Royal Brompton Hospital, London, UK

Authors: Anika Naeem, Imtiaz Ahmed, *Patricia Silveyra

Pulmonary Immunology and Physiology Laboratory (PIP), Department of Pediatrics, Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA
*Correspondence to pzs13@psu.edu

Disclosure: The authors have declared no conflicts of interest.

Received: 01.06.18

Accepted: 31.10.18

Keywords: Bronchopulmonary dysplasia, experimental therapeutics, mechanical ventilation, prematurely born infant.

Citation: EMJ. 2019;4[1]:20-29.

Abstract

Bronchopulmonary dysplasia (BPD) is a chronic inflammatory lung disease that affects thousands of newborns and infants every year. Although it is accepted that BPD results from lung damage and inflammation triggered by mechanical ventilation and hyperoxia, the causes and molecular events leading to lung damage and arrested development remain unknown. While recent advances in neonatal care have improved the survival of very low-weight infants, the rates of BPD have not improved accordingly. This is mainly due to our limited understanding of the disease's pathogenesis and the effective therapeutic options available. Current therapeutics for BPD involve ventilation management, steroid treatment, and administration of various agents, such as pulmonary surfactant, caffeine, vitamin A, nitric oxide, and stem cells. However, the efficacy of these agents in preventing and ameliorating BPD symptoms varies depending on the populations studied and the disease stage. As the field moves towards personalised therapeutic approaches, this review summarises clinical and experimental studies conducted in various models, aiming to increase understanding of the cellular and molecular mechanisms by which these agents can prevent or treat BPD. Due to the increasing number of extremely premature infants, it is imperative that we continue to work towards understanding the mechanisms of BPD pathogenesis and generating more effective therapeutic options.

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a chronic inflammatory lung disease of prematurely born infants characterised by impaired lung development and requiring mechanical ventilation with supplemental oxygen. The disease was initially defined by Northway et al.[1] in 1967 as a disorder resulting from the combined effects of oxygen exposure and mechanical ventilation in premature infants with severe respiratory distress syndrome. This initial definition was based on time spent on ventilatory support and oxygen concentration, which were thought to induce damage to the lung epithelium, smooth muscle hyperplasia, atelectasis, and vascular hypertension, resulting in an immature and surfactant-deficient lung. As a result of significant improvements in neonatal care, including the use of antenatal steroids, ventilation, and nutritional management, the definition of BPD has been modified in the past few decades to include additional criteria reflecting symptoms of arrested alveolar and vascular lung development, fibrosis, and chronic inflammation.[2-4] Despite these changes, some investigators still argue that a definition based on the disease's pathophysiology should be developed.[5] Moreover, recent studies using a variety of biomarkers have provided additional information that could complement current diagnostic strategies and help in the development of a more comprehensive definition.[6] The current National Institute of Child Health and Human Development (NICHD) and National Heart, Lung, and Blood Institute (NHLBI) criteria for the BPD definition encompasses gestational age (GA), supplemental oxygen requirement, and chest X-ray changes, categorising BPD by degree of severity.[7] A summary of the current diagnostic criteria for BPD severity is presented in Table 1. According to the NICHD and NIH guidelines, BPD is defined as mild, moderate, or severe. In infants with a GA <32 weeks, mild BPD is considered when there is a requirement for at least 28 days of supplemental oxygen, together with termination of supplemental oxygen or discharge by 36 weeks post-menstrual age (PMA). Mild BPD is also considered if termination of ventilation or discharge is achieved by 56 days postnatal age in infants with a GA >32 weeks. In contrast, moderate BPD is considered in infants with a GA ≤32 weeks when there is a requirement of at least 28 days of supplemental ventilation with <30% oxygen at 36 weeks PMA, or by 56 days postnatal age (in infants with a GA ≥32 weeks).

Table 1: Definition of bronchopulmonary dysplasia: Current diagnostic criteria.

Gestational age	**<32 weeks**	**≥32 weeks**
Time point of assessment	36 weeks PMA or discharge to home, whichever comes first.	>28 days but <56 days postnatal age or discharge to home, whichever comes first.
	Treatment with >21% oxygen for ≥28 days	
Mild BPD	Breathing room air at 36 weeks PMA or discharge, whichever comes first.	Breathing room air by 56 days postnatal age or discharge, whichever comes first.
Moderate BPD	Need for <30% oxygen at 36 weeks PMA or discharge, whichever comes first.	Need for >30% oxygen at 56 days postnatal age or discharge, whichever comes first.
Severe BPD	Need for ≥30% oxygen and/or positive pressure (PPV or NCPAP) at 36 weeks PMA or discharge, whichever comes first.	Need for ≥30% oxygen and/or positive pressure (PPV of NCPAP) at 56 days postnatal age or discharge, whichever comes first.

BPD: bronchopulmonary dysplasia; NCPAP: nasal continuous positive airway pressure; PMA: post-menstrual age; PPV: positive pressure ventilation.

In the case of severe BPD, the diagnosis is defined as requiring >30% supplemental oxygen for at least 28 days at 36 weeks PMA or at discharge (for infants with a GA <32 weeks), or at 56 days postnatal age or discharge (for infants with a GA ≥32 weeks).

However, experts argue about the practicality of the current definition of BPD, which fails to consider the newer modes of ventilation adopted in current practice. They also advocate for a more subjective grading of the severity of BPD that would justify interventions with higher risk–benefit profiles for babies predicted to develop more severe cases of BPD or more severe respiratory morbidity after discharge.[8] The field is moving towards developing an updated definition of BPD based on prior definitions and current care practices. Recent research has shown that the best predictability of lung disease and neurodevelopmental injury at 18–21 months are oxygen requirement levels and/or respiratory support status at 40 weeks. Despite these considerations, there is still inconsistency among the definitions of BPD currently used for clinical trials and studies examining long-term outcomes.[7]

PATHOPHYSIOLOGY OF BRONCHOPULMONARY DYSPLASIA

BPD is the most common respiratory morbidity in preterm infants, affecting >10,000 babies in the USA each year.[2] The aetiology of BPD is multifactorial and involves exposure to antenatal and/or postnatal factors that disrupt pulmonary development.[9,10] In addition, the disease's pathogenesis involves a complex interplay between environmental and host factors, including respiratory infections, genetic predispositions, intrauterine growth restriction, chorioamnionitis, oxidative stress, pulmonary fluid overload, and postnatal nutritional deficits.[5] Both low GA and birth weight (BW) are commonly associated with higher rates of BPD. Patients who develop BPD are usually low BW infants (BW <2,500 g), very low BW (VLBW) infants (BW <1,500 g), or extremely low BW (ELBW) infants (BW <1,000 g). Hyperoxia, mechanical ventilation, nosocomial infection, male sex, patent ductus arteriosus, and congenital conditions are also risk factors for BPD. Some of these are known to induce lung injury, activation of inflammatory gene expression, and induction of the regulatory pathways involved in alveolar and vascular development.[6]

Understanding the pathogenesis and factors involved in BPD development is of significant importance in neonatal care, not only to design better preventative strategies for the disease but also because BPD is often associated with several comorbidities, which range from severe complications to conditions that significantly increase the disease mortality risk; these include complications such as retinopathy of prematurity, impaired bone growth, intraventricular haemorrhage, periventricular leukomalacia, and neurodevelopmental abnormalities,[11-15] as well as pulmonary hypertension of the newborn, which occurs most often in infants with severe BPD and increases the mortality risk by almost 40%.[16-19]

EXPERIMENTAL THERAPEUTICS: UPDATE ON ANIMAL STUDIES

Multiple studies involving large (sheep, lambs, pigs, and nonhuman primates) and small (rabbits, rats, and mice) animal models have been conducted to date.[20] These have provided useful information on the disease's pathogenesis and potential therapeutic targets.[21,22] Although there is currently no standardised single model that fully captures disease pathogenesis, several animal models using a variety of lung injury stimuli through exposure to hyperoxia, activation of the inflammatory response, and/or mechanical ventilation appear to be widely accepted.[23] These have been used to identify key players in the mechanisms leading to the disease's development and progression, identify genetic and epigenetic contributions, and to test potential therapeutic targets.[24-29]

Effective experimental therapeutics reported to date in animal models range from maternal diet supplementation using lipids (e.g., omega-3/6 polyunsaturated fatty acids [PUFA w-3, PUFA w-6]), which confer prevention against hyperoxia-induced inflammation and pulmonary hypertension in a rat model,[30] to nutritional interventions in mechanically ventilated newborn animals (Table 2).[30-37] Among these experimental therapeutics, parenteral treatment with lipid emulsion preparations, including SMOFlipid® (Fresenius Kabi, Bad Homburg, Germany) and

Intralipid® (Fresenius Kabi) (which vary in PUFA w-6 content), have been proposed in clinical studies. However, in a BPD model consisting of hyperoxia-exposed newborn guinea pigs, SMOFlipid administration induced greater oxidation and had pro-apoptotic effects, indicating the need for additional studies before its adoption in the clinical setting.[31]

Table 2: Recent experimental therapeutics for bronchopulmonary dysplasia tested in animal models.

Therapeutic agent(s)	Laboratory model	Results	Reference
PUFA w-3 PUFA w-6	Pregnant rats treated at embryonic Day 15. Pups exposed to 85% oxygen after birth.	PUFA w-3 reversed and reduced inflammatory gene expression and leukocyte infiltration, improved survival, and decreased pulmonary hypertension caused by hyperoxia.	Zhong et al.,[30] 2018
SMOFlipid® or Intralipid® parenteral administration	3-day old guinea pigs.	SMOFlipid induced greater oxidation, apoptosis, and hypoalveolarisation.	Lavoie et al.,[31] 2018
Quercetin (20 mg/kg)	Newborn mice (85% oxygen).	Intraperitoneal administration of quercetin attenuated hyperoxia-mediated lung injury by reducing inflammation and improving alveolarisation.	Maturu et al.,[32] 2018
Vitamin D (1 ng/g)	Intra-amniotic LPS injection of pregnant Sprague-Dawley rats.	Treatment with vitamin D alleviated alveolar simplification and decreased expression of inflammatory factors.	Liu et al.,[33] 2017
Pioglitazone plus synthetic lung surfactant	1-day old Sprague-Dawley rat pups (95% oxygen).	Treatment with pioglitazone increased markers of alveolar epithelial/mesenchymal maturation and reduced hyperoxia-induced lung injury.	Sakurai et al.,[34] 2018
Deferoxamine (17 mg/kg/day for 14 days)	Newborn mice (75% oxygen).	Deferoxamine-treated animals showed better weight gain, improved vascularisation and alveolarisation, and reduced pathological severity than controls.	Chen et al.,[35] 2018
Human amniotic fluid stem cells	Preterm rabbit pups (95% oxygen).	Treatment with stem cells expressing high levels of VEGF attenuated lung inflammation and hyperoxia-induced parenchymal and vascular structural or functional damage.	Jiménez et al.,[36] 2018
Exosomes from MSC	Newborn mice (75% oxygen)	Treatment with exosomes ameliorated alveolar simplification, fibrosis, pulmonary vascular remodelling, and pulmonary hypertension.	Willis et al.,[37] 2018

LPS: lipopolysaccharide; MSC: mesenchymal stem/stromal cells; PUFA: polyunsaturated fatty acids; VEGF: vascular endothelial growth factor.

Regarding natural products such as flavonols, which are compounds derived from fruits and vegetables that possess anti-oxidant and anti-inflammatory properties, a recent study of postnatal intraperitoneal administration of the flavonoid quercetin in mice showed it conferred protection to hyperoxia-induced lung injury via reduction of inflammation and oxidative stress marker expression, and improved alveolarisation outcomes, indicating the potential therapeutic value of flavonoids in BPD.[32]

Treatment with vitamin D has also proven effective at preventing BPD in a rat model.[33] A study using intra-amniotic lipopolysaccharide (LPS) administration in pregnant rats, which is known to induce BPD in pups, showed that treatment with vitamin D for 7 days starting at postnatal Day 0 alleviated structural alveolar lung simplification induced by BPD and suppressed LPS-induced lung and spleen inflammatory gene expression, particularly IFN-γ.[33] This study also explored the association of maternal vitamin D exposure during pregnancy and neonatal IFN-γ levels in a prospective birth cohort, finding a similar trend to that observed in the animal model.

Regarding pharmacological treatments, aerosolised administration of the iron-chelating drug deferoxamine for 2 weeks resulted in improved weight gain, reduced BPD severity, increased hypoxia-inducible factor-1α expression, and activation of vascular endothelial growth factor (VEGF)-induced angiogenesis in a mouse model of hyperoxia-induced BPD.[35] Similarly, a combination of the peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist pioglitazone with a synthetic lung surfactant mix effectively accelerated lung maturation and prevented hyperoxia-induced lung injury when nebulised in a mouse model of hyperoxia-induced BPD.[34] Experimental studies have also revealed that rosiglitazone (PPAR-γ) treatment can enhance pulmonary vascular development, restore alveolar function, and combat pulmonary hypertension by affecting VEGF and its receptor. PPAR belong to the superfamily of nuclear receptors; these are ligand-regulated transcription factors that control gene expression by binding to specific response elements within promoters and regulate energy homeostasis, lipid and glucose metabolism, and inflammation. PPAR-γ is predominantly found in white adipose tissue and has a role in storing triglycerides and fatty acids; however, the role of PPAR in pulmonary hypertension and alveolar function is still not known.

Finally, more recent experimental strategies to prevent and treat BPD have incorporated cell therapeutic approaches, including administration of mesenchymal stem cells (MSC), and cell-free therapeutics, such as the use of extracellular vesicles, including exosomes.[37-44] For example, a recent study of postnatal intraperitoneal injection of amniotic fluid stem cells in a rabbit model of hyperoxia-induced BPD showed attenuated inflammation and improvement in parenchymal and vascular structure and function, particularly when using cells in which VEGF expression was upregulated.[36] A more recent study used exosomes purified from human MSC culture media in a murine model of BPD and showed significant improvement in the lung inflammatory phenotype and pulmonary hypertension.[37] Taken together, these recent studies indicate that BPD management and prevention could potentially be achieved by a combination of nutritional, pharmacological, and cellular therapeutic approaches, with promising results. Future work on the characterisation of mechanisms associated with the observed outcomes in animal models will help in the design of new clinical studies assessing the effectiveness of these experimental agents and/or nutritional management strategies in preventing or ameliorating BPD symptoms.

EXPERIMENTAL THERAPEUTICS: RECENT CLINICAL STUDIES

In the past few years, a number of prospective and retrospective clinical studies aimed to prevent and treat BPD have been conducted using a variety of agents and ventilation management approaches. A summary of the most recent studies using these compounds and the most significant outcomes are shown in Table 3.[45-59] These studies have provided useful information on the effectiveness and pharmacological properties of various therapeutic agents, as well as other treatments for sepsis, inflammation, and concurrent infection in BPD patients.[57,58,60] Of the most frequently used therapeutic agents for BPD management, the two most common compounds are caffeine and vitamin A. These

are used either alone or in combination with ventilation management strategies, inhaled nitric oxide administration, systemic corticosteroid treatment, and other nutritional interventions.

Caffeine is one of the most widely used drugs in the neonatal intensive care unit. Initially used as a potent respiratory stimulant to treat apnoea of premature infants, as well as intermittent hypoxaemia and complications of extubation in mechanically ventilated infants, caffeine has been used to treat BPD and patent ductus arteriosus for many years. In addition, multiple studies have shown that its use has a positive effect on BPD outcomes due to its anti-inflammatory and diuretic properties, resulting in a shorter duration of mechanical ventilation.[61] However, evidence regarding the timing of caffeine treatment initiation is unclear, since several observational studies have reported differential outcomes resulting from early and late caffeine use.[46] For example, early caffeine use is considered a controversial practice because the benefits are limited to observational data, and associations of cerebellar injury with high-dose caffeine started early after birth have been reported.[62] In contrast, late caffeine use has been associated with refractory respiratory failure, increased duration of mechanical ventilation, and increased requirement of postnatal steroids.[45] Common current practice involves starting caffeine therapy in preterm infants early, often before the second day after birth.[47] This practice may reduce extubation failure and the incidence of adverse neurodevelopmental outcomes.[63] However, the dosage should be considered carefully, as caffeine use outside of the therapeutic range has also resulted in proinflammatory pulmonary outcomes.[47] In the absence of concrete evidence, the optimal timing and dosage of caffeine administration for maximal benefit remain unclear.

Either alone or in combination with caffeine treatment, vitamin A supplementation has also been used as a preventative practice against BPD due to its proposed role in lung maturation. Its use is also often justified because preterm infants are particularly predisposed to vitamin A deficiency, and evidence from *in vitro* studies has showed vitamin A can promote DNA repair and alveolarisation. However, despite initially promising results, vitamin A supplementation is not currently widely implemented due to recent concerns regarding sepsis, discomfort (from repeated intramuscular administration), and necrotising enterocolitis (in small-scale studies).[64,65] Although the most recent Cochrane review on the topic showed a modest reduction in the risk of BPD in VLBW infants,[52] evidence from a nationwide decline in vitamin A supplementation during a recent drug shortage showed that BPD rates remained stable, thus leading investigators to question its efficacy.[66] In this regard, the ongoing multicentre trial NeoVitaA[53] aims to assess the ability of high-dose oral vitamin A supplementation to reduce BPD rates in infants weighing <1,000 g at birth and treated for 28 days. The results from these trials will hopefully provide clarification on the use of oral vitamin A as an effective treatment to prevent BPD in small, preterm infants.

Regarding postnatal corticosteroid therapy, including dexamethasone and budesonide, the evidence remains controversial. While recent clinical trials have shown that when administered systemically corticosteroid use results in significant improvements in short-term lung function and a reduction of BPD in infants at increased risk,[51] these results have only been repeated in combination with surfactant therapy in a separate cohort,[50] or showed no significant improvement in other studies.[48,49] Moreover, systemic corticosteroid administration has also been associated with serious short and long-term adverse effects,[67] making this practice less appealing due to safety concerns. Therefore, additional multicentre randomised controlled trials with proper follow-up studies are needed to further validate the benefits of prophylactic or therapeutic corticosteroid use in BPD patients.

Nutritional supplementation therapies have also been implemented in BPD clinical trials. In a recent review and meta-analysis of 31 randomised controlled trials and observational studies, investigators found that administration of donor human milk conferred protection against BPD in VLBW preterm infants.[56] This meta-analysis had several limitations, including the observational nature of many of the studies included; that the studies displayed substantial heterogeneity in duration of intervention, timing of initiation, and population; and that none of the included randomised controlled trials were powered to detect the effects of donor human milk (HM) on BPD. In a prospective study of very

preterm infants who received HM-derived cream, it was found that those receiving a HM-derived cream supplement were discharged earlier than the control group and, among them, infants with BPD benefitted the most.[68] In another cohort study, it was found that increased doses of the mother's own milk reduced the odds of BPD in VLBW infants.[69] This notion has also been supported by a meta-analysis of multiple studies showing breast milk feeding in VLBW infants lowers the risk of BPD and associate comorbidities.[70] On the other hand, vitamin D supplementation remains controversial in its efficacy and dosage recommendation from various contradictory studies,[54,55] although deficiency of this vitamin has been directly associated with increased severity of BPD in an African-American preterm cohort.[71] Furthermore, ongoing clinical studies evaluating the effectiveness of lipid formulations, such as SMOFlipid, in parenteral nutrition for preterm infants have shown anti-inflammatory properties that can benefit BPD patients,[59,72,73] although recent animal studies have indicated a potential pro-apoptotic and pro-oxidant effect of these preparations.[31] Finally, as promising preclinical studies have shown effective therapeutic potential of various preparations of mesenchymal stem cells to reduce the risk and severity of BPD,[38] at least three ongoing early Phase I clinical trials (NCT03378063,[74] NCT01297205,[75] and NCT01632475[76]) are exploring their effectiveness in the prevention and treatment of BPD in various prematurely born infant cohorts.

Table 3: Clinical studies assessing potential therapeutic agents for bronchopulmonary dysplasia in neonates.

Therapeutic agent	Type of study	Sample size	Results	Reference
Caffeine	Single-centre, retrospective cohort study	138 infants	Early caffeine treatment reduced the odds of BPD or all-cause mortality and reduced the rate of patent ductus arteriosus requiring treatment and the likelihood of discharging home on oxygen. Patients receiving a high-dose of caffeine had improved secondary outcomes but no difference in BPD was noted.	Shenk et al.,[45] 2018
	Systematic review of five observational studies	63,049 infants	Earlier initiation and high-dose caffeine were associated with a decreased risk of BPD but the overall quality of this evidence is low.	Pakvasa et al.,[46] 2018
	Systematic review of six randomised control trials	620 infants	A higher caffeine dose resulted in a significant decrease in BPD, combined outcomes of BPD/mortality, and failure to extubate, and may provide neurodevelopmental benefits. Earlier initiation of caffeine and/or use of high-dose caffeine may reduce the risk of BPD compared with later initiation or the standard dose of caffeine.	Vliegenthart et al.,[47] 2018
Budesonide/ postnatal corticosteroids	Randomised controlled trial	70 neonates	No significant differences in BPD with corticosteroid alone. Budesonide showed effectiveness only in combination with two doses of surfactant.	Sadeghnia et al.,[48] 2018
	Observational study	863 preterm infants	No difference in developmental disability at 2 years of age among survivors. Higher mortality rate in the group receiving budesonide.	Bassler et al.,[49] 2018
	Randomised controlled trial	265 infants with severe respiratory distress syndrome	The intervention group had a significantly lower incidence of BPD or death and required fewer doses of surfactant.	Yeh et al.,[50] 2016
	Single-centre retrospective study	165 infants born preterm	Risk estimates for BPD or death were significantly greater in infants treated with steroids compared with controls.	Cuna et al.,[51] 2018

Table 3 continued.

Therapeutic agent	Type of study	Sample size	Results	Reference
Vitamin A	Systematic review of two randomised controlled trials	N/A	Vitamin A administration resulted in a statistically significant reduction in the incidence of BPD and a borderline significant reduction in combined outcomes of mortality/BPD.	Garg et al.,[52] 2018
	Prospective multicentre randomised controlled trial	914 (target)	Ongoing	Meyer et al.,[53] 2017
Vitamin D	Prospective randomised controlled trial	138 (target)	Ongoing	Kołodziejczyk et al.,[54] 2017
	Systematic review of eight randomised control trials	N/A	Daily supplementation of vitamin D at high or low disease resulted in no significant difference for BPD.	Yang et al.,[55] 2017
Donor human milk	Meta-analysis of 31 randomised control trials	859 infants	Overall results show that donor human milk protected against BPD in very low weight birth infants.	Villamor-Martínez et al.,[56] 2018
High-flow jet ventilation	Case control study	100 preterm infants	Death before discharge was more frequent in cases. High-flow jet ventilation was not associated with reduced odds of death or discharge home on oxygen.	Anvekar et al.,[57] 2018
Antifungal therapy (for suspected fungal infection)	Cohort study	13,343 very low birth weight infants from 54 centres	Antifungal treatment increased risk for BPD.	Fortmann et al.,[58] 2018
SMOFlipid® for parenteral administration	Retrospective EPOCH study	222 infants	Use of SMOFlipid as the primary lipid emulsion was well tolerated in preterm infants, with minimal side effects or effects in neonatal outcomes.	Choudhary et al.,[59] 2018

BPD: bronchopulmonary dysplasia; N/A: not applicable.

CONCLUSION AND FUTURE DIRECTIONS

The effective management and prevention of BPD continues to be challenging despite significant advances in neonatal care, and the information obtained from animal models and clinical studies remains controversial. In addition, the efficacy of currently available therapeutic and preventative strategies varies depending on the populations studied and disease stage. Work conducted in animal models shows promising results on the potential use of pharmacological interventions, ventilation and nutritional management, and stem cell therapeutics to prevent and treat BPD. These studies have also provided information that could help identify the mechanisms behind the disease development and progression. As the results from clinical studies on the most commonly used BPD therapeutics remain contradictory and ambiguous, additional randomised controlled trials, together with longitudinal evaluation of potential beneficial or negative effects, are essential to optimise disease management and preventative strategies. As the rates of BPD continue to increase due to increased survival of VLBW preterm infants, it is imperative that we continue to investigate the mechanisms of BPD pathogenesis as well as alternative approaches for its treatment and prevention.

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Why Do People Misdiagnose Themselves with Food Hypersensitivity? An Exploration of the Role of Biopsychosocial Factors

Authors: *Rebecca C. Knibb

Department of Psychology, School of Life and Health Sciences, Aston University, Birmingham, UK
*Correspondence to r.knibb@aston.ac.uk

Disclosure: The author has declared no conflicts of interest.

Received: 08.03.18

Accepted: 30.10.18

Keywords: Aversions, biopsychosocial, conditioning, food allergy, food hypersensitivity, food intolerance, psychosomatic, self-diagnosis, stress.

Citation: EMJ. 2019;4[1]:30-37.

Abstract

Up to 35% of people self-diagnose food allergy or intolerance (food hypersensitivity [FH]), or diagnose it in their child, and self-manage the condition rather than seek a clinical diagnosis. This is much higher than the latest FH prevalence rate, estimated to affect 2–5% of the general population. The actual prevalence rate may be underestimated due to the lack of diagnostic services; however, this can only account for a small proportion of the discrepancy because only a small percentage of self-reported FH can be clinically confirmed. Many people are therefore misdiagnosing their or their child's symptoms as FH and needlessly removing foods from their or their child's diet. There are a number of possible reasons for this misdiagnosis, which can be considered from a biopsychosocial perspective. Psychological factors include a confusion over the diagnosis, coincidental pairing of food and symptom, psychological or psychosomatic reactions, and taste aversions. There are also biological mechanisms that have not been fully considered in food allergy research that may be relevant, such as conditioning of the immune system or stress responses. A social context pertains to a greater awareness of FH due to media coverage and changes in food labelling laws. Any of these theories are plausible, but the research to date has a number of methodological issues. Most studies report on small self-selected samples recruited from clinics and there is a lack of general population data. Studies also tend to be cross-sectional, which does not allow cause and effect to be established. Future research needs to include longitudinal designs that incorporate qualitative elements to enable a detailed exploration of reasons why people self and misdiagnose FH.

INTRODUCTION

Food hypersensitivity (FH), which includes food allergy and intolerance, is an adverse reaction to food causing unpleasant and sometimes life-threatening symptoms.[1] Recent estimates put the prevalence rate of food allergy at around 2% of adults and 5% of children in the general population,[2] and there is evidence that the prevalence is increasing.[3] There is currently no cure for FH and management requires constant vigilance to avoid the food in question. Having this condition or caring for someone with FH

has therefore been associated with stress, worry, anxiety, and depression, and can impact quality of life.[4-6] Allergy service provision in primary and secondary care is limited across Europe,[7] with the number of certified allergists as low as 1 per 25 million of the population in some areas.[8] There is also a paucity of knowledge about FH in primary care[9-10] and physicians experience difficulties in arriving at correct diagnoses,[11] meaning that FH could go unrecognised and undiagnosed for some years.[12] However, FH is well known in the public domain, with widespread media coverage on the topic in recent years and more awareness due to changes in food labelling laws for unpackaged food.[13]

It may therefore be unsurprising that many people self-diagnose FH or diagnose it in their child and self-manage, rather than attaining a clinical diagnosis. Recent meta-analyses have reported that up to 35% of people self-report food allergy or intolerance or report it in their child.[3,14] Nwaru et al.[3] found a pooled lifetime prevalence of self-reported FH of 17.3% but only 2.7% prevalence as confirmed by skin prick tests and 0.9% confirmed by food challenges. This discrepancy is not just confined to FH, but it is also seen in other allergic conditions. Two large randomised controlled trials found that self-reported allergic triggers were not confirmed by skin prick testing in 41% of a paediatric population[15] and 78% of an adult population[16] with asthma or rhinitis.

It is unclear why there is such a large discrepancy between perceived self-reported FH and that which can be confirmed clinically. The actual FH prevalence rates may be underestimated because of a lack of diagnostic services,[3] but this may only account for a small proportion of this discrepancy. Many people may therefore be misdiagnosing their symptoms as FH and needlessly removing foods from their or their child's diet. There are a number of possible reasons for this misdiagnosis, which can be considered from a biopsychosocial perspective. Psychological factors contributing to misdiagnosis include confusion over definitions, coincidental pairing of food and symptom, taste aversions, and psychosomatic reactions. Biological factors encompass mechanisms that have been given less consideration in FH research, such as conditioning of the immune system or stress responses. This is set within a social context, where there is greater public awareness (but not necessarily knowledge and understanding) of FH due to media coverage of fatal reactions and changes in labelling laws. This paper takes a biopsychosocial approach to explore possible reasons for the discrepancy between self-diagnosed and clinically diagnosed FH and provides new directions for research.

COINCIDENCE AND CONFUSION

For many years, the academic literature used different terms to refer to food allergy or intolerance, and there was a lack of consensus over the definitions of food allergy, intolerance, hypersensitivity, and aversion. To address this, a position statement was published that gave definitions for food allergy (both IgE and non-IgE mediated) and food intolerance (which does not involve the immune system).[17] However, these definitions were not clearly transmitted to the public and often people do not understand the difference between the two, with knowledge about FH being poor even in those with a clinical diagnosis.[18,19] As a result, people may label themselves as allergic to a food when they have a food intolerance or even have another condition entirely. A high prevalence of irritable bowel disease has been found in people with self-reported FH,[20] and, although up to 70% of those with irritable bowel disease have been found to have immune activation, this was not typical of an IgE-mediated reaction.[20] Therefore, these cases would not be diagnosed by standard skin prick testing for IgE. Coeliac disease and rarer diseases, such as hydatid disease,[21] also have symptoms in common with FH and may be misdiagnosed as such. A proportion of the discrepancy between self-reported and clinically confirmed FH may, therefore, be due to a lack of knowledge resulting in a misdiagnosis of food-induced symptoms.

More commonly, a misdiagnosis could be due to coincidence or confusion. People eat food regularly throughout the day and there are many reasons why symptoms are exhibited. Food poisoning may be experienced or a short-term virus that causes gastrointestinal symptoms. There are also a range of agents that can cause allergic reactions similar to food allergy, such as aeroallergens (e.g., pollen), reactions to animal dander, washing powders and latex, or

low molecular mass chemicals (e.g., salicylates, benzoates, and sulphites).[22] It is often difficult for a trained practitioner to properly diagnose food allergy or intolerance based on recalled history alone.[11] Kelsay[23] stated that patients and families could more accurately diagnose food allergy by flipping a coin rather than relying on symptoms. Histories can be unreliable,[24] and often people cannot recall much detail about past experiences regarding reactions to food.[25]

Thus, it can be extremely easy to make a mistake and think an innocent food has caused symptoms and that it will cause symptoms again if eaten.[26] In a large study, where 300 people were interviewed about their reasons for self-diagnosing FH, Knibb et al.[25] found that a vague recollection of instances where food was followed by symptoms correlated negatively with pathophysiological plausibility of actual FH, based on an assessment of their recalled clinical history. Many of the histories did not match typical clinical presentations of food allergy and could be explained by other factors, such as food poisoning, taste aversion, or a concomitant illness. In addition, approximately a fifth of those interviewed had decided that they had FH after experiencing symptoms just once after eating the food; they avoided the food after this incident and so never tested their assumption that the food was responsible for their symptoms.

This illustrates an important issue: people do not behave like scientists when testing a hypothesis that food causes a symptom. In a study exploring scientific decision-making, Croker and Knibb[27] provided adults without food allergy in the USA and the UK with a hypothetical situation where a person avoided peanuts because they thought they had an allergy to it. When asked what the person should do to find out if they were, in fact, allergic to peanuts, people were more likely to advise the person to continue avoiding peanuts rather than try eating peanuts to see if symptoms occurred. Thus, coincidental pairing of food and symptom on one occasion, coupled with an absence of symptoms on food avoidance and a reluctance to try the food again to see if symptoms reoccur, could explain a large proportion of misdiagnosed FH.

Judgement about the cause of symptoms may also be affected by a confirmation bias,[28] where the person reaches the view that they have FH and ignores additional information that conflicts with this view (e.g., they have eaten the food before and never experienced symptoms or they experience similar symptoms again even though they are now avoiding the food). As these people do not seek a clinical diagnosis for their perceived FH, these misattributions could lead to long-term food avoidance and reductions in health-related quality of life seen in those with clinically confirmed FH.[4-6] More qualitative research exploring reasons why people decide they have a FH is needed to determine the extent to which confusion and coincidence may be a causative factor.

TASTE AVERSIONS

A coincidental pairing of food and symptom leading to an erroneous assumption that the food caused the symptom may result in the development of an aversion to that food, which may perpetuate the belief that the food causes symptoms. Aversion to the taste of food occurs when the taste of food is paired with an unpleasant physiological reaction, most commonly nausea and vomiting.[29] Thereafter, the implicated food is often avoided and the sight or smell of that food can induce nausea without having to actually ingest it. However, aversions are not just related to nausea and vomiting; they have been reported in people with self-diagnosed FH.[30]

Knibb et al.[30] found that just over a third (35%) of the people interviewed with self-reported FH who avoided the food completely stated an acquired dislike for the food after they decided that consuming it caused symptoms. Aversion to the taste of the food occurred most often after nausea or vomiting; however, aversion also occurred equally as often to other symptoms, including behavioural and emotional states, such as hyperactivity, irritability, anxiety, and depression. Aversion to the flavour of alcoholic and non-alcoholic beverages (possibly due to the strong taste of these compared to other foods implicated in self-reported FH) occurred most often.

Interestingly, Knibb et al.[30] found that taste aversion was reported significantly more often in people who had less plausible recalled histories for FH. Those who reported fear of the symptoms as opposed to an aversion to the

taste were more likely to give more plausible accounts. This pattern of results was evident for foods inducing nausea and vomiting, as well as behavioural symptoms. The authors suggest that nausea and vomiting in particular (due to these symptoms' ability to provoke thoughts of the oral region) may bring sensory aversions to mind without any foundation for a real contingency between eating the food and suffering the illness.[30] Little empirical research has been conducted on taste aversions in those with self-reported FH and is needed to further explore its potential role in being a cause of misdiagnosed FH.

PSYCHOSOMATIC OR PSYCHOLOGICAL REACTIONS

One reason for misdiagnosis of FH that has received a lot of attention in the literature is a psychosomatic or psychological reaction. It may be that some people are more prone to misinterpret bodily sensations as an adverse reaction to food. Anxiety can increase vigilance for bodily symptoms and people with high trait anxiety tend to report more symptoms and be more concerned about those symptoms.[31] This bias may mean that patients are reluctant to see a connection between symptoms, anxiety, and stress, and this may partly be due to the stigma associated with psychological factors as a cause of symptoms.[32]

In the UK in 1984, the Royal College of Physicians (RCP) defined food intolerance as an unpleasant reaction to food caused by emotions associated with the food rather than being caused by the food itself.[33] Research published around this time suggested that people reporting FH that could not be clinically confirmed were likely to be suffering from psychosomatic reactions (subjective symptoms with a psychological rather than medical or biological explanation). Their evidence was that these people were more likely to score higher for hypochondria, hysteria, somatisation, and symptom distress than patients with clinically confirmed FH.[34-36] It was also reported that people with self-diagnosed FH complained of more subjective somatic symptoms or symptoms related to the central nervous system (CNS), such as headaches; hyperactivity; learning problems; behavioural problems; insomnia; and emotional symptoms, such as anxiety, fatigue, and general aches and pains. Objective symptoms, such as skin symptoms or angioedema were reported less often.[37] At the time, there was no proof of a consistent relationship between the CNS and food allergy;[38] however, more recent research on conditioning of the immune system is providing evidence that the CNS may indeed play a role. It has now been demonstrated that substances do not need to cross the blood–brain barrier to affect emotions and behaviour but can affect the CNS through neural pathways.[39]

More recently, Nekam et al.[40] have looked at food allergy symptoms as a possible consequence of a subconscious response to stress and anxiety in a small sample of 14 female patients where double-blind placebo-controlled food challenges could not establish food allergy beyond any doubt. Compared to a control group of patients with anxiety and social phobia with no physical disease, the patients reporting food allergy had significantly lower stress scores, elevated state anxiety (but not trait anxiety) scores, and moderately serious depression. The Szondi test (a projective test of subconsciously perceived stress and anxiety) showed that patients had a high level of anxiety related to a feeling of guilt, losing attachments, and feeling inhibited. The authors concluded that these patients do not consciously perceive their own stress and may convert their anxiety into somatic symptoms, which the patients attribute to food allergy.[40] Polloni et al.[41] have also reported higher levels of alexithymia (difficulty in recognising and expressing emotions) in children and young adults with confirmed food allergy compared to healthy controls.

The research focussing on a psychosomatic or psychiatric explanation for misdiagnosis has often been limited to small self-selecting samples of people attending an allergy clinic. These people may be very different to a general population sample self-diagnosing FH. Larger scale studies have been conducted and found that those with self-reported FH often have more self-reported depression, anxiety, somatisation, and subjective health complaints than those with no self-reported FH.[42,43] In a large, general population survey, Knibb et al.[44] found significantly higher levels of neuroticism and psychological distress in people with self-reported FH compared to healthy people. However, there was no greater prevalence of psychiatric disorders than that

seen in a reference sample of NHS and university staff and no differences in neuroticism compared to normative data. In a similar study, Peveler et al.[45] could find no evidence for greater psychological symptoms in a self-reported FH group compared to a control group. A recent study has also shown that parents who have diagnosed FH in their child do not have higher levels of stress, depression, or anxiety than those with a clinical diagnosis.[46]

Many of these larger studies do not verify the self-reported diagnosis through clinical testing and rely on self-report of psychological distress. One study to overcome these limitations involved the conduction of diagnostic interviews with 76 patients with self-reported FH and found that 57% of patients met the DSM-IV criteria for at least one psychiatric disorder, but only 8% of FH cases could be confirmed by double-blind placebo-controlled food challenges.[47] The authors noted the possibility of somatisation being involved in the presentation of FH, suggesting that the symptoms may be a result of stress, which can affect gastrointestinal function and increase gut motility, or depression, which has been associated with constipation. Conversely, they also suggested that suffering from prolonged symptoms could cause psychological distress.[47]

The limitation of all of these cross-sectional studies is the inability to determine the causal pathways between somatic symptoms, psychological distress, and self-diagnosed FH. Chida et al.[48] have reported evidence of a bi-directional relationship between stress, anxiety, depression, and allergy. The meta-analysis showed a small, but statistically significant, positive association between psychological distress and future atopic disorders, as well as between atopic disorders and future psychological distress.[48] Therefore, it is important for future research to separate the role of psychological factors in the cause of supposed FH from the consequences of such a condition. Longitudinal research is needed to determine this.

THE IMMUNE SYSTEM, STRESS, AND CONDITIONING

Allergic reactions to food involve the immune system; thus, studies investigating the conditioning of immune responses and evidence from the field of psychoneuroimmunology, exploring how the immune system responds to psychological stress, may provide further explanations for misattributed FH.

Early animal research has shown how conditioning can elicit an allergic reaction. In a study by Williams et al.,[49] rats were conditioned with audiovisual stimuli (a loud noise and flashing lights). The rats were sensitised to egg albumen and were then trained on three successive occasions to associate the injection of antigen with the audiovisual cue. Subsequently, the rats demonstrated significant rises in serum rat mast cell protease when exposed to the audiovisual cue alone. This rise was comparable to animals challenged with the antigen without a cue. It has been suggested that this mast cell activation may be partly triggered by the CNS via the peripheral nerves in the respiratory and GI tracts.[50] Histamine release (which is the cause of unpleasant allergy symptoms) as a response to stress has also been demonstrated in guinea pigs in a conditioning paradigm.[51]

As a result, food could act as a conditioned stimulus in humans if paired with something that stimulates an immune response, such that a conditioned response develops when a person is exposed to the food on subsequent occasions. Psychological stress affects the immune system and has been shown to reduce the rate of wound healing and affect responses to infectious diseases and vaccines.[52] In relation to allergic conditions, chronic stress can dysregulate the hypothalamic pituitary adrenal system, resulting in blunted cortisol release and increased eosinophil counts leading to reduced lung function in patients with asthma.[53,54] Active and passive stressors have also been shown to increase sympathetic nervous system activity, cortisol and inflammatory responses, and induce mild bronchoconstriction.[55] Similar immune responses to stress have been seen in patients with atopic dermatitis.[56]

In addition, when experiencing acute stress, activation of the sympathetic nervous and hypothalamic pituitary adrenal systems and an increase in adrenaline and cortisol induces symptoms, such as increased heart rate, blood pressure, and respiration rate, which can cause redness of the skin and irritation of the gastrointestinal tract.[31] If food has been paired

with symptoms in the past that caused distress, subsequent presentation of the food could induce anxiety or stress-related symptoms, which could be misinterpreted as being caused by the food.

These hypotheses offer possible psychopathophysiological pathways that may go some way to explain why certain people have real allergy-like symptoms for which they blame food but do not react to double blind placebo-controlled food challenge where they do not have the food as a cue. The lack of food cues may result in an absence of a stress-related or conditioned response; thus, leading to null or equivocal food challenge results. These hypotheses require full examination in human participants with self-reported FH to explore their potential as reasons for the discrepancy between self-reported and double-blind placebo-controlled food challenge confirmed FH.

THE SOCIAL CONTEXT OF FOOD HYPERSENSITIVITY

The factors explored in this paper are likely to be reinforced or influenced by a social context, which may increase the likelihood that someone attributes the symptoms that they have experienced to the food consumed. FH is now more in the public consciousness; people with FH have reported greater awareness of FH when eating out and increased confidence in communicating with catering staff about FH.[13] This is partly due to the change in European labelling laws meaning that catering establishments must publish information about the main allergens in the food they serve (European Union [EU] Food Information for Consumer Regulation No 1169/2011),[57] making FH more salient when people eat out or buy non-prepacked food. It is also partly due to widespread media coverage of fatal food allergy related reactions, some of which have led to successful prosecutions for manslaughter where food has been served containing allergens despite the customer declaring an allergy. It has also become much easier for people to obtain health and illness related information from the internet, where there is a plethora of FH related websites, online discussion boards, and support groups. The availability of information related to FH could increase the chances of people attributing symptoms to food due to availability bias,[28] which increases the perception of an event's risk (therefore leading a person to believe they have developed a FH) for which an example can be easily recalled. The social context of increased awareness of FH requires further investigation as a reason for misdiagnosis to explore these hypotheses.

CONCLUSIONS

It is clear that one factor alone cannot explain the large discrepancy between self-diagnosed and clinically diagnosed FH. A biopsychosocial approach to health and illness recognises the interaction of factors in the cause of illness and a bidirectional relationship across them.[31,58] Thus, it is likely that more than one factor is responsible for misdiagnosed FH in any single case and that often symptoms are not merely due to a psychosomatic reaction, but have a plausible biological basis, whether those symptoms are caused by FH or not. How these factors interrelate and the direction of cause and effect requires further exploration to provide greater insight into why people misdiagnose not just FH, but other allergic conditions too. A mixed design longitudinal approach using quantitative and qualitative methods investigating biological, psychological, and social factors would be beneficial in furthering our knowledge in this important area.

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Clinical Conditions that Masquerade as Urticaria

Authors: Nofar Kimchi,[1] *Jonathan A. Bernstein[2]

1. The Baruch Padeh Medical Center, Poriya, Israel
2. Department of Internal Medicine, Division of Immunology, Rheumatology, and Allergy, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

*Correspondence to jonathan.bernstein@uc.edu

Disclosure: Dr Kimchi has declared no conflicts of interest. Dr Bernstein has worked as a principal investigator, speaker, and consultant for Novartis and Genentech.

Received: 17.08.2018

Accepted: 24.12.2018

Keywords: Adult-onset Still's disease (AOSD), autoinflammatory, cryopyrin-associated periodic syndromes (CAPS), cutaneous mastocytosis (CM), erythema marginatum (EM), erythema multiforme (EMu), hereditary angioedema (HAE), systemic-onset juvenile idiopathic arthritis (SoJIA), urticaria, urticarial vasculitis (UV).

Citation: EMJ. 2019;4[1]:39-47.

Abstract

Chronic urticaria is one of the most commonly diagnosed dermatoses. Following diagnosis, correct identification and proper treatment significantly reduces disease activity, thereby improving the patient's quality of life. However, there is an extensive differential diagnosis for chronic urticaria that, if missed, can lead to life-threatening sequelae. Many of the diseases that masquerade as urticaria are rare and often have a significant delay in diagnosis. This paper aims to fill the gap in the literature by clearly characterising the cutaneous eruptions and atypical findings in many of the most common mimickers of chronic urticaria. Conditions such as erythema marginatum seen in conjunction with hereditary angioedema, urticaria vasculitis, autoinflammatory cryopyrin-associated periodic syndromes, adult-onset Still's disease and systemic onset juvenile arthritis, Schnitzler syndrome, erythema multiforme, and cutaneous mastocytosis will be discussed.

INTRODUCTION

Urticaria is one of the top ten most commonly diagnosed dermatoses, with up to 20% of the population at risk of developing an acute eruption.[1,2] Urticaria is a heterogeneous disorder that, in most cases, is divided into acute or chronic severity of an inducible or spontaneous disease subtype. Urticaria is considered acute (AU) if the lesions last <6 weeks, and potential causes include contact urticaria, food allergies, adverse reactions to drugs, viral infections, and insect bites. If the flare lasts >6 weeks, it is classified as chronic urticaria (CU). CU is characterised by the sudden appearance of wheals or angioedema, and, in up to 40% of cases, both. Hives are evanescent, meaning they usually resolve within 24–48 hours, although sometimes lesions can take longer to dissipate.[1-6]

CU can be further divided into two major subgroups: spontaneous and inducible. The former occurs without the presence of an exogenous stimulus and can be persistent or intermittent, whereas inducible hives are physical urticarias that can be reproduced by physical stimuli, including cold, sunlight, water, and exercise, or emotional triggers, such as cholinergic or adrenergic urticaria. Hives are considered idiopathic when the history and supportive testing fail to identify an underlying cause.[4]

Box 1: Common and less common or uncommon causes of urticaria angioedema and urticaria-like dermatoses.

Common	Less common or uncommon
Anaphylaxis	Angiolymphoid hyperplasia with eosinophilia
Atopic dermatitis	Autoimmune progesterone-associated dermatoses
Autoimmune thyroid disease	Autoinflammatory syndromes: - Familial cold-autoinflammatory syndrome - Muckle–Wells syndrome, NOMID - Hyper-IgD syndrome, TRAPS, PFAPA, PAPA, FMF
Bullous pemphigoid	Blepharochalasis
C1-inhibitor deficiencies	Cheilitis glandularis
Contact dermatitis	Cheilitis granulomatosa
Contact urticaria	Cryoglobulinaemia
Cutaneous and systemic lupus erythematosus	Drug-related eosinophilia with systemic symptoms
Cutaneous mastocytosis	Episodic angioedema with eosinophilia
Dermatitis herpetiformis	Oestrogen-induced angioedema
Erythema multiforme (infection, drugs)	Complement factor I deficiency
Exacerbation of physical urticaria	HES
Food/insect allergies	Schnitzler syndrome/malignancies
Adverse medication reactions - Angioedema with ACE inhibitors - Fixed drug eruptions	SM
Parasite or bacterial infections	Urticaria-like dermatoses of pregnancy: - Gestational pemphigoid - PUPPS, prurigo of pregnancy
Polymorphous light eruption	Wells syndrome
Recall urticaria	
Scabies, insect bites	
Urticarial vasculitis (e.g., hepatitis)	
Viral infections	

ACE: angiotensin converting enzyme; FMF: familial Mediterranean fever; HES: hypereosinophilic syndrome; NOMID: neonatal-onset multisystem inflammatory disease; PAPA: pyogenic arthritis, pyoderma gangrenosum, and acne syndrome; PFAPA: periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis; PUPPS: pruritic urticarial papules; SM: systemic mastocytosis; TRAPS: tumour necrosis factor receptor associated periodic syndrome.

There are a number of conditions, presented in Box 1, that can look like CU; therefore, a high index of suspicion must be present to differentiate these rare mimickers from true CU. Early diagnosis of these other conditions increases the chance of successful treatment and unnecessary morbidity and, in some cases, the related potential mortality. Furthermore, proper identification and treatment of CU greatly reduces disease activity, thereby improving patients' quality of life.

Thus, the aim of this paper is to define and discuss the presentation, pathomechanism(s), complications, and treatments of clinical conditions that may be incorrectly diagnosed as

CU. These include erythema marginatum (EM) seen in conjunction with hereditary angioedema (HAE); urticarial vasculitis (UV); autoinflammatory cryopyrin-associated periodic syndromes (CAPS); adult-onset Still's disease (AOSD); systemic-onset juvenile arthritis; Schnitzler syndrome (SchS); erythema multiforme (EMu) cutaneous mastocytosis (CM); and dermatitis artefacta.

ERYTHEMA MARGINATUM

EM is a skin condition commonly seen as a prodromal symptom in patients with a diagnosis of HAE. A recent Danish study by Rasmussen et al.[7] found that 56% of HAE patients present with EM as a prodrome.

HAE is a rare autosomal dominant disease that is the result of a dysfunctional C1 esterase inhibitor (C1-INH) protein leading to dysregulation of kallikrein, which is essential for preventing the unimpeded breakdown of high molecular weight kininogen to form bradykinin, a potent vasoactive peptide that binds to bradykinin B2 receptors leading to vascular permeability and recurrent attacks of angioedema.[8-16]

Several studies have found that physicians struggle to recognise this sentinel rash and other prodromal symptoms of HAE leading to a delay in diagnosis ranging from 8–20 years in >50% of patients.[7-9] This can have serious consequences as patients who are misdiagnosed, and therefore not optimally treated, can experience increased mortality related to asphyxiation secondary to laryngeal angioedema.[8]

A thorough physical exam, as well as patient and family history, provide the subtle clues needed for differentiating CU (Figure 1A) from EM. The EM rash (Figure 1B) is usually asymptomatic, more reticular and serpiginous, less widespread, and typically non-pruritic compared with CU.

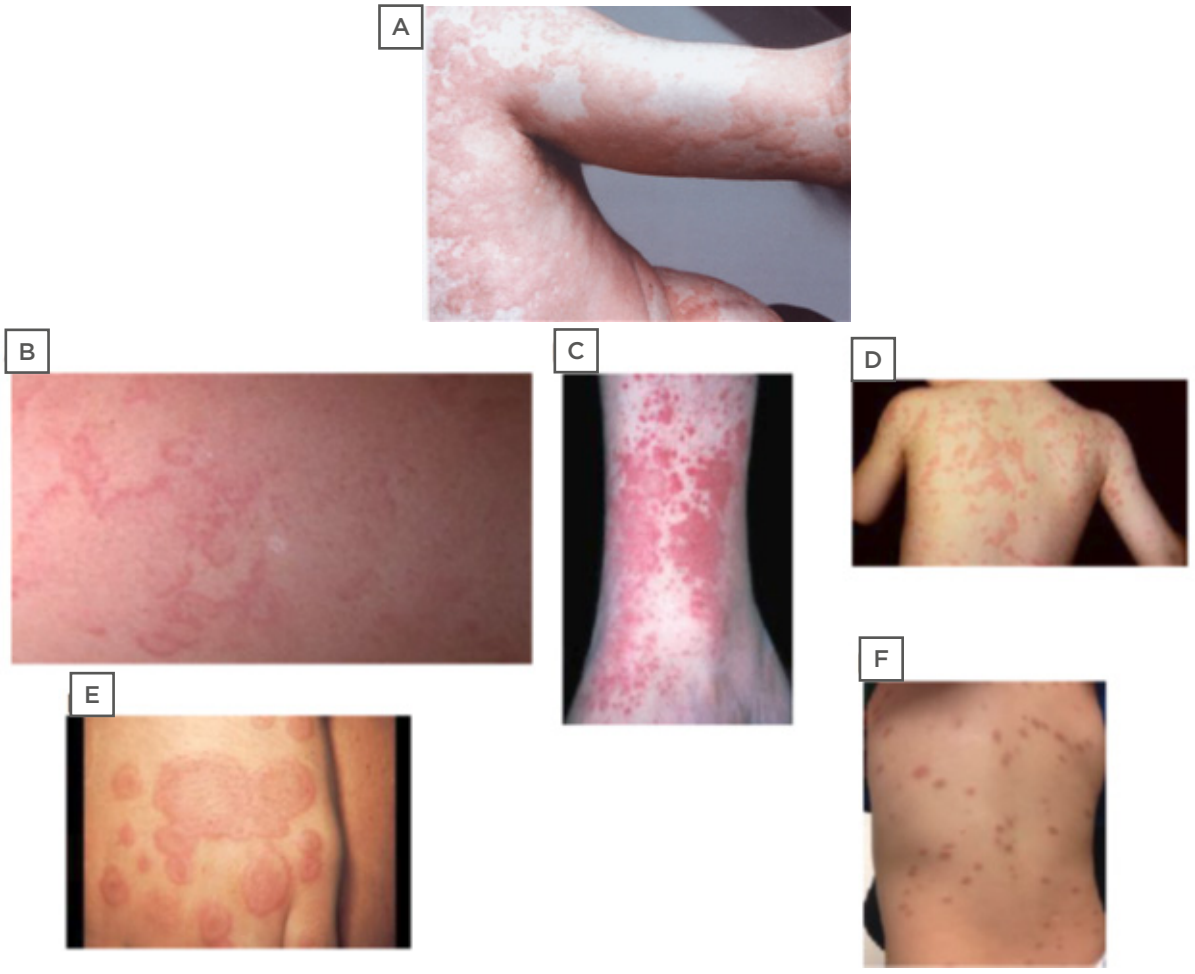

Figure 1: Photographs depicting chronic urticaria and commonly misdiagnosed rarer conditions.

A) Chronic urticaria; B) erythema marginatum; C) urticaria vasculitis; D) familial cold autoinflammatory syndrome; E) erythema multiforme; F) cutaneous mastocytosis.

Table 1: Characterisation of urticaria versus autoinflammatory syndromes.

	Urticaria	Schnitzler syndrome	Familial cold autoinflammatory syndrome	Muckle–Wells syndrome	Neonatal-onset multisystem inflammation disease	Systemic juvenile idiopathic arthritis and adult-onset Still's disease
Cutaneous manifestation	Sudden appearance of wheals with central swelling and surrounding erythema, lasting 24-36 hours.	Pale rose papules of plaques.	Urticaria that is minimally or non-pruritic.	Urticaria that is minimally or non-pruritic.	Urticaria that is minimally or non-pruritic.	Evanescent salmon-coloured rash.
Associated symptoms	Pruritus, angioedema	IgG or IgM gammopathy. Recurrent fever, lymphadenopathy, organomegaly, and arthralgia.	Cold-induced	Cold-induced fevers, sensorine ural hearing loss, chronic meningitis, migraines, conjunctivitis, and arthralgia.	Dysmorphic features, chronic meningitis, sensorine-ural hearing loss, end-organ damage, and rapidly progressive arthropathy.	Spiking fevers (>39°C) and arthralgia. Linear dermatographism, splenomegaly, hepatomegaly, lymphadenopathy, serositis, and arthritis. Some AOSD patients also complain of a sore throat.
Sequelae	Decreased quality of life, long-standing refractory disease.	Lymphopro-liferative disorders, AA amyloidosis.	Renal failure due to amyloidoses.	Intracranial hypertens-ion, fatigue, social isolation, renal failure due to amyoloidosis.	CNS damage and skeletal deformaties. Renal failure due to amyloidosis.	Macrophage activation syndrome.
Treatment	Avoidance of triggers and physical stimuli. Anti-histamines, leukotriene modifying agents, omalizumab, or cyclosporine.	Anti IL-1	Anti IL-1	Anti IL-1	Anti IL-1	Glucocorticoids, anti IL-1, and anti IL-6.

AA: amyloid A; AOSD: adult-onset Still's disease; CNS: central nervous system.

The most frequently involved sites are the abdomen, axillae, and extremities. Similarly to CU, EM lesions tend to fade within a few hours, but they can persist for as long as 2–3 days; however, unlike CU, which leaves no residual scarring or pigmentation, EM lesions often leave pale or slightly pigmented macules. The EM rash can recur daily over several weeks at different sites of the body, making EM confusing to differentiate from CU. In these patients, a serum C4 level, C1-INH functional and quantitative levels, and a C1Q should be measured to confirm a diagnosis of HAE.[7] The exact pathophysiology of EM is not well understood, but it may involve abnormal humoral and cellular immune responses to undefined antigens.[7-17]

URTICARIAL VASCULITIS

Up to 15% of refractory urticaria is due to UV, which is estimated to occur in approximately 1% of all treatment refractory CU cases, although the prevalence does vary based on the population studied.[17] Urticaria vasculitis includes normocomplementemic UV and hypocomplementemic UV, as well as hypocomplementemic urticarial vasculitis syndrome.

Unlike AU or CU, the rash associated with UV can be described as either painful or burning and lasts longer than 24–48 hours;[4,18,19] however, they may have a similar evanescent pattern to typical CU lesions (Figure 1C). The UV lesions differ from CU as they often leave associated bruising or hyperpigmentation and may be associated with a broad range of systemic involvement after resolution of the rash. The extent of extracutaneous manifestations, such as renal and pulmonary involvement, frequently correlates with decreasing levels of complement. Furthermore, up to 50% of patients with hypocomplementemic UV may develop or have coexistent systemic lupus erythematosus or other connective tissue disorders.[19,20]

Diagnosis of UV requires a consistent clinical presentation and histologic confirmation. Histopathology reveals small-vessel leukocytoclastic vasculitis with fibrinoid, complement, and immunoglobulin deposits perivascularly.[18,21] Additional laboratory tests include complete blood count; complete metabolic panel; erythrocyte sedimentation rate (ESR); C reactive protein (CRP); hepatitis profile; urinalysis; and C3, C4, and anti-C1q antibody assays. Depending on the presentation, further tests for connective tissue diseases may be indicated.[19,21]

Although both CU and UV activate the complement pathway, the latter is a Type 3 hypersensitivity reaction mediated by the deposit of antigen-antibody complexes on the vascular endothelium.[21] These antigen–antibody complexes activate the complement pathway, leading to increased anaphylatoxins (C3a, C5a) capable of activating mast cells, resulting in the release of bioactive mediators (i.e., histamine) and urticarial plaques. Just as in CU, the eliciting factor is usually not discovered, but possible aetiologies include medications, infections, autoimmune diseases, and malignancy.[18]

There are no formal guidelines for the treatment of UV, but antihistamines are often effective to control the pruritus. Other immunosuppressive, anti-inflammatory, or biologic treatments as recommended by the Joint Task Force Urticaria guidelines may be necessary and have been reported to have variable effectiveness.[18-21]

AUTOINFLAMMATORY SYNDROMES

Autoinflammatory conditions are a group of rare disorders that result from the inappropriate activation of the innate immune system (Table 1).[22,23] These conditions include SchS, CAPS, systemic onset juvenile idiopathic arthritis (SoJIA), and AOSD, which all can be easily mistaken for urticaria. However, the rash seen in autoinflammatory syndromes consists of predominantly flat wheals.[22] Their exact pathophysiology was not well understood until the recent discovery of IL-1 antagonists, most notably anakinra. The strong response of these conditions to anakinra solidified the notion that these are autoinflammatory conditions mediated by IL-1. A high index of suspicion is needed to recognise the subtle variations of these conditions upon clinical presentation.

Schnitzler Syndrome

The clinical picture of SchS can be very difficult to diagnose. Since its first description in 1972, the condition remains relatively underdiagnosed with only 250 known cases and a 5-year delay in diagnosis.[24] The mean age of presentation is 51 years, with a slight male predominance.[25]

SchS can be differentiated from urticaria by the presence of either pale rose macules or papules and plaques that, unlike in urticaria, may range from non to slightly pruritic, completely resolve within 24 hours, and commonly occur in the absence of angioedema. The hallmark feature is the presence of a skin rash with a monoclonal gammopathy, most commonly an IgM gammopathy with very few cases manifesting as an IgG gammopathy.[26] Initially, the IgM levels are low and may increase at a rate of roughly 0.5–1.0 g/L/year.[25,26] Similarly to the physical urticarias, these skin lesions can be induced by the same triggers, such as cholinergic, cold, and aquagenic stimuli.[26]

Other common symptoms include fever, arthralgia, and organomegaly. Most patients will develop recurrent fevers either with or separately from the skin flare. Eighty percent of cases exhibit musculoskeletal involvement, with significant bone pain being the predominant complaint and confirmed by radiologic findings in 30–40% of cases. Hepatomegaly and splenomegaly occur in an estimated 30% of SchS patients. Palpable lymph nodes occur in roughly 45% of cases. A definitive diagnosis is made if two obligate criteria (which include a chronic urticarial rash and either an IgG of IgM monoclonal gammopathy) and at least two minor criteria (recurrent fever >39°C, abnormal bone remodelling, neutrophilic dermal infiltrate on skin biopsy, leukocytosis, increased CRP or ESR, hepatomegaly or splenomegaly, lymphadenopathy, arthralgia, or arthritis) in the presence of an IgM gammopathy are met. If the monoclonal component is IgG then three minor criteria are required. Collectively, these requirements for diagnosis are referred to as the Strasbourg Criteria.[24-28]

Biopsied plaques reveal a perivascular and interstitial neutrophilic inflammation with leukocytoclasia, without vasculitis or dermal oedema, and therefore SchS is classified as a neutrophilic dermatosis. In addition, immunofluorescent studies have reported IgM deposits in 30% of patients.[26]

In addition to the physical and aesthetic impairments patients suffer, an estimated 20% will develop a lymphoproliferative disorder, such as a monoclonal gammopathy of undetermined significance, and there is a 15% 10-year risk of developing Waldenstrom's macroglobulinaemia. Other complications include severe inflammatory anaemia and amyloid A amyloidosis.[24-28] In the same way that SchS occurs through a distinct mechanism from that of AU or CU, it also requires a different treatment. Recent case studies demonstrate complete resolution of symptoms with treatment using IL-1 inhibitors.[21,24-28]

Cryopyrin-associated Periodic Syndromes

CAPS encompass three autoinflammatory syndromes: familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disease (NOMID). Like other orphan diseases, its true prevalence is hard to estimate and there is a delay in diagnosis. In fact, a study by Stych and Dobrovolny[29] found that 44% of FCAS cases carried a different primary diagnosis.

The severity of presentation is variable but includes daily urticaria, fever, and arthralgia. The mildest form is FCAS and the most severe form is NOMID.[22,28] CAPS initially presents in infants <6 months. The rash, an atypical urticaria, develops in a circadian pattern as the rash develops throughout the day, and has minimal or no pruritus, hence making it easily confused with UV. However, the dermatologic flare in CAPS, unlike UV, usually resolves within 24 hours without any leftover bruising or hyperpigmentation.[18,12-22,28]

Each of the three CAPS has its own unique features in addition to the aforementioned presentation. FCAS, similarly to cold-induced urticaria, presents after exposure to cold temperatures (Figure 1D). Both MWS and NOMID have associated sensorineural hearing loss, which is more severe in NOMID patients. Patients with MWS also present with cold-induced fevers and conjunctivitis.[22,28-30] NOMID newborns present with dysmorphic features, a myriad of CNS impairments, pseudo-urticaria lesions, end-organ damage, and rapidly progressive arthropathy. The extent of joint involvement can mimic SoJIA in addition to urticaria. The CNS changes include chronic aseptic meningitis, papilloedema, headaches, vomiting, static diplegia, and impaired cognitive function. Brain MRI shows central nervous inflammation, brain atrophy, and enlarged ventricles due to the continuous increase in intracranial pressure.[18,20-22,28-30]

Just as with NOMID, MWS patients can also present with headaches. In up to 90% of cases, patients complain of headaches due to migraines, chronic meningitis, and intracranial hypertension. In addition, 40–80% of patients suffer from severe fatigue leading to social isolation. Some also experience heaviness of the lower limbs with oedema.[22,28-30]

A definitive diagnosis of CAPS requires a clinical picture consistent with clinical history and genetic analysis confirming a mutation in the *NLRP3* gene. Lab test results usually reveal increased inflammatory markers (CRP and

ESR), elevated eosinophils and neutrophils, and low haematocrit. Patients also require frequent urinalysis due to their increased risk of developing systemic amyloid A amyloidosis as a common complication of the disease.[20,21,28-30]

For treatment of CAPS, IL-1 antagonists have been U.S. Food and Drug Administration (FDA)-approved and are the treatment of choice. Patients treated with these agents are able to live a normal, mostly symptom-free life.[18-20,22,28-30]

JUVENILE IDIOPATHIC ARTHRITIS AND ADULT ONSET STILL'S DISEASE

SoJIA and AOSD are two similar conditions. SoJIA, a class of juvenile idiopathic arthritis (JIA), occurs before the age of 16 with an age range of presentation between 1 and 16 years. It comprises 10% of JIA cases in northern Europe.[31]

The triad of a salmon-coloured rash, spiking fevers >39°C, and arthralgia are characteristic of both SoJIA and AOSD. In addition to the classic triad, other clinical manifestations include linear dermatographism, splenomegaly, hepatomegaly, lymphadenopathy, serositis, and arthritis. AOSD patients may initially complain of a sore throat.[31-39]

Most patients present with an evanescent rash, but some may present with a refractory cutaneous lesion, similar to that seen in CU. This finding can make it difficult to distinguish one from the other. In such cases, the onset of the rash in the evenings concurrently with fever and arthralgia should raise the possibility of either SoJIA or AOSD.

The prognosis of SoJIA and AOSD is complicated by clinical or subclinical macrophage activation syndrome in 10% and 30–40% of patients, respectively. SoJIA and AOSD have a mortality rate of 10–22% and it is therefore imperative that the diagnosis be determined as soon as possible.[31-40]

With respect to treatment, both AOSD and SoJIA respond well to anti IL-1 or IL-6 therapies and have better outcomes than treating with the traditional disease-modifying antirheumatic drugs. Glucocorticoids are used to treat acute flare-ups.[22,24,32-36]

Overall, it is often difficult both for the patient and physician to differentiate CU from autoinflammatory syndromes. Table 1 highlights the key characteristics of each of the cutaneous manifestations and associated symptoms seen in the different conditions. Important red flags that may hint at an inflammatory syndrome include inflammation of the anterior eye, uveitis, periorbital oedema, serositis, stomatitis, ulcers, meningeal inflammation leading to headaches, abdominal complaints, arthralgia, myalgia, CNS changes, lymphadenopathy, and fever.

ERYTHEMA MULTIFORME

EMu is a rare, immune-mediated mucocutaneous condition that is most often associated with the herpes simplex virus (HSV) Type 1. Other infectious causes are HSV Type 2, as well as mycoplasma pneumoniae, hepatitis C, and vulvovaginal candidiasis.[3,41]

Mycoplasma pneumoniae seems to be a more common eliciting factor of EMu in the paediatric population. It may also be drug-induced by non-steroidal anti-inflammatory drugs, sulfonamides, antiepileptics, and antibiotics.[41]

Unlike CU, EMu presents with targetoid lesions, which appear over 3–5 days and require 1–2 weeks for resolution.[15,41] The cutaneous lesions are fixed, as opposed to evanescent. The primary lesions are erythematous and oedematous round papules, surrounded by areas of blanching. These papules enlarge into the characteristic targetoid lesions composed of three concentric colour zones, seen in EMu.[41] The outermost ring is erythematous. Within it, there is an oedematous ring that surrounds a red, inflammatory zone, enveloping a dusky centre or blister due to epidermal necrosis (Figure 1E). EMu most commonly presents on the acral extremities in a symmetric distribution with a preference for the extensor surfaces.

These features help differentiate EMu from fatal conditions, such as Stevens–Johnson syndrome and the more widespread toxic epidermal necrolysis. These initially appear with macular EMu-like target lesions on the trunk.[41] Therefore, correct and early diagnosis allows for prompt and effective treatment, in addition to halting progression to Stevens–Johnson syndrome and to toxic epidermal necrolysis.

An acute episode of EMu is usually treated with antihistamines and topical corticosteroids to

achieve symptomatic relief. Antiviral therapy for HSV infection, administered after the appearance of EMu, does not shorten the course of disease and is therefore not indicated.[41] However, in patients affected with recurrent EMu associated with HSV, continuous prophylaxis with antiviral therapy for at least 6 months is indicated. Most patients require 1–2 years of prophylaxis therapy. If nonresponsive, the antiviral dose can either be doubled or substituted with a different antiviral agent.[41]

Mucosal involvement is seen in 25–60% of EMu cases.[3,41] The extent of mucosal involvement also helps to guide treatment. Minimal mucosal involvement is treated with a corticosteroid gel. For patients whose ability to eat or drink is impaired, oral corticosteroids are prescribed. An ophthalmology consultation is required for cases presenting with ocular involvement.[41]

CUTANEOUS MASTOCYTOSIS

Mastocytosis is a heterogeneous disorder that is characterised by the accumulation of mast cells within different organs, including the skin, bone marrow, gastrointestinal tract, liver, spleen, and lymphatic tissues. Cutaneous mastocytosis is most easily confused with CU.[8,15,19,41] It can be distinguished from CU by its characteristic deep brown-orange pigmentation (Figure 1F).[15,19,42-44] Often a wheal-and-flare reaction can be elicited by stroking or rubbing the lesion. This is known as a positive Darier sign. It is different from dermatographism in that it affects both clear and lesioned skin.[42-45]

The exact cause of CM is not known; however, c-Kit mutations (mast stem cell growth factor receptor, also referred to as proto-oncogene c-Kit or tyrosine-protein kinase Kit or CD117) have been identified in biopsies of affected skin.[44]

Although CM is a relatively benign condition, it can be associated with systemic mastocytosis, especially in adult patients.[15,45] If active systemic mastocytosis cases are not treated, it can lead to life-threatening anaphylactoid reactions.

Over 65% of CM cases occur in the paediatric population, in whom this condition is usually transient and resolved by puberty;[42,43] however, it can persist and be very disfiguring. In adults who are affected by CM, the treatment depends on disease severity and includes antihistamines, topical corticosteroids, topical calcineurin inhibitors, phototherapy with or without psoralen, and, more recently, certain tyrosine kinase inhibitors (midostaurin).[41-45] Water-soluble cromolyn sodium cream and aqueous-based cromolyn sodium skin lotion have been reported to alleviate pruritus and flares with variable success.

DERMATITIS ARTEFACTA

Although ultimately a diagnosis of exclusion, dermatitis artefacta (DA) should also be considered in the differential diagnosis of urticaria, especially in cases where the past medical history is inconsistent with CU. DA is a factitious disorder, which is also part of a spectrum of psychocutaneous disorders. While urticaria can be elicited by psychosocial stressors as well, DA is self-inflicted and is usually differentiated from other dermatologic conditions via a strong anamnesis and patient–physician rapport. The lesions are non-healing, located in areas that can be easily accessed by the patients' dominant hand, and surrounded by intact skin. Upon presentation, the patient may seem indifferent to the lesions, while the family is concerned. Moreover, the patient may have other psychiatric illnesses, such as eating disorders and personality disorders.[41-43] Patients with DA are at increased risk of harming themselves further and therefore it should not be taken lightly.

CONCLUSION

The conditions discussed in this paper have been historically confused for CU. Although these mimickers are relatively rare, patients' prognoses can be profoundly affected if they are misdiagnosed. Maintaining a high index of suspicion, especially in cases of refractory urticaria, allows for better treatment and, in many cases, remission of symptoms.

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Rocking Pneumonia and the Boogie Woogie Flu

Authors: *Ger Rijkers, Sophie Croon, Thuc Anh Nguyen

Science Department, University College Roosevelt, Middelburg, Netherlands
*Correspondence to g.rijkers@ucr.nl

Disclosure: The authors report no conflicts of interest.

Received: 11.06.2018

Accepted: 07.11.2018

Keywords: Colonisation, conjugate vaccines, influenza virus, pneumococcal pneumonia.

Citation: EMJ. 2019;4[1]:48–54.

Abstract

The relation between pneumonia and influenza is regularly addressed in modern music. Epidemiological data obtained during influenza pandemics, as well as during seasonal influenza, illustrate and underscore this association. Even though the number of pneumonia cases are generally under-reported and blood tests show a lack of sensitivity, a clear link between influenza and pneumonia can still be observed. In fact, the majority of mortality during influenza pandemics is due to pneumonia caused by a bacterial superinfection, in most cases *Streptococcus pneumoniae*. Vaccination is a powerful tool to prevent the development of both influenza and pneumonia in children, as well as in the elderly. Cellular and molecular data show that influenza can lead to changes in the integrity of lung epithelial cells, including desialysation of carbohydrate moieties, which favour attachment and invasion of *S. pneumoniae*. Further elucidation of these mechanisms could lead to targeted intervention strategies, in which universal influenza vaccines could play a role.

INTRODUCTION

The epidemiological association between influenza and pneumonia is well known. In this paper, the authors review the cellular and molecular mechanisms that underlie this association.

The association between influenza and pneumonia is also well known to the general public, as reflected in pop culture. Previously, the authors have analysed the context in which pneumonia (the disease) is dealt with in rock songs.[1] One of the findings was that most songwriters and singers have little idea about the infectious nature of pneumonia.[2] Taking into account that the causes of pneumonia, as well as its symptoms, treatment, and prognosis, are poorly described in songs, it is remarkable that the association between pneumonia and influenza is rather adequately depicted in music.[1] The best example may be from Huey 'Piano' Smith: "Young man rhythm's got a hold of me too, I got the rockin' pneumonia and the boogie woogie flu," from the 1957 record "Rockin' Pneumonia and the Boogie Woogie Flu". Followed later in 1960, The Genies released their song "Twisting Pneumonia": "I've got the twistin' pneumonia, the shimmy shimmy flu, the doctor is gonna tell you that there ain't no cure." Because the relationship between fantasy forms of pneumonia (rockin', twistin') and fantasy forms of influenza (boogie woogie, shimmy shimmy) is so evidently described in music, the epidemiological and molecular mechanisms underlying this relation warrant closer analysis. Hopefully this will lead to a moment in time when 'the doctor is gonna tell you that there *is* a cure'.

THE EPIDEMIOLOGICAL ASSOCIATION BETWEEN INFLUENZA AND PNEUMONI

In the 20th century, the world experienced three major influenza pandemics. Namely, the Spanish influenza in 1918 (H1N1), Asian influenza in 1957 (H2N2), and Hong Kong influenza in 1968 (H3N2). The 1918 pandemic had an especially large impact, with conservative estimates of 40–50 million deaths following H1N1 infection.[3] Many studies have shown that bacterial superinfection causing pneumonia following an influenza infection leads to severe disease and mortality. It has been estimated that >95% of deaths during the 1918 pandemic were complicated by bacterial pneumonia, most commonly by *Streptococcus pneumoniae*.[4] However, other common respiratory pathogens, including *Staphylococcus aureus, Haemophilus influenzae,* and *Streptococcus pyogenes* were all identified as predominant pathogens in various individual studies.[5] It has been predicted that due to the ever-increasing world population and global mobility, both work related and recreational, a future influenza outbreak with a strain with similar virulence to the 1918 Spanish influenza strain may cause even higher mortality rates, with an estimate of between 50 and 80 million deaths.[6] Such an outbreak would primarily affect developing countries, because of the limited preparedness and availability of healthcare facilities. Additionally, an epidemiological risk factor is the higher carriage rates of *S. pneumoniae* among children, as well as adults, in combination with limited availability of pneumococcal conjugate vaccine and antibiotics.[7] Generally, people living in sub-Saharan countries experience the highest rates of influenza-associated respiratory deaths, whereas eastern Mediterranean and southeast Asian countries have lower, but still high, death rates.[8,9] These findings underline the importance of understanding the synergy and copathogenesis between influenza and subsequent infection by bacterial pathogens, leading to bacterial pneumonia.

An influenza outbreak does not have to be a pandemic to cause substantial mortality. Annual seasonal influenza episodes are associated with significant morbidity. The Centre for Disease Control and Prevention (CDC) estimates that there are between 3 and 5 million annual cases of severe illness due to influenza, and between 291,000 and 646,000 deaths that are a direct consequence of seasonal influenza-related respiratory illnesses.[9] Children and the elderly are especially susceptible, particularly those who are <4 years and >65 years of age. Children <1 year of age account for >50% of all influenza-associated deaths in young children.[10] Influenza numbers reach their peak during the winter months. The incidence is highest during this time of year because the aerosol spread of the influenza virus depends on both the humidity and temperature of the air. Both cold and dry conditions favour transmission; thus,[11] the environmental conditions favouring influenza transmission are relatively well known.[12-14] For *S. pneumoniae,* the transmission mechanisms are known but not the environmental conditions.

Seasonal outbreaks of influenza, rather than pandemics, also are associated with a higher incidence of pneumonia. In the Netherlands, a clear rise in pneumonia can be observed during the peak of the influenza season (Figure 1).[15] However, influenza-related pneumonia is most likely highly under-reported, because in cases of community-acquired pneumonia there is often no culture made of the disease-inducing bacterium. Furthermore, blood cultures in patients with pneumonia lack sensitivity, providing accurate results in only 10–15% of cases. However, even when including sputum cultures, urinary tests, and serotype specific antibodies responses, the aetiological agent responsible for community acquired pneumonia often cannot be established.[16-18]

Pneumonia poses a significant risk for morbidity and mortality in young children as well as the elderly. In 2008, approximately 156 million children worldwide were affected by pneumonia, with an approximate 1.3 million deaths, accounting for 18% of all deaths of children <5 years old.[19,20] Furthermore, in people >65 years of age, 40% of pneumonia cases are of a severity that requires hospital admission.[21]

Vaccination is a primary strategy to reduce the burden imposed by influenza. Currently, the CDC recommends the annual influenza vaccine for everyone ≥6 months of age.[9] Live-attenuated influenza vaccines were shown to be most effective in those between 6 months and 7 years of age.[22]

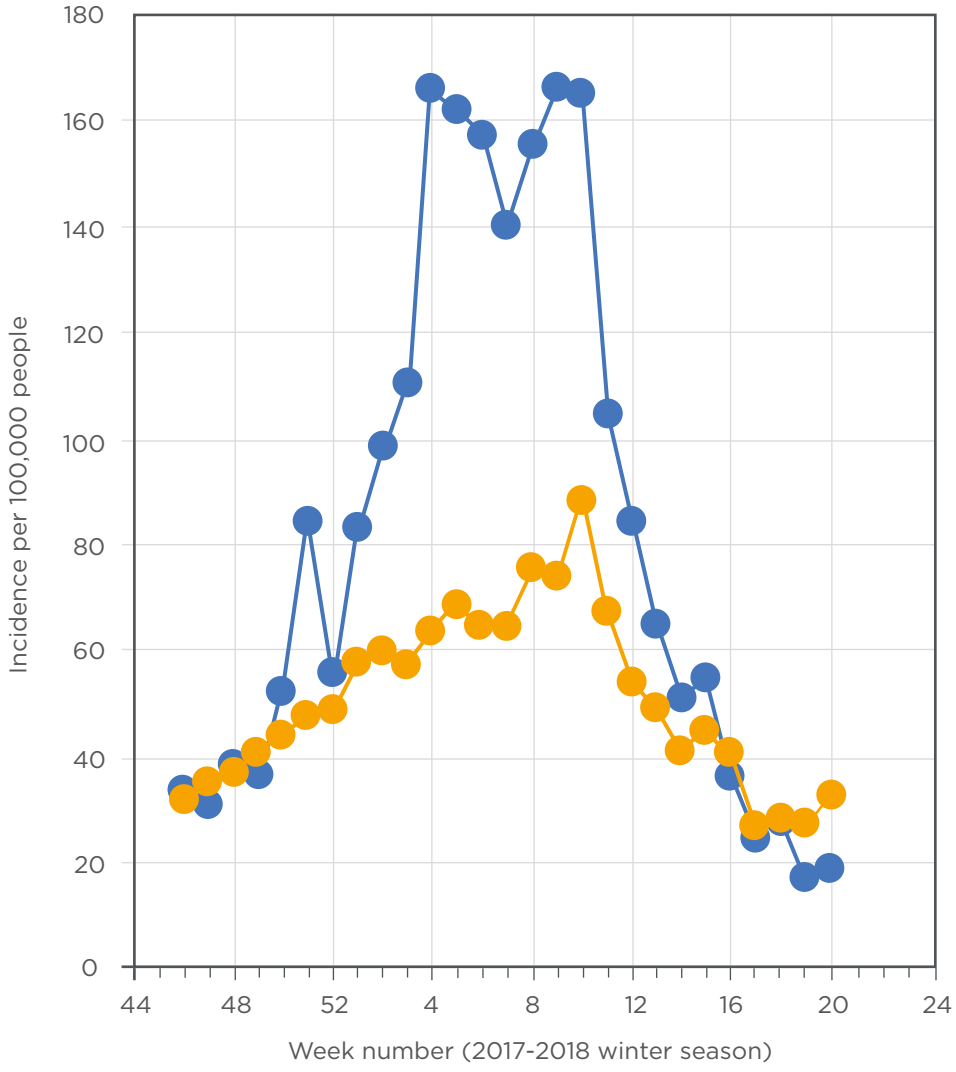

Figure 1: Seasonal trends in incidence of influenza-like illness (blue line) and pneumonia (orange line) as reported by general practitioners in the Netherlands during the 2017-18 winter season.

Data derived from the public available weekly surveillance reports published by NIVEL.[15]

The influenza vaccine's efficacy is 56% (95% confidence interval [CI]: 39–68%) for preventing respiratory illness, 53% (CI: 35–66%) for preventing pneumonia, 50% (CI: 28–65%) for preventing hospitalisation, and 68% (CI: 56–76%) for preventing death.[23] Seasonal influenza vaccination thus shows efficacy in reducing pneumonia morbidity and mortality.

Following the introduction of pneumococcal conjugate vaccination of children in the USA and elsewhere, the incidence of clinically diagnosed all-cause pneumonia declined by 39% (95% CI: 22–52%).[24] Additionally, pneumococcal conjugate vaccines have played an important role in reducing influenza-associated morbidity, preventing 31% (95% CI: 15–43%) of pneumonia cases associated with any of seven respiratory viruses in hospitalised children.[25] It was also shown that the children that received the vaccine and subsequently developed laboratory-confirmed influenza were at a 45% lower risk (95% CI: 14–64%) of hospitalisation due to the influenza-associated pneumonia.[26] It would be highly unlikely that vaccination with pneumococcal conjugate vaccines (composed of pneumococcal polysaccharides conjugated to bacterial carrier proteins) would have a direct protective effect against infection with respiratory viruses. Therefore, the interpretation of these data would be that recipients infected with respiratory viruses had a lower subsequent

complication rate of pneumococcal infection, due to serotype-specific protection. These children, protected against superimposed pneumococcal pneumonia developing due to the viral (influenza) infection, were less likely to progress to pneumonia requiring hospitalisation.[27]

In elderly people, influenza vaccination lowers the risk of laboratory-confirmed influenza.[28] Additional research in immunocompetent individuals aged ≥65 years examined the effects of influenza and pneumococcal vaccination in the prevention of hospital admissions. The influenza vaccine has an efficacy of 30% (95% CI: 17–42%) for high-risk and a 40% (95% CI: 1–64%) efficacy for median-risk elderly persons in preventing influenza and pneumonia hospitalisations and that the pneumococcal vaccine has a 75% (95% CI: 57–85%) efficacy in preventing invasive pneumococcal disease in people >65 years of age.[29,30] These studies suggest that the vaccination of both infants and the elderly may greatly reduce influenza-related morbidity and, combined with the data on herd immunity, suggest that immunisation of children may additionally protect adults as well.[26] This has been demonstrated both for influenza[31] as well as pneumococcal vaccination;[32] in both instances, childhood vaccination had an indirect protective effect on the elderly.

CELLULAR AND MOLECULAR MECHANISMS UNDERLYING THE INTERACTION BETWEEN INFLUENZA AND PNEUMONIA

As indicated and discussed above, during and after the influenza pandemic in 1918, the association of a respiratory virus predisposing to a bacterial infection was already recognised.[33] Multiple mechanisms could explain this association between influenza and pneumococcal infections, including impairment of host immune responsiveness, exacerbation of inflammatory mediators, and biofilm dispersion. The influenza A virus impairs both the host's innate and adaptive immune systems by downregulating IFN-γ production and by other means.[34] Infection with influenza H1N1 leads to a decrease in CD4+ T lymphocytes and B lymphocytes.[35] Furthermore, viral infections of the airways can induce a 'cytokine storm' and the production of various other mediators, including histamine and leukotriene E4, that damage local lung tissue indirectly;[36-38] thus, influenza infected patients would become more susceptible to diseases such as pneumonia. In the nasopharynx of patients already colonised, *pneumococci* have formed biofilms. Influenza virus infection can trigger biofilm dispersion and active release of *pneumococci*.[39] It has been observed, both *in vitro* and *in vivo*, that during influenza infection, pneumococcal colonisation of respiratory epithelial surfaces can increase. *In vitro* data indicate that desialylation of airway epithelial cells, which occurs during influenza virus infection, can enhance pneumococcal adhesion via galectin binding.[40] Influenza-virus-infected mice failed to kill *S. pneumoniae in vivo*.[41] Additionally, similar relationships have been demonstrated in other viral respiratory viruses and bacteria that can cause pneumonia in humans. One of the first studies in that respect was conducted by Sanford et al.[42] by means of an adherence assay. In this experiment, group B *Streptococcus* and several other streptococcal species were found colonising only on the cells infected with influenza virus. A more recent study showed that cells infected with swine influenza virus have enhanced bacterial adherence and invasion of deeper tissues of *Streptococcus suis,*[43] a bacterium that can also cause pneumonia in humans.[44] Evidence has shown that viruses might promote bacterial infection by facilitating the colonisation, adherence, and translocation of bacteria through the epithelium. There are a number of mechanisms determining these processes, which involve changes and damage to host epithelial cells, several specific viral virulence determinants, and extracellular molecules, which are detailed below.

Viruses Damage Host Respiratory Epithelial Cells Facilitating Bacterial Adhesion

Infection with influenza virus results in multiple changes in the lung epithelial cells. Upon infection, inflammatory reactions are initiated, aiming to neutralise the virus but at the same time inevitably resulting in damage to the epithelial cells that mediate respiratory gas exchange. As the respiratory mucosa (the physical and chemical barrier to infection by producing

mucus, surfactants, and other inflammatory mediators) is damaged, opportunistic bacteria, such as *S. pneumoniae,* can more easily invade and colonise the lung tissue.[45] The deposition of dead cells and cell fragments into the airways also provides a rich source of nutrients for bacterial growth.[46] Furthermore, cilia are damaged and ciliary motion becomes unco-ordinated, causing impairment of their cleaning function.[47] Both the respiratory epithelial damage and impairment in protective mechanism caused by (influenza) viruses facilitates the adherence of bacteria to host cells. Evidence for this mechanism was reported in pathologic examination of patients who died of community acquired *S. aureus* pneumonia in the 1957 influenza pandemic. Autopsies showed that *Staphylococci* were adhered in regions of the tracheobronchial tree where the epithelial layer had been destroyed and sloughed off.[48] However, most seasonal influenza strains do not result in severe lung damage but can still facilitate bacterial superinfection.[49]

VIRULENCE DETERMINANTS OF INFLUENZA INCREASE RECEPTOR AVAILABILITY FOR BACTERIA

While viruses expose sites for attachment as previously outlined, bacteria also possess a wide range of virulence factors, such as pneumococcal surface protein A, choline-binding protein A, and pneumococcal serine-rich repeat protein.[50] These factors can be used for adherence to the basement membrane or extracellular matrix constituents of the host, such as fibrin, fibrinogen, and collagen.[51] *S. pneumoniae* illustrates this point clearly, as it was found to bind strongly to fibronectin. Viral infection also upregulates expression of fibronectin. Bacterial binding was thus enhanced proportionally to the increase of fibronectin and duration of exposure.[52] Moreover, influenza viruses can increase viral fitness and cytotoxic potential by antigenic variation and mutations, thus enhancing epithelial damage and subsequently facilitating bacterial infection.[53,54]

In addition to changing their antigenic surface, influenza infection can increase receptor availability for bacterial adherence and infection through three other mechanisms. First, influenza viruses insert their glycoproteins, neuraminidase and haemagglutinin, into the host membrane, which subsequently acts as a potential receptor for bacteria.[55] Furthermore, viral neuraminidase cleaves sialic acid residues, including the sialic that cover bacterial receptors on cell surface carbohydrates, thus promoting bacterial adherence and invasion.[56] Indeed, it was found that treatment with neuraminidase inhibitor (oseltamivir) was associated with a reduction in mortality of at-risk patients diagnosed with the influenza A H1N1pdm09 virus infection.[57] In fact, this type of treatment was also widely used during the 2009–2010 influenza A H1N1 pandemic. However, neuraminidase and haemagglutinin inhibitors only inhibit streptococcal adherence to influenza-infected cells, not of *Staphylococci*, suggesting the presence of other cellular receptors for bacteria.

A second mechanism shows how viral infections can contribute to bacterial superinfection. The virus triggers a host inflammatory response, altering the cell membrane, and thus contributes to bacterial adherence. Influenza virus infection upregulated platelet activating factor receptor expression in lung epithelia, which promotes adhesion of *S. pneumoniae.*[58] Other respiratory viruses, such as respiratory syncytial virus, upregulate receptors that bacteria can bind to, such as CD14, CD15, and CD18, and receptors mediated by outer membrane protein P5 homologous fimbriae.[59,60] It is interesting to note that cigarette smoke and e-cigarette vapour both increase the expression of platelet activating factor receptor.[61,62]

A third and final mechanism, more general in nature, is that areas of incomplete wound healing (induced by influenza or otherwise) might be avid binding sites for bacteria. Fibrinogen expression is increased in incomplete wound healing.[62] The surface adhesin PfbA of *S. pneumoniae* binds to fibrinogen.[64] Furthermore, bacteria, including *S. pneumoniae,* can also adhere to extracellular matrix components involved in tissue repair, including laminin and type I and type IV collagen.[5]

CONCLUSION

The authors have elaborated on the special interaction between (a rocking, or whatever cause) pneumonia and the (boogie woogie) flu. There are arguments to propose that other respiratory viruses can pave the airways for bacterial pneumonia.[25,27] As indicated above,

in a substantial number of patients with community-acquired pneumonia, a causative agent has not been found, so these could also be pneumococcal pneumonia.[17,18] In cases where influenza virus, or any other respiratory virus for that matter, is found, it can be debated whether this infection would be sufficient argument for the classification of viral pneumonia.

The interaction between influenza, other respiratory viruses, and *S. pneumoniae* is complex and based on multiple mechanisms. Full understanding of these mechanisms, as well as their mutual interplay, is relevant for a better estimation of the burden of pneumonia in young children. Nasopharyngeal carriage with *pneumococci* is high during the first 3–5 years of age;[65,66] mucosal pneumococcal infections, such as otitis media, are prevalent during this period. Fortunately, most children do not develop a pneumococcal pneumonia. The triggering event for this development could be influenza infection. From such a perspective, it could be speculated that universal influenza vaccines could offer the broadest possible coverage against pneumococcal pneumonia in young children.[67,68] The moment these vaccines become available, clinical studies should include their effect on pneumococcal pneumonia.

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Persistent Cough: Changes in Prevalence, Impact, and Beliefs From 2006–2015 in Italy

Authors: *Roberto Walter Dal Negro,[1] Massimiliano Povero,[2] Alessandro Zanasi,[3] Paola Turco[4]

1. National Centre for Respiratory Pharmacoeconomics and Pharmacoepidemiology, Verona, Italy
2. Research and Clinical Governance, Verona, Italy
3. Italian Association for Cough Study, AIST, Bologna, Italy
4. AdRes Health Economics and Outcome Research, Torino, Italy

*Correspondence to robertodalnegro@gmail.com

Disclosure: The authors have declared no conflicts of interest.

Received: 04.06.18

Accepted: 10.09.18

Keywords: Computer-assisted telephone interview (CATI), national survey, people's beliefs, persistent cough, public opinion, questionnaire.

Citation: EMJ. 2019;4[1]:55-62.

Abstract

Background: Persistent cough is one of the most common conditions affecting quality of life. The aim of this study was to assess people's beliefs regarding the impact of, and changes in the prevalence of, persistent cough in the Italian general population over a 10-year time period.

Methods: Two telephone surveys were conducted in 2006 and 2015, and the answers were compared. In 2015, the same questionnaire was also randomly distributed in paper form to another cohort. Sample sizes were precalculated for their representativeness and comparability (Chi-square test).

Results: In total, 1,251 subjects in 2015 and 1,334 in 2006 completed the interviews. The corresponding completion rate for the interviews was 23.0% and 21.8%, respectively; 5,056 individuals completed the paper-form questionnaire. A substantial proportion of respondents stated that persistent cough should be regarded as a disease and not merely as a symptom. This belief increased from 38.8% to 46.4% ($p<0.03$) over the study decade. The prevalence of persistent cough recorded through computer-assisted telephone interview was 14.2% and 18.4% in the 2006 and 2015 surveys, respectively ($p<0.02$), and 35.5% in individuals answering the paper-form questionnaire ($p<0.01$). General practitioners (69.6%) and lung physicians (16.2%) were among the most frequently consulted medical professionals for cough. The majority of respondents disagreed with first-line antibiotic and/or systemic steroid use, while antitussive drugs and mucolytics were highly valued. The willingness of the patient to pay for their own treatment increased, with >40% of responders willing to pay >€10 at the pharmacy in 2015.

Conclusion: These surveys showed the prevalence of persistent cough is increasing, together with the willingness of the patient to pay out of their own pocket for treatment. However, the methodology for data collection should be carefully considered before data are generalised.

INTRODUCTION

Persistent cough is one of the most frequent respiratory symptoms causing patients to seek a medical consultation worldwide; thus, the condition represents a true challenge in daily practice.[1] Acute cough (cough lasting for <3 weeks) is often due to the common cold or upper respiratory tract infections and, in the vast majority of cases, results in life-limiting effects in the absence of significant comorbidities. Chronic cough (cough lasting >8 weeks) has several different causes.[2-4]

Persistent cough, which is a cough lasting 3–8 weeks or longer,[5] represents a grey area that is difficult to define because it can have an aetiology similar to that of acute or chronic cough.[2] The most frequent causes of persistent cough include bronchial asthma, tobacco smoking, inhalation of pollutants and environmental irritants, gastro-oesophageal reflux disease, post-nasal drip, and the use of some antihypertensive drugs.[2-4] Other possible causes include subacute infections, airway cancer, mediastinal occupation of different aetiology, chronic bronchitis, chronic obstructive pulmonary disease, bronchiectasis, foreign bodies, cystic fibrosis, lung fibrosis, extrathoracic causes, obstructive sleep apnoea, laryngeal dysfunction, and psychological disorders.[4-17]

While the impact of persistent cough on a patient's life ranges from minimal discomfort to disabling symptoms, physical impairment is a frequent complication of the condition, together with depression, school or work absenteeism, and a substantial economic burden.[18-20] The prevalence of persistent cough has been estimated in different international studies and ranges from 11–18%, with higher values reported in Europe than in the USA.[21-24] The impact of persistent cough was specifically assessed in episodic studies,[18,21,24] but, to the authors' knowledge, the prevalence of persistent cough has not been investigated in Italy to date.

AIM

The aim of this study was to assess and compare the prevalence and the impact of persistent cough among Italian adults from the general population over a 10-year time period and to monitor the beliefs of the patients regarding the condition. The parallel use of a paper-form questionnaire and a telephone survey was to compare the characteristics of responses from respondents reached by the two different tools of investigation.

METHODS

A cross-sectional telephone survey was conducted between 21st and 29th July 2015 in individuals aged ≥18 years. The results of this 2015 survey were compared to the results from an identical survey carried out between July 17th and 25th July 2006.

The investigational tool for collecting data was the same validated, anonymous questionnaire consisting of 20 predetermined questions on cough: 16 of the 20 questions were closed (80.0%), while Questions 5, 6, 16, and 20 were open questions (Box 1). Possible answers for closed Questions were 'Yes', 'No', and 'Doubtful'. For Questions 5 and 6, respondents had to indicate their own opinion, while Questions 16 and 20 asked the patients for their age (in years) and the region where they lived, respectively. According to consolidated validation procedures, the original version of the questionnaire was previously given to a sample of 25 randomly selected individuals with varying educational levels (the usual sample size for a pilot test) to check the comprehension of the questions included. When linguistic errors and/or misunderstandings occurred, the corresponding items were reworded until full comprehension was obtained.[25]

In both editions of the survey, all interviews were performed according to the computer-assisted telephone interview (CATI) methodology[26] by the same agency of professional interviewers. The distributions of all answers were calculated in the overall samples. The interviewers were provided with one work station consisting of a personal computer connected to a central processing unit. The central unit was also equipped with specific software for the random selection of individuals to contact. Compared to a conventional telephone interview, the CATI technique allowed the randomisation of question order. Moreover, this system worked as a supervisor of the interviewer's activity: if the interviewer forgot some questions or even an entire section of the questionnaire, the software would alert them, thus avoiding errors due to missed questions.

Box 1: The questions asked of each of the participants in the 2006 and 2015 computer-assisted telephone interview and the 2015 paper-based questionnaire.

Please note that the questionnaire was administered in Italian and this is an English translation.

Introduction

Hi, how are you? We are carrying out a national survey and your opinion is quite important. May I ask you some simple questions concerning your beliefs on cough? It will take a maximum of 5 minutes of your time. The interview will remain anonymous and data collection is conducted according to the present law on privacy. If you agree, we can start (If 'Yes', go on; if 'No', then thank you, and have a good day).

Questions

1. In your opinion, is cough a disease? ☐ Yes ☐ No ☐ Doubtful
2. What do you do after 2–3 days of cough?
   ☐ Wait ☐ Domestic remedies ☐ Ask the pharmacist ☐ Ask the doctor
3. After when do you start to be worried about your cough? ☐ 7 days ☐ 15 days ☐ 30 days
4. Is cough merely a symptom of any disease? ☐ Yes ☐ No ☐ Doubtful
5. After 4 weeks of cough, what do you think about its cause? .....
   .....
6. Which specialist do you presume will be the best to refer to?.....
   .....
7. Can persistent cough only be effectively treated with antibiotics? ☐ Yes ☐ No ☐ Doubtful
8. Are present antitussive drugs effective? ☐ Yes ☐ No ☐ Doubtful
9. Are systemic steroids needed in persistent cough? ☐ Yes ☐ No ☐ Doubtful
10. Are domestic aerosols the right option against cough? ☐ Yes ☐ No ☐ Doubtful
11. How much are you worried if cough affects a child?
    ☐ Not at all ☐ As in adults ☐ More than in adults ☐ Much more than in adults
12. How much are you willing to pay in your pharmacy for an effective antitussive drug?
    ☐ <€10 ☐ €10–20 ☐ >€20
13. How many episodes of cough do you have over 12 months? ☐ Never ☐ 1-2 ☐ 3-5 ☐ >5
    (If Never, skip to question 16)
14. What is the overall duration of these episodes? ☐ Never ☐ <10 days
    ☐ 10–30 days ☐ >4 weeks
15. In general, does your cough produce any sputum? ☐ Yes ☐ No ☐ Doubtful
16. Age: _____ (years)
17. Sex: ☐ Male ☐ Female
18. Smoking status: ☐ Active ☐ Never ☐ Ex-smoker
19. Job:
    ☐ Worker ☐ Employee ☐ Manager ☐ Professional/entrepreneur
    ☐ Student ☐ Retired ☐ Unemployed ☐ Housewife
20. Region where you live: .....

As previously mentioned, the sampling strategy adopted in the present survey was the computerised random selection of an adequate number of subjects from the national pool of home and mobile telephone numbers. A minimum number of 1,226 respondents in 2006 and 1,178 respondents in 2015 were required to achieve an accurate representation of the Italian population in terms of age, sex, education, smoking habit, and geographical distribution (by 3% maximum error and 95% probability).

A short explanation, with a mean duration of 5 minutes, concerning the aim of the survey preceded all interviews. Interviews were only conducted after having recorded the respondent's informed consent to the interview itself and to the possible use of their information for scientific purposes.

In 2015, the same questionnaire was randomly distributed in a self-managed, anonymous paper form to individuals from the general population. A minimum of 5,010 respondents were required to achieve representativeness of the general population (by 3% maximum error and 95% probability). maximise the national distribution and completion rate, the National Centre for Respiratory Pharmacoeconomics and Pharmacoepidemiology (CESFAR) and the Italian Cough Association (AIST) involved their affiliate members actively in the distribution and collection of the questionnaires across Italy. The organisations also shipped the questionnaires to the operational centre for calculations.

In the present paper, only data concerning persistent cough were considered, reported as frequencies. Statistical comparisons were calculated by a Chi-squared test, and $p<0.05$ was assumed the minimum level of statistical significance. Each survey was approved by the CESFAR Ethical Committee, Verona, Italy, in 2005 and 2016, and all subjects were required to provide their informed consent to the interview before participating.

RESULTS

A total of 1,251 individuals (mean age: 49.0 years; 44.2% females) completed the interview in 2015, while 1,334 individuals (mean age: 47.8 years; 43.7% females) took part in 2006. The overall telephone contacts were 5,362 and 6,109 in 2015 and 2006, respectively. The corresponding interview completion rate (e.g., the proportion of calls properly completed and providing reliable data for the investigation) was 1 in every 4.3 calls (23.3%) in 2015, and 1 in every 4.6 calls (21.8%) in 2006. A total of 5,056 paper-form questionnaires were also collected. The data that characterise the respondents from the two telephone surveys and the paper survey are reported in Table 1. The data proved comparable over the 10-year period and equally representative of the general population.

The questions included in the questionnaire were originally divided according to five main sections: basic opinion on cough, the general impact of cough on life, approach to cough, therapeutic expectations, and willingness to pay for treatment.

Respondents' Basic Beliefs

The majority of respondents in both surveys had the opinion that cough should be considered a symptom of several diseases, but 46.4% of respondents in 2015 and 38.8% in 2006 claimed that persistent cough should be regarded as a disease. This proportion increased significantly over the 10 year time period ($p<0.03$). Moreover, the number of survey non-responders dropped by >50% over the same period ($p<0.01$). Data from the 2015 paper-form questionnaire showed different results to the 2015 CATI, such as a lower belief of cough as a disease (36.6%, similar to that seen in the 2006 CATI survey) ($p<0.02$) and a much higher proportion of non-responders ($p<0.01$) (Figure 1A).

General Clinical Impact

The prevalence of persistent cough was 18.4% in 2015 and 14.2% in 2006 ($p<0.02$), and increased to 35.5% among respondents to the 2015 paper-form questionnaire ($p<0.01$) (Figure 1B). Productive cough was consistently slightly more frequent than persistent cough: 55.9% in the 2015 CATI; 58.8% in the 2006 CATI; and 57.1% when data were collected via the 2015 questionnaire paper-form. The majority of respondents (71.7%) started to worry about their own cough 1 week after cough initiation, while a greater number of respondents (76.9%) stated that they were much more concerned when persistent cough affected a child.

Table 1: General characteristics of the 2006 and 2015 cohorts investigated via computer-assisted telephone interview, and of the 2015 cohort investigated via paper-form questionnaire.

	CATI 2006	CATI 2015	p value	Paper-form questionnaire 2015	p value versus CATI 2015
Number of participants	1,334	1,251	-	5,056	-
Age (years, mean±standard deviation)	47.8±14.8	49.0±16.1	0.88	49.4±15.4	0.84
Sex (% female)	43.7	44.2	0.83	44.9	0.75
Geographical distribution within Italy (%)	-	-	0.61	-	0.58
› northern	43.1	42.6	-	46.1	-
› central	15.6	14.5	-	13.8	-
› southern	41.3	42.9	-	40.1	-
Tobacco smokers (%)	22.8	21.4	0.43	20.6	0.33
Job distribution (%)	-	-	0.76	-	0.81
› white collar	35.4	36.0	-	35.7	-
› retired	24.1	24.8	-	25.1	-
› homemaker	14.3	14.6	-	14.0	-
› blue collar	15.3	14.5	-	15.3	-
› students	10.1	9.7	-	9.9	-

Dashed boxes indicate where data are not applicable.
CATI: computer-assisted telephone interview.

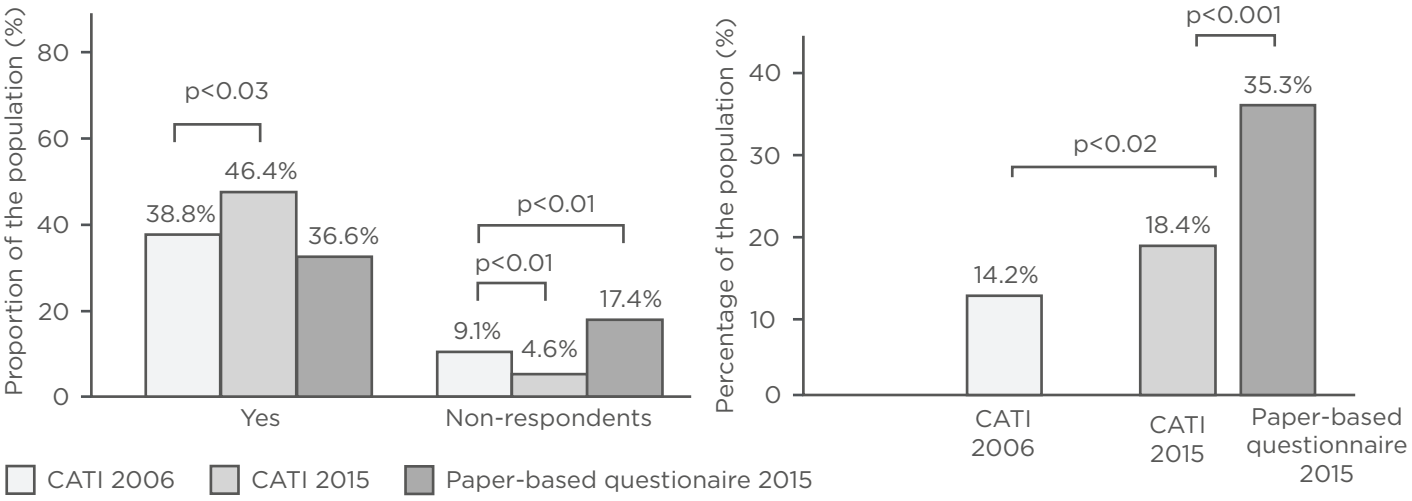

Figure 1: A) The proportion of respondents perceiving persistent cough as a disease. B) Prevalence of persistent cough (lasting >4 weeks) identified by the three surveys.

CATI: computer-assisted telephone interview.

Respondents' Approach to Cough

General practitioners (69.6%), lung physicians (16.2%), and otorhinolaryngologists (10.6%) were consulted most often following the initiation of persistent cough. Moreover, chronic airway disease (48.2%), pneumonia (25.7%), persistent allergic troubles (7.8%), and cancer (2.3%) were the four most feared causes of persistent cough.

Respondents' Therapeutic Expectations

The majority of respondents stated that antibiotics are not the only effective treatment for persistent cough and that steroids were not needed in the treatment of persistent cough (56.4% and 51.6% in 2006, and 61.2% and 58.2% in 2015, respectively), while antitussive drugs were generally perceived as an effective option. Data from the 2015 paper-form questionnaire further confirmed these beliefs (62.8% concerning antibiotic use, 60.1% concerning steroid use, and 68.6% concerning the perceived therapeutic value of antitussive drugs).

Willingness to Pay

The willingness of a patient to pay out of their own pocket for their treatment increased significantly over the 10-year survey. While 63.4% of respondents agreed to pay up to €10, 26.8% up to €20, and 1.0% >€20 for an effective antitussive remedy in 2006 (8.8% non-responders), the corresponding willingness to pay in the 2015 CATI was 46.3% up to €10, 27.8% up to €20, and 13.3% >€20 (12.6% non-responders). The willingness to pay out of their own pocket proved even higher when calculated via the 2015 paper-form questionnaire: 20.7% up to €10; 51.5% up to €20, and 27.8% >€20. The role of inflation was considered. Under the assumption of gamma distribution, the mean willingness of patients to pay out of their own pocket was €8.51 in 2006 and €14.01 in 2015 (also including the results of the paper-form questionnaire). As the willingness to pay in 2006 cannot be compared directly with that of 2015 due to inflation, it was inflated to 2015 applying a discount rate of 1.235 (Harmonised Indices of Consumer Prices).[27] Taking this correction for inflation into account, the willingness to pay out of pocket was still considerably higher in 2015 than in 2006 (€14.01 and €10.51, respectively).

DISCUSSION

Persistent cough is one of the most frequent events reported in respiratory medicine; nevertheless, its impact remains difficult to define in real life. The beliefs of the general public do not always fit the aetiology or the severity of cough, and subjects tend to be equally worried about their cough even if it is not disabling, particularly when long-lasting. From this point of view, the widespread lack of specific knowledge[19] is a contributing factor to these beliefs. On the other hand, only 50% of patients with persistent cough receive a diagnosis and care.[18,26]

Despite the consolidated belief that cough represents a non-specific symptom shared by several diseases, >38% of respondents in 2006 and >46% of respondents in 2015 believed that persistent cough may represent a disease. This opinion increased significantly over the 10-year period, in parallel with a substantial drop in non-respondents or doubtful subjects. This unexpected result is particularly interesting because it corresponds to the recent scientific view on cough, which regards persistent cough as the clinical effect of a cough hypersensitivity syndrome, such as a condition due to the exalted status of airway sensory nerves occurring independently of the aetiology of cough.[28,29] It is highly presumable that the vast majority of the public were unaware of this scientific view of cough by experts.

Persistent cough showed a significantly elevated prevalence in the general population over the 10-year interval between the two phone surveys (14.2% in 2006 versus 18.4% in 2015; $p<0.02$), by an increase of 29.6%. Although these data may be biased by the method for collecting data and may overstate the true prevalence of persistent cough, they are in general agreement with other European and extra-European studies on the global epidemiology of chronic cough in adults.[21-24,26,30-32]

Despite the good concordance of the CATI surveys, the proportion of respondents to the 2015 paper-form questionnaire who agreed with the hypothesis of cough being a disease was much lower, while the prevalence of persistent cough (35.3%) was much higher compared to the CATI interview results (18.4%). These large differences are likely due to the different methods for collecting data. In other words, even if comparable for age, sex, and national distribution, it is probable that the CATI responders operated in a different setting (with a more limited time for their responses) than those individuals who completed the paper-form questionnaire, meaning that their psychological approach to the questions was different.

The results of the study emphasise that the prevalence of persistent cough increased significantly over the 10-year study period in Italy, independently of the method adopted for the survey. Environmental conditions have contributed progressively to cough development over the same period in Western countries,[32] including Italy,[33] due to uncontrolled urbanisation and exposure to industrial, occupational, and traffic irritants. These factors support and explain the increasing prevalence of persistent cough observed during the last decade in Italy.

The effective treatment of persistent cough still represents a critical issue in many cases. With this in mind, a further interesting result was revealed from analysis of the beliefs of the respondents regarding the therapeutic approach to persistent cough. The majority of respondents disagreed with the first-line use of antibiotics and/or systemic steroids for managing persistent cough. Consequently, the general population, even if generally unaware, seem to share the same opinion of the most updated scientific community.[28,29] Nevertheless, despite this widespread belief, the prescription of antibiotics and/or systemic steroids still remains diffuse and consolidated in Italy, even if not recommended by national and international evidence.[3,33-35] However, it is important to note that around 40% of respondents were convinced of the primary role of antibiotics and steroids in the treatment of cough.

The attitude in favour of symptomatic drugs proved high within the Italian general population. Antitussives and mucolytics were confirmed as the most preferred and used drugs among the over-the-counter medications, either for self-medication or for regular prescription after a medical consult. If easy accessibility contributes to the widespread use of over-the-counter products, other factors can influence the high prescribing rates of antibiotics and steroids: doctors' uncertainty on the causes of persistent cough, which are infrequently investigated in clinical practice; patients' expectations (increasingly supported by the widespread attitude to the self-research for medical information via social media);[36,37] the shifting doctor–patient relationship;[38] and the ever-increasing role of defensive medicine.

According to the literature,[3,4] a significant component of the cost of persistent cough is related to work or school absenteeism; therefore, the authors also assume this to be the case in the present study. The general dissatisfaction for the present therapeutic approach to treatment of persistent cough is clearly mirrored by the progressively higher and ever-increasing willingness of patients to pay out of their own pocket to obtain effective antitussive remedies in the pharmacy. Over the 10-year time period, the general population's attitude regarding spending on medication changed dramatically, independently of the method for data collecting (the phone survey rather than the paper-form questionnaire), and clearly shifted in favour of a higher level of personal, direct expenditure (2–3-fold higher) compared to the last decade.

The present study has limitations. Firstly, it was not designed to define the aetiology of persistent cough, but instead focus on the overall crude prevalence of persistent cough in the general population. Secondly, individuals were questioned with the same questionnaire but using two different methods, via phone contacts (data of 2015 compared to those of 2006) and via a self-administered paper-form questionnaire. This study trait should not solely be considered as a weakness because this parallel procedure proved that results may be dependent of and affected by the method adopted for investigation; how carefully should the results obtained be considered and how can their generalisation be at risk. Finally, the precise expenditure of patients for the treatment of persistent cough was not calculated, despite data on the willingness of patients to spend out-of-pocket emphasising that several patient needs are still unmet. Further studies are needed for investigating this particular issue more deeply.

CONCLUSION

A large proportion of individuals regarded persistent cough as a disease and not as a symptom common to several diseases. This proportion has increased significantly over the last decade in Italy. The prevalence of persistent cough increased significantly from 2006–2015, independently of the different method of investigation. Usually, subjects' concerns tend to increase when their cough lasts for >1 week,

while persistent cough in children is much more feared than in adults. The majority of Italians oppose the first-line use of both antibiotics and steroids for the treatment of persistent cough, while symptomatic antitussive drugs are highly valued within the general population. Finally, the willingness to pay for an effective antitussive remedy has increased over the last decade. Data collected via different methods (CATI versus the paper-form questionnaire) can affect the homogeneity of results, likely due to the uneven targeting of individuals participating in the surveys. This difference in data gathering methodology should, therefore, be carefully considered before data are generalised.

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Obesity: The Impact on Host Systems Affecting Mobility and Navigation through the Environment

Authors: *David A. Hart,[1,2,3,4] Walter Herzog,[1,3] Raylene A. Reimer,[1,5] Jaqueline L. Rios,[1] Kelsey Collins[1,6]

1. Faculty of Kinesiology and the Human Performance Laboratory, University of Calgary, Calgary, Canada
2. Department of Surgery, University of Calgary, Calgary, Canada
3. McCaig Institute for Bone & Joint Research, University of Calgary, Calgary, Canada
4. Alberta Health Services, Bone & Joint Health Strategic Clinical Network, Calgary, Canada
5. Department of Biochemistry & Molecular Biology, University of Calgary, Calgary, Canada
6. Department of Orthopaedic Surgery, Washington University at St. Louis, St. Louis, Missouri, USA

*Correspondence to hartd@ucalgary.ca

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: Studies from the laboratories of the authors were supported by the Alberta Health Services Strategic Clinical Network Program, the Canadian Institutes for Health Research, the Canada Research Chair Program, the Killam Foundation, and the Ministry of Education-Brazil (CAPES Foundation Grant).

Received: 19.02.18

Accepted: 19.10.18

Keywords: Activated fat, bone, diet, joint damage, metabolic syndrome (MetS), mobility and navigation, muscle, obesity, tendons, vision.

Citation: EMJ. 2019;4[1]:63-70.

Abstract

Obesity is known to affect a high percentage of both adults and children in developed countries. Individuals with obesity are at risk of developing a number of comorbidities, as well as metabolic syndrome, which can create a low-grade systemic inflammatory state that further exacerbates the risk of developing comorbidities. Two systems that are susceptible to obesity-related effects are the musculoskeletal system, which contributes to mobility via the bones, muscles, tendons, and joints, and the eye, which contributes to mobility via fidelity of navigation through the environment. Subsequently, the loss of integrity in these systems can lead to sedentary behaviour, inability to exercise, and increased risk of developing cardiovascular and respiratory diseases, loss of cognition, and falls. This review focusses on the impact of obesity on elements of the musculoskeletal system and the eye, with particular focus on the involvement of inflammation and how this may affect mobility and navigation. Finally, the use of prebiotics in altering the inflammatory state associated with obesity via the gut microbiome is discussed as one approach to address issues related to mobility and navigation.

INTRODUCTION

Obesity has become an epidemic according to many, with >35% of individuals defined as being obese or having obesity in the developed world.[1] This epidemic affects adults as well as children and adolescents, and the increasing numbers of affected individuals have led to the condition being officially recognised as a disease. However, it is a highly heterogeneous disease, as a large number of genes have been implicated in obesity or the risk of obesity.[2-5] Although the root of this epidemic, which has appeared and gained traction over the past 40 years, is not clear, diets high in fat and sugar have been implicated, as well as sedentary behaviour and genetics and epigenetics.[1,6,7]

A central issue related to obesity is not obesity itself, but the consequences of the condition on multiple host systems; these include insulin resistance and Type 2 diabetes mellitus (T2DM), elevated risk for cardiovascular disease, increased risk of osteoarthritis (OA) and joint damage, decline in cognitive integrity, sarcopenia, fatty liver disease, and loss of gut integrity.[1,7,8] The realisation of these obesity-related risks often also depends on other genetic or epigenetic risks associated with specific tissues or organs.[9-12]

The question then arises as to whether all or most of the aforementioned obesity-related risks are independent risk factors, or whether there are common elements associated with obesity that could impact a diverse set of target tissues or organs to mediate much of this risk. While not definitive, one possible explanation is the development of an obesity-associated metabolic syndrome (MetS) with an accompanying low-grade inflammatory state.[7,8] This inflammatory state may reside in alterations to the host regulation of systemic inflammatory processes and activation of fat depots by excessive energy or fat intake from the diet, but it may also be a consequence of diet-induced alterations to the gut microbiome, with the release of mediators, such as bacterial lipopolysaccharide (LPS), which translocate to the systemic circulation via a 'leaky' gut as a result of the diet.[7,8] Thus, the inflammatory state of an individual with chronic obesity is the result of a combination of direct dietary effects on host systems, in addition to its impact on the microbiome and associated gut tissues.

These two contributors to disrupted inflammatory regulation can be separated using preclinical models, in which short-term responses to an obesity-inducing environment can be evaluated. Thus, very short-term exposure of rats to a high-fat high-sucrose (HFS) diet can lead to inflammatory changes in some muscles within days,[13] before detectable alterations to plasma LPS concentrations can be detected. Over time, the gut-associated parameters and the host parameters may contribute to a chronic state of altered inflammatory regulation. Whether this latter state is completely reversible remains an open question, because with chronicity also comes the risk of epigenetic alterations that may preclude complete reversibility.

As obesity and its associated dysregulation of inflammatory processes is complex, disruption of some systems may be interactive and have synergistic sequelae over time (e.g., T2DM and inflammation during muscle or cardiovascular repair), and not everyone has the same components of MetS disrupted.[7,8] However, to address all of the potential areas affected is beyond the scope of this review. Therefore, this review is focussed on those systems that are impacted by inflammation associated with obesity and related to a common and fundamental process of human life: mobility and navigation through the environment. Thus, the loss of integrity of muscles, bones, the cardiovascular system, articulating joints, and cognition have all been implicated as risks of ineffective mobility. With loss of mobility comes an accelerated loss of the integrity of these systems, an inability to lose weight and restore function through exercise, and a downward spiral to early death for many. Humans have evolved to be a highly mobile species, and sedentary behaviour and loss of mobility is detrimental to all systems. Therefore, to combat this obesity epidemic, one needs to be active, mobile, and achieve metabolic regulation or control and restore function.

MUSCLE INTEGRITY WITH OBESITY

Chronically obese individuals often demonstrate muscle damage and loss, termed sarcopenia of obesity.[14-16] Muscles are a major target for

glucose metabolism and the combination of T2DM and fat accumulation in muscle with subsequent fibrosis and loss of contractile material can lead to the loss of specific muscle function,[7] particularly of the lower extremities, with consequences for the integrity of motion segments responsible for mobility.

As it is difficult to assess the path to this chronic state in humans with obesity, many researchers have turned to preclinical models to gain insights into better understanding the sequence of events leading to muscle compromise and whether different muscles are at varying risks of dysfunction. In a rat model of HFS-induced obesity, it has been demonstrated that alterations to the glycolytic stabilising muscle, the vastus lateralis, incur alterations in fat content, fibrosis, and macrophage activation that can be detected as early as 3–7 days after exposure to such a HFS diet, with an appreciable consolidation of changes by 12 weeks on the diet.[7,17] In contrast, the oxidative soleus muscle, a postural muscle in the calf, is more resistant to such changes.[18] In fact, the soleus muscle can adapt to the HFS diet and exhibit enhanced ability to react to the oxidative stress of metabolic overloading (e.g., induction of superoxide dismutase-2 and succinic acid dehydrogenase). Whether power muscles, such as the medial gastrocnemius, are affected in a unique manner or similar to the vastus lateralis remains to be determined. Thus, not all muscles, even of a motion segment, such as a leg, may respond similarly to a diet-induced challenge leading to obesity and one cannot generalise from the results of studies on a single muscle. However, inflammation via activation of fat in the muscle or via mediator release from activated macrophages can lead to fibrosis and compromise of susceptible muscles.

Another issue regarding obesity, inflammation, and muscle integrity relates to whether the inflammation directly or indirectly affects the integrity of the neuromuscular control systems. As muscles require neural input to affect function, disruption of this control could lead to atrophy and, in the case of obesity, systemic loss of integrity. Loss of the neural component of muscle function, using botulinum toxin injections directly into muscles of the quadriceps complex, leads to loss of muscle integrity with development of fatty deposits and fibrosis.[19,20] Therefore, more research related to the neural impact of obesity-associated inflammation on function is warranted.

BONE AND TENDON CHANGES WITH OBESITY

Obesity can lead to infiltration of fat into the bones of both rodent models and humans,[21-24] which can contribute to a loss of bone integrity. Of interest, sedentary behaviours, such as prolonged bedrest, can also lead to fat in bone marrow, which can be prevented by exercise,[25,26] even in the absence of obesity. Similarly, fat can accumulate in the bone marrow of astronauts on the International Space Station, and thus prolonged exposure to microgravity can also lead to changes in the absence of obesity, possibly due to the default differentiation of bone marrow mesenchymal stem cells.[27] Therefore, obesity does impact bones; however, the exact role of inflammation in the process in humans still remains to be clarified.[7,22]

In preclinical models of diet-induced obesity, deposition and activation of fat within subchondral bone has been detected.[21] Thus, bone integrity is put at risk as a result of changes and alterations to tissues closely associated with bone (e.g., the bone marrow). Obesity, likely through low-level systemic inflammation, can also influence bone health via development of diseases such as osteoporosis.[23,28] Thus, through direct impact of fat within bone and the marrow cavity, or indirectly via the inflammation associated with obesity and concomitant expression of adipokines and cytokines that can facilitate bone resorption, obesity can affect bone health in multiple ways. As females are more at risk of developing osteoporosis in the absence of obesity, this population may be more adversely impacted than the male population. Second to osteoporosis is the risk for falls and fractures, and, with impairment issues related to gait control,[29,30] this risk may be compounded.

Tendons play a critical role in mobility via transmitting muscle forces to move bones, and the integrity of tendons in obesity is also essential in maintaining the function of a motion segment. Studies in humans have shown that ankle tendons of those with higher levels of obesity exhibit more features associated

with tendinopathies.[31] Recently, it has been reported that, based on ultrasound analysis, asymptomatic disturbances to Achilles tendon integrity can be correlated with obesity.[32] Fairley et al.[33] reported links between patellar tendinopathy and obesity, and concluded the link was mechanical because it correlated with BMI and not with fat mass. However, these authors did not assess the patients for MetS. Thus, obesity-related disruption of the integrity of multiple components of the motion segment (muscle, tendon, and bone) may contribute to compromised mobility and stability.

DEVELOPMENT OF JOINT DAMAGE IN OBESITY

Individuals with obesity are at higher risk of developing joint diseases, such as knee osteoarthritis, than non-obese individuals.[7,34,35] This risk could reside in the increased mechanical load placed on joints like knees, in the dysregulated inflammatory state, or a combination of both factors. However, some reports indicate an increased risk of developing hand OA in individuals with obesity, and the hand may not be subjected to increased loading, so perhaps the risk in obesity resides more with the inflammatory dysregulation.[7,8]

Recently, it has become apparent that OA does appear to have an inflammatory component related to its progression,[36-38] and, thus, a dysregulated inflammatory state in chronic obesity could potentially contribute to the initiation and progression of joint damage and degeneration along with the associated pain.[39,40] Relevant to this point are findings from preclinical rat studies, which indicate that diet-induced obesity leads to both systemic alterations to inflammatory mediator expression and local alterations in the synovial fluid of the knee.[7] Furthermore, recent studies have indicated that the risk for joint damage is not restricted to just the knee, because the shoulders of the obese rats also experienced damage.[41] However, for reasons currently unknown, in these 12-week studies, the hip displayed less joint damage than the knee or shoulder.[41] Whether the shoulder risk is related to the fact that rats are quadrupeds is also unclear at this point. However, it is known that obesity is also a risk factor for OA[42,43] and pain[44] in the shoulder of humans, so issues of obesity and joint involvement in motion and mobility disorders may cross species. Similarly, compared to the knee and shoulder, the hip is a ball and socket joint, a configuration that may protect its integrity in some manner.

POTENTIAL ALTERATIONS TO VISION WITH OBESITY

Individuals with obesity are reported to be at risk of developing some ocular diseases,[45] such as macular degeneration,[46,47] cataracts,[48,49] retinal diseases[50] (possibly related to sugar consumption),[51] and others.[52] Regarding obesity and cataract risk, the findings are still somewhat controversial, because some reports have indicated a negative correlation for a Korean population.[53]

It is also not clear whether this risk is related to a direct effect of the fat, the dysregulated inflammatory state, or a secondary consequence of obesity related to T2DM and refined sugar consumption. The eye is an immune privileged site,[54,55] and disruption of homeostasis via a dysregulated inflammatory system could contribute to the loss of such privilege and allow for inappropriate inflammatory or immune activities within the eye (e.g., via the vitreous humour [VH]), as well as at the level of the cornea. Recently, the authors reported that following the induction of diet-induced obesity, gene expression patterns are altered in VH cells, and that based on a protein array methodology, the protein homeostasis of this fluid is disrupted with the elevated presence of inflammatory mediators.[56] While these results have not yet correlated with the ocular changes associated with overt disease processes in the preclinical model or disrupted navigation, this is an area of active research.

In addition, it is not yet possible to directly attribute the changes in the VH of the diet-induced obese rats to the systemic inflammatory alterations detected. Such changes could also be an indirect effect of the joint damage occurring after induction of diet-induced obesity.[21,41] In addition, since the recent rat studies used only the 12-week time point post diet-induced obesity to assess joint damage and the VH alterations, it is not yet known whether the joint damage preceded

the alterations in the VH of the eye, whether they developed in parallel, or whether the eye alterations preceded the joint damage. Resolution of this issue is the subject of ongoing research.

This issue of eye involvement in obesity is likely to be clinically relevant because humans use their eyes to navigate through their environment. As such, mobility is critical but navigation possibly even more so. However, it is not yet known whether injury to the ankle or the hip also leads to changes in the eye, or whether there is a specific knee–eye or mobility–navigation axis.[57] This is an area of active research, as is determining whether this potential axis is unidirectional (knee to eye) or bidirectional (also eye to knee). Thus, mobility is intrinsically integrated with navigation. Furthermore, as skeletally mature adults, humans define where they are in space while they are mobile without overtly thinking about it, but may require more active thinking while learning to be mobile as a young child, or during older age when the integrated systems decline in integrity. Interestingly, postural control in the elderly is adversely affected by vision impairments.[58] This latter aspect of mobility likely also involves brain elements associated with memory or specific areas of the brain, such as the hypothalamus[59] or other centres.[60]

The assessment of both normal body weight and individuals with obesity either blindfolded or with uninhibited vision has shown that such interruption of this putative knee–eye axis leads to abnormal gait patterns, with the alterations more pronounced in those with obesity.[61-64] Such alterations in gait with obesity have also been observed in dogs.[65] Furthermore, Lam et al.[66] have recently reported that individuals with obesity have an elevated intraocular pressure compared to sex and normal body weight-matched individuals, and the intraocular pressure of the individuals with obesity declined to more normal levels after weight loss via bariatric surgery. In these studies, which were correlative in nature and did not define cause and effect, it was not determined whether the obese individuals exhibited MetS characteristics or whether their visual acuity was affected by the bariatric surgery; these factors constitute important limitations of the study. While the basis for these increases in patients with obesity remains to be determined, it could potentially be related to increased fluid due to the oedema related to a low-grade inflammation in the eye; further investigation is required to identify the mechanisms involved. While not directly related to obesity, it has been noted that astronauts flying long-duration missions on the International Space Station do experience visual impairments that are linked to globe deformations and increased intracranial pressure due to vascular fluid shifts,[67] possibly supporting the concept that increased intraocular pressure in patients with obesity could also lead to visual compromise.

Therefore, potential compromise of ocular integrity via chronic inflammatory changes, such as those associated with obesity, may have more impact on the fidelity of mobility for both new and established patterns of movement, even in the context of walking in a familiar environment with brain elements intact. This may reflect the central nature of a possible knee–eye–brain/neural axis[57] that needs to be intact to optimise both mobility and navigation. As all knee joint tissues (and other joints of the body, except for articular cartilage) are innervated, and muscles are dependent on neuroactivation to initiate force generation, the integrity of such an axis of control and regulation may be necessary for optimal mobility and navigation, and obesity and the associated inflammatory state can be considered a threat to such integrity at several points (i.e., muscles, joint integrity, loss of visual integrity). Currently, the evidence for the impact of obesity on navigation suffers somewhat from a paucity of well-designed studies to assess the type and extent of the alterations, as well as mechanistic implications, and, thus, while a potentially important area, it is one that requires further investigation to better understand the impact of obesity on the integration of mobility and navigation.

ADDRESSING THE NEED FOR EFFECTIVE INTERVENTIONS TO PREVENT OR INHIBIT OBESITY-RELATED RISK OF LOSS OF MOBILITY INTEGRITY AND NAVIGATION FIDELITY

It is clear from the previous discussion that obesity, and the inflammatory state associated

with it (i.e., MetS), likely impacts host tissues that contribute to effective and energy-efficient mobility and navigation. These include tissues directly involved in mobility (muscles, tendons, and bone), as well as others that contribute to the fidelity of mobility, such as the eyes and the peripheral neural system/brain (via memory control of movement, neural control of muscles, and integration of the functioning of the diverse tissues involved in motion via proprioception, bilateral co-ordination, and avoiding risk).

Potential interventions to re-establish metabolic control include weight loss via diet or bariatric surgery; exercise, alone or in combination with diet and nutrition changes; or the use of prebiotics (substrates that are selectively used by host micro-organisms conferring a health benefit),[68] alone or in combination with other modalities. Some options may work better for some patients than others, likely due to the variation in the factors contributing to risk. The conclusion that there is no 'quick fix' for obesity is supported by the large number of genes that have been implicated in obesity, and the fact that epigenetic mechanisms also play a role in the development and progression of obesity and MetS-associated inflammation.[9-12] It is also unclear to what extent damage and alterations due to obesity or MetS are reversible in musculoskeletal and/or eye tissues. Thus, obesity is likely complex in its control at multiple levels, and, as such, more individualised approaches may be required.

It is also clear that use of prebiotic compounds (usually oligofructose and inulin) can help re-establish metabolic regulation.[69-72] Such prebiotics are not metabolised by humans but are used by the gut microbiota to re-orient the microbial community by altering the bacterial species present and correcting the leaky gut syndrome, which allows bacterial LPS to pass into the host.[68,73] As LPS is a proinflammatory molecule, such changes could impact the inflammatory component of MetS directly. Such an impact could re-establish muscle integrity, insulin resistance, and better mobility control. It remains to be determined whether such prebiotics can influence a return of the VH to a more normal state and potentially re-establish navigation integrity. Secondly, use of prebiotics by bacteria leads to the generation of short chain fatty acids that can be absorbed and impact host systems,[74-76] and whether such metabolites can influence the eye or the proposed circuitry remains to be clarified. Thus, this prebiotic approach has a relatively low cost and could be a beneficial avenue to address some aspects of the impact of obesity.

CONCLUSION

For those with obesity, options are available to help address the impact of obesity on host systems (e.g., diet, exercise, prebiotics, bariatric surgery, and medications), including those related to mobility. However, as with many conditions or diseases, prevention is better than having to deal with the problem once it has become medical. Therefore, an active lifestyle with good nutrition, likely starting very early in life, is the best option to prevent the development of obesity and loss of mobility, which can become a vicious cycle of increasing obesity contributing to increasing loss of mobility and the fidelity of navigation through the environment. While the above is a general approach to prevention, for those with specific genetic or epigenetic risk factors, precision health with better understanding of the risks associated with specific genetic contributions could lead to targeted preventative interventions and protocols to make such interventions more specific and effective.

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Inflammation and Thrombosis in Coronary Atherosclerosis: Pathophysiologic Mechanisms and Clinical Correlations

Authors: *John A. Ambrose, Amarbir S. Bhullar

University of California, San Francisco, San Francisco California, USA
*Correspondence to jamambrose@yahoo.com

Disclosure: The authors have declared no conflicts of interests.

Received: 16.07.18

Accepted: 07.11.18

Keywords: Atherothrombosis, coronary atherosclerosis, high sensitivity C-reactive protein (hsCRP), hypercoagulability, inflammation, myocardial infarction, plaque, thrombosis.

Citation: EMJ. 2019;4[1]:71-78.

Abstract

Inflammation and thrombosis are interrelated processes that are important in the pathogenesis of atherothrombosis. Inflammation is important in both the early and late stages of atherosclerosis, and it involves elements of immune system activation. Low density lipoprotein (LDL) is an important initiator but is not the only one. LDL enters the cell membrane, is modified, and sets into motion a series of events that stimulate the ingress of specific proinflammatory mononuclear cells through the vessel wall. These cells imbibe lipids and form foam cells. Proinflammatory mediators secreted by these cells can eventually lead to intimal thickening and lipid accumulation, forming atherosclerotic plaques. A complex interplay between inflammation, platelet function, and hypercoagulability is a major contributor to the progression from stable to unstable plaque and an acute coronary event. In the later stages of atherosclerosis, inflammatory cells can destabilise certain lipid-rich lesions contributing to symptomatic coronary thrombosis. Thus, thrombosis is the final common pathway for most atherosclerotic complications. Thrombi may also contribute to the asymptomatic rapid progression of atherosclerotic lesions. While antithrombotic agents are important in the treatment of acute coronary syndromes, as well as preventive therapy in high-risk primary prevention and in secondary prevention, the role of specific anti-inflammatory agents is not currently established. If such therapies are to become routine, these anti-inflammatory drugs must significantly reduce events while not adversely affecting a patient's natural immunity to an extent that erases any potential benefit. This article reviews these two processes with an emphasis on coronary atherosclerosis and its sequelae.

INTRODUCTION

The importance of inflammation and thrombosis in the pathophysiology of atherosclerosis was debated in the 19th century by two prominent pathologists, Virchow and Rokitansky, who had opposing views. In the 20th century, the role of thrombosis in the pathogenesis of acute myocardial infarction remained unsettled until the 1970s.[1] Studies over the last 40 or more years have improved the understanding of these two processes and explained the role of inflammation in the initiation and progression of atherosclerosis. There is a close interrelationship between inflammation, which is a driver of the atherosclerotic process, and most thrombotic lesions, the final pathway that leads to many of

the complications triggered by inflammation. This article reviews some of these data and considers the clinical importance of antithrombotic and anti-inflammatory agents in mitigating these processes. The emphasis of this review will be on coronary atherosclerosis and thrombosis.

INFLAMMATION AND ATHEROSCLEROSIS

Dr Russell Ross, a pioneer in the field of atherosclerosis pathogenesis in the second half of the 20th century, considered atherosclerosis to be a chronic inflammatory disease of the intima.[2] Atherosclerosis principally affects large and medium-sized elastic and muscular arteries, and it may lead to ischaemic complications in the muscles served by these arteries. It is well known that early atherosclerotic lesions can be found in adolescents and young adults decades prior to any clinical sequelae. Pathologic studies in children at autopsy, autopsy studies in young adults during war time, and intravascular ultrasound studies of the coronary arteries in adolescent and young adult donor hearts soon after transplant attest to the common presence of fatty streaks or intimal thickening, particularly if risk factors are present.[3-5] The next section will consider a brief description of the role of inflammation in early atherosclerosis. For a more detailed discussion, the reader is referred to the report by Libby et al.[6]

The inflammatory response in atherosclerosis involves elements of both the innate (e.g., the most prominent cells are certain proinflammatory monocytes) and the adaptive (e.g., T lymphocyte subtypes) arms of the immune system. Considerable evidence supports the importance of oxidised phospholipids from modified low-density lipoproteins (LDL) as an important, but not the only, initiator for the ingress of monocytes into the intima by eliciting the expression of adhesion molecules and chemoattractant molecules, including chemokines, such as monocyte chemoattractant protein-1.[7] Pathologic studies of the coronary arteries in young adults dying from noncardiac causes indicate that lipid accumulation in the intima precedes intimal inflammation.[8] Once in the intima, monocyte-derived macrophages imbibe oxidised lipids to form foam cells. These cells secrete chemoattractant chemokines that encourage the migration of smooth muscle cells from the media into the intima. An intimal accumulation of smooth muscle cells in a proteoglycan-rich matrix (secreted by the smooth muscle cell) with extracellular lipid forms a progressive lesion termed pathologic intimal thickening. It is generally accepted that advanced atherosclerotic lesions responsible for the development of acute coronary events develop from intimal masses and not necessarily from fatty streak, which often regress.[9]

A discussion of coronary atherosclerosis must also consider the role of altered haemodynamics and flow patterns in the development of focal atherosclerotic lesions within the coronary arteries. Local haemodynamic forces and, in particular, low endothelial shear stress, significantly contribute to early lesion development and progression of atherosclerosis.[10] Low shear stress modulates endothelial gene expression, inducing an atherogenic phenotype to the endothelium. Low shear stress also promotes inflammation by activating nuclear factor κB and by reducing the bioavailability of endothelial nitric oxide.[11]

As lesions progress, a fibrous cap forms, composed of smooth muscle cells with an extracellular matrix of collagen and elastin infiltrated by inflammatory cells (macrophages and T lymphocytes to a varying degree). Underlying the cap, there may be a mass of lipid composed of extracellular lipid and necrotic cells derived, in part, from the apoptosis of macrophage-derived foam cells; these come together to form the necrotic core. The lipid can be found as either cholesterol esters, free cholesterol, or monohydrate cholesterol crystals.

Vasa vasorum grow into the intima from the adventitia as plaques enlarge to provide nourishment to the thickened vessel wall. Intraplaque haemorrhage from these thin-walled vessels with faulty endothelial cell junctions is a potential source of additional lipid and inflammatory cells allowing for asymptomatic, accelerated growth of the plaque.[12] Not all plaques contain an abundance of lipids. Other plaques are fibro calcific, with little or no lipid core, and they are potentially not as dangerous as the lipid-rich plaques, particularly those with a thin fibrous cap that are classified as thin-capped fibro atheromas (TCFA).

INFLAMMATORY MARKERS AND CORONARY ARTERY DISEASE

There is abundant literature concerning the role of biomarkers in atherosclerosis. High sensitivity C-reactive protein (hsCRP) is the most widely studied. It is a sensitive but non-specific marker of inflammation that, when elevated to >3 mg/L, has been shown to be associated with a significant increase in coronary events compared to a normal value.[13] HsCRP has been added to conventional risk scores in the Reynolds Risk Model and improved prediction above the Framingham Risk Score.[14] In the JUPITER Study, patients with an elevated value ≥2 mg/L and LDL levels <130 mg/dL were randomised to rosuvastatin 20 mg versus standard care. These patients had a 44% reduction in a composite endpoint, including myocardial infarction, stroke, and cardiovascular death.[15] More recently, in an analysis from the FOURIER trial,[16] LDL cholesterol reduction with the PCSK-9 inhibitor, evolocumab, reduced cardiovascular events across hsCRP strata in patients with clinically stable cardiovascular disease with greater absolute risk reductions in patients with higher baseline hsCRP. Furthermore, in patients with LDL levels as low as <20 mg/d L on therapy, 3-year event rates were higher in those with an elevated hsC-RP compared to those with a level <1 mg/L.[16]

The CANTOS trial assessed the role of canakinumab, a monoclonal antibody targeting IL-1β in patients with a prior myocardial infarction and an elevated hsCRP.[17] This was a test of the inflammatory hypothesis in atherosclerosis, because the drug does not alter lipid levels. At a median follow up of 37 months, the drug reduced the primary endpoint of myocardial infarction, stroke, or cardiovascular death by a small, but significant, amount compared to placebo. However, the residual event rate was high and canakinumab was associated with a significant increase in fatal infections versus placebo.

FROM LESION PROGRESSION TO SYMPTOMATIC THROMBOSIS

Asymptomatic progression of atherosclerosis can be linear or non-linear, the latter caused by processes that acutely (or subacutely) accelerate plaque burden. This is generally related to two processes. The first has already been alluded to: intraplaque haemorrhage from rupture of the vasa vasorum. The other process is asymptomatic thrombosis. Pathologic studies in patients that died as a result of sudden cardiac death frequently show multiple prior episodes of asymptomatic thrombosis that can contribute to plaque growth.[18] Furthermore, thrombectomy specimens from primary percutaneous interventions in ST-elevation myocardial infarction (STEMI) patients show partially organised thrombus in about 50% of the cases suggesting silent lesion progression with thrombus formation prior to the symptomatic acute event.[19]

Symptomatic thrombosis is responsible for nearly all STEMI incidences and probably about half of non-ST elevation infarcts. The percentage of non-STEMI (NSTEMI) depends somewhat on the definition of the condition and whether you include an elevation of cardiac enzymes immediately post coronary intervention, which is not usually caused by thrombosis. In general, type 1 myocardial infarctions by the universal definition of myocardial infarction are caused by an acute obstructive process in an epicardial coronary artery and this process is usually thrombus formation.[20] Thrombus formation in myocardial infarction or sudden cardiac death has been shown to be secondary to three pathologic processes. The most common is plaque rupture followed by plaque erosion (Figure 1). The least common is a calcified nodule, which occurs in <10% of cases.[21]

Plaque rupture of a thin cap atheroma is the usual lesion responsible for most thrombotic events, particularly in STEMI. The numbers vary from about 60–75% depending on how the diagnosis was made.[22] While pathologic studies originally identified thrombi in those who died suddenly or after myocardial infarction, more recently, intravascular imaging, particularly with optical coherence tomography imaging during primary percutaneous intervention for STEMI or NSTEMI, can differentiate plaque rupture from other mechanisms, including plaque erosion.[23]

Inflammation is an important mechanism in plaque rupture for a thin cap atheroma with an abundant inflammatory substrate. Thinning and disruption of the cap leading to thrombus formation is precipitated by macrophages and T lymphocytes that are abundant in these lesions.

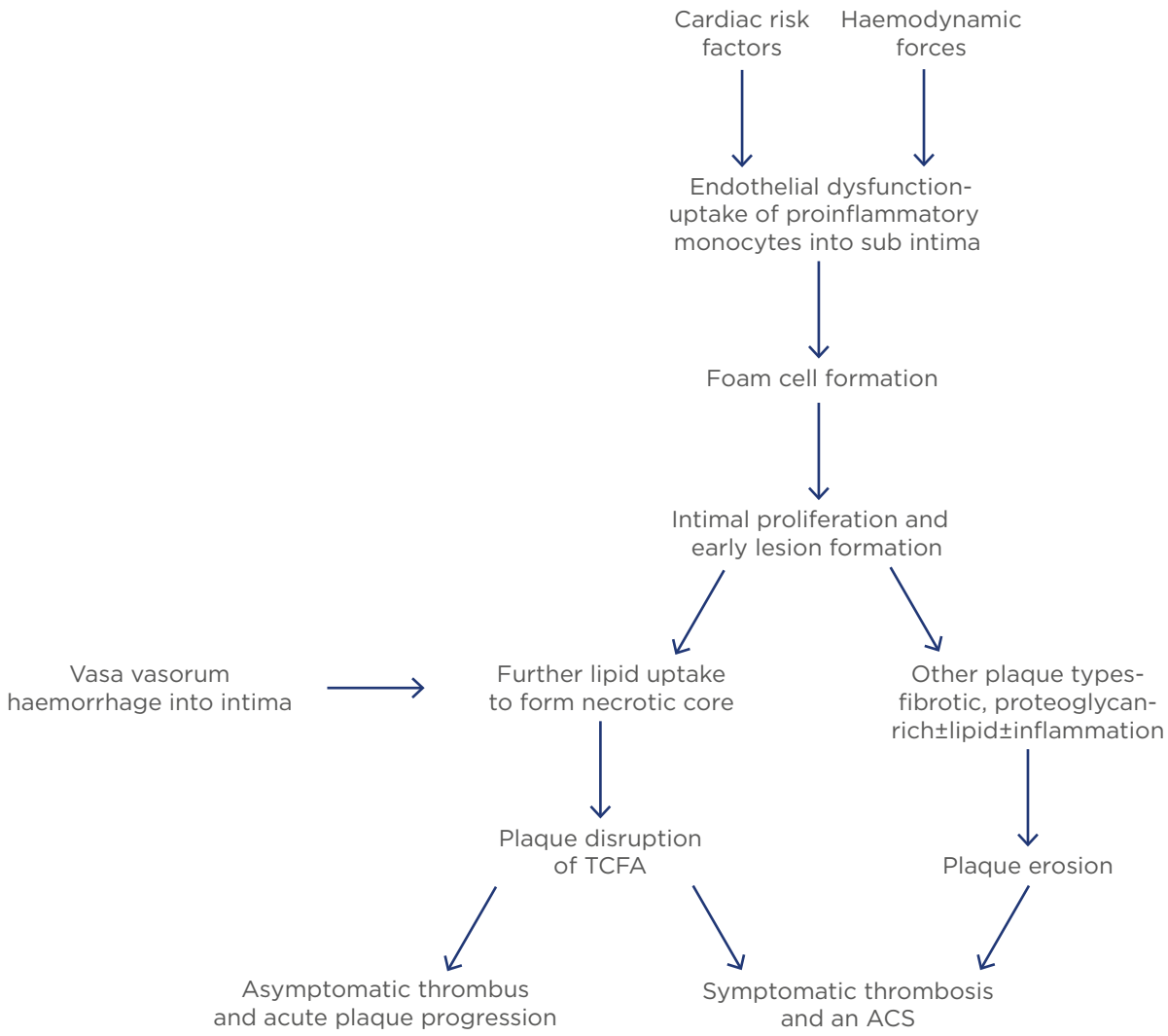

Figure 1: From atherosclerosis to atherothrombosis.

ACS: acute coronary syndrome; TCFA: thin-cap fibroatheroma.

Macrophages also furnish the bulk of the enzymes that catabolise collagen, a key constituent of the fibrous cap of the plaque. Overexpression of interstitial collagenases (MMP-1, MMP-8, MMP-13) in human atheromata are colocalised with macrophages bearing these proteinases.[24] Ultimately, thinning of the cap leads to rupture of the cap. Flowing blood is exposed to the under surface of the plaque, which is highly thrombogenic. Platelet deposition is followed by tissue factor activation and the thrombus, if it occludes the lumen (which occurs about 85% of the time in STEMI), will have a platelet-rich head and a red cell and fibrin tail.

Plaque erosion is the second leading pathophysiology for thrombosis in acute coronary syndromes. Pathologic studies suggest that an average of 25–44% of fatal events caused by acute coronary thrombosis are related to erosion.[25] Intravascular imaging studies with optical coherence tomography at the time of coronary intervention in myocardial infarction patients have shown erosion as the main mechanism of STEMI in about 25–30%.[23] In a limited number of patients interviewed, erosion was more frequent in NSTEMI Type I than in STEMI. However, all these intravascular observations have been performed in small studies after the removal of the thrombus by thrombectomy and have been reported by very few groups. Thus, how well these data can be generalised to acute myocardial infarction is presently unclear.

The role of inflammation in plaque erosions is controversial. Pathologic studies of plaque

erosion differ on the role of inflammatory cells adjacent to the thrombus.[21,26] While this continues to be debated, inflammation is undoubtedly an important determinant of hypercoagulability, which contributes to thrombus formation in both plaque erosions and plaque disruptions. It is possible to speculate that hypercoagulability of blood may represent a mechanism responsible for inducing or extending the amount of thrombus and thereby converting an asymptomatic thrombus into a symptomatic event.

INFLAMMATION AND HYPERCOAGULABILITY

Inflammation and hypercoagulability promote atherothrombosis. In fact, the complex interplay between inflammation, platelet function, and hypercoagulability is largely responsible for the progression from stable to unstable plaque and an acute coronary event. The effects of inflammation on thrombosis involve both enhanced platelet reactivity, as well as activation of the coagulation cascade from tissue factor expressed by macrophages and other cells. In addition to CRP, other molecules, such as the various IL, TNF, and adhesion molecules, have been associated with increased ischaemic events. For example, IL-6 not only increases CRP, but it also increases platelet reactivity and fibrinogen levels.[27] The inflammatory marker, hsCRP, as already discussed, has been shown to predict future thrombotic complications, such as acute myocardial infarction or stroke. Based on all the data, hsCRP appears to be a marker for, and possibly an active participant in, promoting atherothrombotic complications[28] (Figure 2).

The relationship between inflammation and thrombosis is bidirectional. Inflammation promotes thrombosis and, vice versa, thrombosis promotes inflammation. Platelet granules contain several proinflammatory chemokines and cytokines.[29] Thrombin, a key protein in the coagulation cascade, can also increase the expression of proinflammatory cytokines from vascular endothelial and smooth muscle cells.[30] Furthermore, platelets, in experimental studies, exert a host of proatherogenic activities and create a link between haemostasis, innate immunity, atherosclerosis, and inflammation.[31]

All the standard CAD risk factors are proinflammatory and prothrombotic; they promote endothelial dysfunction and thus interfere with the protective (anti-inflammatory and antithrombotic) effects of nitric oxide. Perhaps the most prothrombotic of these risk factors is exposure to cigarette smoke. The authors and other research groups have published extensively on this subject and a few salient features will be discussed.[32,33] Both active and passive cigarette smoke exposure increase the risk of coronary thrombosis and myocardial infarction. According to the National Cardiovascular Data Registry (NCDR), which tracks coronary interventions in all hospitals in the USA, about 45% of STEMI patients undergoing primary intervention occurred in active or recent cigarette smokers.[34]

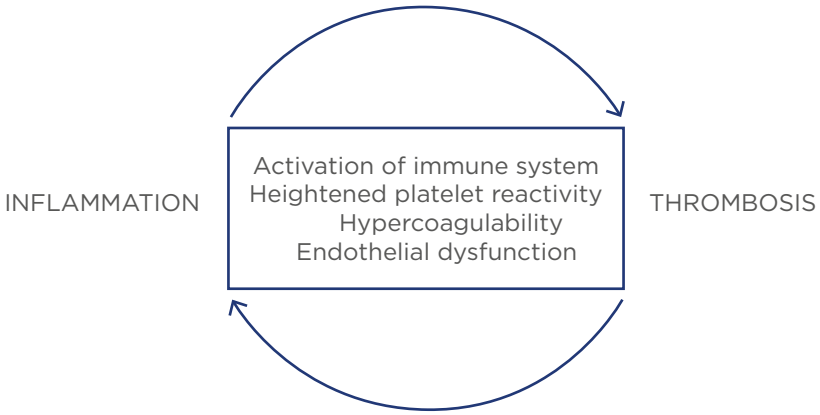

Figure 2: Interrelationship between inflammation and thrombosis.

Cigarette smoke exposure alters the haemostatic process via multiple mechanisms, which include alteration of the function of endothelial cells, platelets, fibrinogen, and coagulation factors. This creates an imbalance of antithrombotic/prothrombotic factors and profibrinolytic/antifibrinolytic factors that support the initiation and propagation of thrombosis. A growing body of evidence supports free radical-mediated oxidative stress and loss of the protective effect of nitric oxide playing a central role in cigarette smoke exposure-mediated thrombotic diseases.[32,35]

Smoke exposure increases inflammatory mediators such as TNF-α; CD40 ligand; and the expression of adhesion molecules on endothelial cells, platelets, and monocytes. Inflammation increases tissue factor expression on monocytes and the activated endothelium, as well as activation of circulating tissue factor-bearing microparticles. In humans, chronic cigarette smoke exposure increased levels of multiple inflammatory markers, including peripheral leukocytosis, hsCRP, homocysteine, IL-6, and TNF-α. Exposure of human umbilical vein endothelial cells to cigarette smoke condensate resulted in increased nuclear factor κB DNA binding activity, increased surface expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and platelet endothelial cell adhesion molecule-1. All the above factors are prothrombotic.[35]

CLINICAL CORRELATIONS

Antithrombotic Agents

Given the importance of thrombosis in myocardial infarction, it is not surprising that antithrombotic agents should have a role both in the prevention and treatment of acute coronary complications. However, there is no clinical evidence that the haemostatic system can attenuate or regress plaque formation. Nevertheless, the importance of antithrombotic agents in coronary disease management was first established in the 1980s when ISIS-2[36] showed a significant reduction in 30 day mortality with aspirin use alone in patients presenting with acute transmural infarction, and the VA Cooperative study showed that 324 mg of aspirin reduced death and nonfatal myocardial infarction by >50% at 12 weeks in patients admitted with unstable angina.[37] Aspirin in low doses irreversibly inhibits COX-1 in the platelet, leading to a significant inhibition of platelet-dependent thromboxane formation, which is responsible for its antithrombotic effect. Aspirin may also inhibit inflammatory cytokines independently of its effect on thromboxane. Its clinical significance is unknown.[38]

Since the 1980s, antiplatelet drugs have been a mainstay of therapy for medically managed and interventional therapy. Most of the recommendations are in secondary prevention, with limited recommendations for primary prevention; even then, primary prevention is recommended only in higher risk patients. In antiplatelet therapy, the data indicate that the more pathways inhibited in the platelet, the greater the antithrombotic (anti-ischaemic) effect but, at the same time, the greater the bleeding risk.[39] Thus, dual antiplatelet therapy is the present class 1 recommendation for stent placement. Use of the more potent ADP receptor blockers, prasugrel or ticagrelor, are more anti-ischaemic with significant reductions in adverse events, such as death, MI, or stroke, compared with the weaker clopidogrel (all with low dose aspirin). However, they are associated with a higher bleeding risk. Likewise, the COMPASS trial[40] assessed the role of the low dose anticoagulant, rivaroxaban, plus low dose aspirin in patients with stable cardiovascular disease without a recent stent. While ischaemic events, such as recurrent myocardial infarctions, were significantly reduced on follow up, it is not surprising that bleeding events were also increased.[40]

Anti-Inflammatory Agents

Up until recently, there was no positive data on specific anti-inflammatory drugs in the management of coronary disease patients. On the other hand, the use of some COX-2 inhibitors, for indications other than heart disease, was associated with increased adverse coronary events.[41] Nevertheless, statins possess anti-inflammatory properties, as indicated by a lowering hsCRP, which have been considered to add to their protective effect regarding the reduction of cardiovascular events. As already mentioned, in JUPITER, apparently healthy patients with mild hyperlipidaemia and

elevated hsCRP had a significant reduction in major cardiovascular adverse events with rosuvastatin therapy.[15]

A proof of targeting specifically inflammation to improve outcomes in atherosclerosis was finally accomplished in the CANTOS trial, as previously discussed. Another trial has been in progress targeting the same IL-1β. CIRT is a double blind, randomised trial of low dose methotrexate versus placebo in diabetic or metabolic syndrome patients with a prior history of myocardial infarction who will be followed up for 3–5 years.[42] Recently, the Data and Safety Monitoring Board (DSMB) recommended that enrolment in this trial should stop, but not for any substantive safety reasons. The National Heart, Lung, and Blood Institute (NHLBI) halted the trial with about 70% of the 7,000 patients randomised and the results will hopefully be available by the end of 2018. Whether the trial was stopped for futility or because there was a significant benefit with methotrexate is presently unknown.

CONCLUSIONS

Inflammation and thrombosis are important interrelated processes in the development of coronary atherosclerosis and the genesis of atherothrombosis. Thrombosis, while the most common cause of acute myocardial infarction, is also a mechanism for the asymptomatic, non-linear progression of atherosclerosis. Inflammation is paramount in both early and late stages of atherosclerosis. Plaque rupture, the major cause of STEMI and sudden coronary death related to coronary thrombosis, is precipitated by inflammatory-mediated degradation of the thinned fibrous cap overlying lipid-rich plaques.

While antithrombotic agents are an important component of care in the treatment of acute coronary syndromes as well as preventive therapy in high-risk primary prevention and in secondary prevention, the authors have explored the potential role of specific anti-inflammatory agents in the clinical care of these patients. If such therapies (other than statins) are to be considered in the future, anti-inflammatory drugs must significantly reduce events while not adversely overwhelming a patient's natural immunity to an extent that erases any potential benefit. However, the authors believe that to reduce substantially the morbidity and mortality associated with atherosclerosis, it will be best to interrupt plaque development early in the natural history of atherosclerosis, years prior to any adverse event. This can be accomplished with standard medical therapies as well as lifestyle interventions.[43]

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Updating the Impact of Lipid Metabolism Modulation and Lipidomic Profiling on Oocyte Cryopreservation

Authors: Eduardo Domingos Borges,[1] *Alessandra Aparecida Vireque[2]

1. Department of Obstetrics and Gynecology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
2. Invitra - Assisted Reproductive Technologies Ltd., Supera Innovation and Technology Park, Ribeirão Preto, Brazil

*Correspondence to invitra.br@gmail.com

Disclosure: The authors have declared no conflicts of interest.

Received: 25.04.18

Accepted: 04.09.18

Keywords: Cryopreservation, lipid metabolism, lipid profile, mass spectrometry, oocyte.

Citation: EMJ. 2019;4[1]:79-87.

Abstract

Oocyte cryopreservation has drastically improved in recent years and is receiving widespread clinical use with increasing demand for fertility preservation and assisted reproduction treatments. However, there are still several points to be reviewed in terms of suppressing sub-lethal damages and improving overall safety, especially when trying to preserve oocytes at the germinal vesicle stage or oocytes matured *in vitro*. The lipid content of oocytes is highly associated with both their competence and cryotolerance. Differences in lipid content are observed not just between different species but also at different developmental stages and when the oocytes are kept under different conditions, including cryopreservation. Many efforts have been made to understand how physiological or *in vitro* alterations in the lipid profile of oocytes impacts cryotolerance and vice-versa; however, the dynamics of cytosolic and membrane lipid involvement in the cryopreservation process remains poorly clarified in the human female gamete. This review presents an updated overview of the current state of cryopreservation techniques and oocyte lipidomics and highlights possible ways to improve cryotolerance, focussing on lipid content modulation.

INTRODUCTION

Oocyte cryopreservation has improved in recent years and is receiving widespread clinical use with increasing demand for fertility preservation and assisted reproduction treatments.[1] From the birth of healthy mouse pups using cryopreserved oocytes for *in vitro* fertilisation (IVF) and embryo transfer in 1977 to now, many advances have been achieved and a wide array of tools made available to preserve genetic material, evaluate oocyte viability biomarkers, and treat many forms of infertility.[2,3] In many mammals, however, oocyte cryopreservation techniques are still poorly developed and, despite advances in oocyte vitrification methods, there are still several points to be reviewed in terms of suppressing sub-lethal damage to oocytes and improving overall safety, especially when trying to preserve immature oocytes or oocytes matured *in vitro*. While cryopreservation of oocytes at metaphase II (MII) (mature oocytes)

is approved for clinical use, cryopreservation of germinal vesicle-stage oocytes, or oocytes matured *in vitro*, are still considered experimental without consistent results.[2-4]

The lipid content of oocytes is highly associated with gamete competence and cryotolerance.[5,6] The oocyte is the largest mammalian cell and has an extensive amount of cytoplasm containing abundant lipid storage. Differences in the lipid content of oocytes are observed not only between different species but also in oocytes from the same ovary and are even noted in the same oocyte under different conditions or at developmental stages.[7] Differential lipid profiling occurs throughout the gamete and its companion cumulus cells under all stages of follicular development. Noticeable changes also occur between immature and matured oocytes,[8] as well as after cryopreservation.[9] Additionally, the presence of various lipid sources in *in vitro* maturation (IVM) systems effects oocyte lipid profile and developmental competence.[8,10,11]

Many efforts have been made to understand how physiological or *in vitro* alterations in the lipid profile of oocytes impact cryotolerance and vice-versa, including studies on the principles of plasma membrane properties[12] and the characterisation of the lipid profile of a single human oocyte.[13] However, the dynamics of cytosolic and membrane lipids involved in the cryopreservation process remain poorly clarified in the human female gamete. This review presents an overview of cryopreservation techniques and oocyte lipidomics, and highlights possible ways to improve cryotolerance, focussing on lipid content modulation.

CRYOPRESERVATION TECHNIQUES

Since its inception 30 years ago, cryopreservation of human oocytes has become an important component of assisted reproduction technology. The increasing success rates of MII oocyte vitrification led to its implementation in IVF clinics worldwide, with the American Society of Reproduction Medicine (ASRM) lifting the experimental status of the practice in 2013.[14] The ascending popularity is being followed by a noted increase in demand, especially among women trying to overcome age-related fertility issues.[15] The rise of 'social egg-freezing' is pushing reproduction scientists to review recent cryopreservation protocols and outcomes, and to pursue new ways of improving the process, to achieve maximum safety.[15-17]

If carried out correctly, the alterations caused by cryopreservation, especially the vitrification technique in MII-oocytes, do not impair gamete function and development. However, the oocyte's unique structure with a large amount of cytoplasm and low hydraulic conductivity, and the sub-lethal damages caused to it by freezing and thawing (temperature, exposure time, and cooling rates) are responsible for the increased difficulty in the cryopreservation of these cells and might explain the observed lower rates of live births when compared to fresh oocytes.[18] In the following section, the authors briefly discuss the known damages caused to the oocyte and the current state of cryopreservation techniques.

Sub-Lethal Damages Caused to the Post-Thaw Oocyte

Cryopreservation-induced stress is a complex and multifaceted mechanism in which different stressors and stress cell responses appear to play important roles. The chilling process affects membrane structure. During freezing, cells are exposed to hyperosmotic solutions and equilibrated by the movement of water across the membranes. During storage and thawing, oocytes are subject to ice recrystallisation episodes.[17,19] Susceptibility of cells to biochemical damage by oxidative stress is also reported during the thawing process.[20]

The most affected structures within the cell are the microtubules, cytoskeleton, lipid droplets, and the membrane system.[21] Studies by Ghetler et al.[12] showed that the oocyte membranes are far more sensitive to the effects of chilling than the membranes of embryos and even zygotes, which are visually very similar to the female mammalian gametes. The membrane of oocytes has a high melting temperature, which means that the lipids are more easily affected by the drop to low temperatures, causing a loss of membrane function. This is a possible explanation for the relatively poor survival rates of cryopreserved human oocytes compared to embryos using the slow freezing method.[12] It has also been observed that lipid droplets are

affected differently between immature oocytes surrounded by cumulus cells and mature oocytes. According to a study by Okotrub et al.,[22] lipid crystallisation occurs gradually in lipid droplets of embryos and mature oocytes and more abruptly in immature oocytes, possibly explaining one of the reasons why vitrification of the latter is less successful in general.

Recently, Xu et al.[23] demonstrated that the vitrification process can also alter phospholipids composition. Comparing fresh and vitrified buffalo oocytes, they observed that five phospholipids were less abundant in the vitrified group. This finding suggests that lipid composition might be a good indicator of the quality of vitrified oocytes.

Cryopreservation also affects communication between oocytes and the surrounding cumulus cells through the disruption of tranzonal processes, microfilaments that maintain the meiotic spindle in the right position during the maturation process.[24] It was recently observed that the number of cumulus cells attached to the oocyte is associated with gamete energy sufficiency, affecting its lipid and ATP content.[25,26] This is thought to be the main cause of low survival and development rates of cryopreserved immature and *in vitro*-matured oocytes.[24]

Current State

Cryopreservation of oocytes can be performed by both slow freezing and vitrification. While the rapid freezing used in vitrification protects the cell from most damages associated with the chilling process, including membrane damages, it requires the use of higher concentrations of cryoprotectant solutions (CPA) that are toxic. In their 2011 review, Saragusty and Arav[27] concluded that due to its lower cost and ease-of-use, vitrification should be the go-to cryopreservation method for human oocytes. More recently, there has been debate as to whether the 'closed-system' vitrification, which prevents the direct contact of gametes with liquid nitrogen, is safer and at least as effective as the traditional 'open-system', but current data are inconclusive regarding safety issues and indicate that the traditional system has better results.[15,17]

There is also discussion as to whether the cryopreservation of oocytes in the germinal vesicle state is advantageous in comparison to oocytes in the MII stage. So far, this debate has not been resolved due to the lack of data on the viability of immature oocytes post-vitrification. It is, however, an undoubtedly useful resource to avoid the risks associated with ovarian stimulation.[24] In these cases, updated protocols suggest that the oocyte should be vitrified in the company of its surrounding cumulus cells for a better chance of surviving and developing after the thaw process.[28]

The adjustment of current protocols, the refinement of CPA composition and delivery, as well as the upgrade of equipment have been identified as possible next steps to further develop the cryopreservation technique. These should take the 'weak links' observed in oocytes (e.g., cellular membrane and intracellular lipid content) into account to achieve maximum effectiveness.[27,29]

Marques et al.[29] recently tested the effects of different cryoprotectants and calcium in the vitrification media on bovine oocytes. Oocytes were exposed to CPA containing either ethylene glycol, dimethyl sulfoxide, and sucrose (EGDMSO), or 1,2-propanediol and sucrose in the presence or absence of calcium. EGDMSO had the best results, independently of Ca^{2+} concentration in the media. The fatty acid (FA) composition of oocytes and cumulus cells was also assessed. Independently of cell type, concentration of vaccenic acid (c11-18:1) was highest in cumulus–oocyte complexes exposed to EGDMSO with Ca^{2+}, while the lowest was present in cumulus–oocyte complex exposed to 1,2-propanediol, sucrose, and Ca^{2+}.[29] This confirms the influence of CPA solutions in oocyte metabolism; however, more studies are needed to understand the precise effects of technical components on oocyte lipid profile and subsequent development.

As for devices, recent studies point to automated systems, such as microfluidic platforms, which keep the oocyte stationary and exposed to an automatic and gradual flow of CPA, as a promising approach capable of lessening many of the known negative effects on the cell. This would be achieved, however, at the expense of important steps that require visual control, such as morphological selection after the equilibration phase.[2,3,17,30]

ALTERING LIPID METABOLISM AND MEMBRANE COMPOSITION OF OOCYTES: A PROMISING ALTERNATIVE TO IMPROVE CRYOTOLERANCE

Apart from the aforementioned changes in current cryopreservation protocols and equipment, another approach to improve cryosurvivability is to induce changes in the properties of the structures subject to freezing. In the case of oocytes, these are usually made through supplementation of IVM media and/or freezing solutions.[31] The intracellular lipid quantities and the lipid composition of membrane systems are two of the main targets of modulation, since it is known that the susceptibility of gametes to freezing is directly associated to membrane properties and lipid content.[2,32]

The use of lipid metabolism regulators appears to be one of the most promising methods to achieve progress in oocyte vitrification, mainly in domestic mammalian species.[33] Centrifugation and delipidation of oocytes,[34] as well as reducing the concentration of fetal bovine serum used in the culture media and concomitant addition of phenazine ethosulfate, a metabolic regulator,[34-36] improved cryosurvivability reducing lipid accumulation without interfering with embryo quality in species with naturally high concentration of lipids like pigs and cattle.[31,37,38]

Options to decrease oocyte lipid content can involve inhibition of lipogenesis or stimulation of lipolysis processes.[33] The ability to control lipid metabolism is valuable for improving cryotolerance of oocytes as well as to improve the quality of the embryo developing *in vitro*.[39,40] Since endogenous lipids participate in energy metabolism and other important functions, lipid content alterations should be managed with caution, especially if conducted through invasive methods such as delipidation.[41] Just as with the cryopreservation technique and *in vitro* manipulation itself, the use of artificial inducers of lipid metabolism may also have a negative impact on oocyte competence. Many of the negative effects sometimes observed are related to an imbalance of reactive oxygen species (ROS) production and endogenous antioxidants, leading to oxidative stress, which is the cause of many conditions related to female fertility.

The superoxide anion, the product of the one-electron reduction of dioxygen, is the precursor of most ROS. Its dismutation reaction produces hydrogen peroxide, which in turn can be cleaved by catalase in water and oxygen or within the Fenton reaction, partially reduced to the extremely reactive hydroxyl radical.[42] Studies have reported that an increase in hydrogen peroxide levels, as well as ROS and a decrease in catalase activity, trigger the meiotic resumption of oocytes in rats, suggesting the importance of ROS in oocyte maturation.[43,44] There are increasing data on the activity of ROS and antioxidants as a driver of oocyte development and maturation; however, extensive research is needed to identify the safe and physiological concentrations of ROS and antioxidants suitable for oocyte development and cryopreservation. There are currently no studies quantifying how much oxidative stress can affect lipid composition via analysis of the lipid profile, but the use of antioxidant supplementation is recommended.[38]

Further research is needed to comprehend the precise mechanisms of lipid modulation.[41] In the subsequent sections, cases where lipid modulators have positively impacted the outcome of cryopreservation are discussed. In addition to phenazine ethosulfate, other examples of lipid modulators include: carnitine, forskolin, and isomers of linoleic acid. Updated information on the use of these compounds is presented.

Forskolin

Forskolin is a labdane diterpene that activates adenylate cyclase and increases intracellular levels of cAMP. Forskolin has a retarding effect on spontaneous maturation of oocytes and, therefore, this substance is being used to achieve greater synchronicity between nuclear and cytoplasmic maturation *in vitro* leading to improvements of development rates.[45] Monteiro et al.[46] showed the ability of forskolin and other cAMP modulators to minimise the damaging effects of vitrification on the cytoskeleton of immature oocytes after exposure in short term culture; however, it was not enough to improve blastocyst and embryo development.

Forskolin also stimulates lipolysis and was shown to have positive effects on the cryopreservation

of oocytes matured *in vitro*.[47] Paschoal et al.[48] confirmed its lipolytic action on tests with bovine embryos but it failed to improve their cryosurvivability and development potential. Recently, Meneghel et al.[49] reported interesting results. While forskolin supplementation on culture media had no effect on blastocyst yield, pre-treatment with 5.0 µM forskolin for 24 hours before vitrification was shown to decrease intracytoplasmic lipid content and improve the cryotolerance of bovine embryos. Its precise mechanism of action and how it affects cryotolerance is currently unknown.[50]

L-Carnitine

In animal cells, the ammonium compound L-carnitine acts as an enhancer of lipid metabolism due to its primary role in FA transport from the cytoplasm to the mitochondria. Triacyglycerols are metabolised by lipases from both cumulus cells and the oocyte. Therefore, FA generated by lipolysis are metabolised by β-oxidation in the mitochondria for the production of ATP.[39] The use of L-carnitine led to greater development of blastocysts in mice,[51] increased mitochondrial activity, and reduced intracellular lipid content and levels of ROS, which improved nuclear maturation and cleavage of porcine oocytes.[52] A brief exposure of porcine MII oocytes to 3 mM L-carnitine shortly before IVF improved post-warming survival rate of blastocysts.[53] In cattle, this led to higher hatchability rates on fresh oocytes and protected vitrified oocytes from spindle damage; however, L-carnitine exposure failed to improve the development of either fresh or vitrified oocytes.[54] The effects were attributed to the capacity of the compound to reduce cellular lipid content and provide antioxidant protection. Despite the incongruent results, it is a candidate reagent for non-invasive improvement of oocyte cryotolerance and developmental competence in oocytes of domestic animals with high lipid concentration. Its action is likely caused by an improvement of mitochondrial function.[23] Acetyl-L-carnitine, an ester of L-carnitine, has been used in some studies with similar results. Further studies are needed to determine the precise mechanism of action of carnitine compounds and the optimal protocol to replicate the improvements on human oocytes.[23,53,55,56]

Conjugated Isomers of Linoleic Acid

The conjugated isomers of linoleic acid (CLA) have been identified as promising lipid modulators. Tests with CLA in cattle demonstrated its ability to improve cryotolerance of embryos cultured in medium with the addition of 100 µM CLA, and improve the overall quality of the blastocyst.[57] These effects were able to overcome the possible negative effects caused by the presence of fetal bovine serum in culture media.[57] The positive effects of CLA were also observed in sheep embryos when used in a lower concentration.[38] The addition of CLA in bovine oocyte maturation medium was able to reduce its lipid content without interfering with the progression of meiosis.[58,59] The precise mechanism of action of CLA has not yet been fully clarified; it is currently believed that CLA undergoes β-oxidation in the mitochondria for energy production or synthesis of FA used in development, similar to other polyunsaturated FA. This increased mitochondrial activity may cause excess ROS production, leading to oxidative stress. An alternative to overcome the negative effects of ROS is the addition of antioxidants to production media.[38] A study by Leão et al.[60] showed a significant improvement in post-vitrification re-expansion of bovine embryos produced in media with CLA supplementation, especially when used in both maturation and culture media. In addition, this study helped to elucidate the mechanism of action of CLA analysing the lipid content of embryonic plasma membrane with a matrix-assisted laser desorption/ionisation-mass spectrometry technique (MALDI-MS). It was observed that CLA not only reduces intracellular lipid content, but it may also cause changes in the composition of membrane phospholipids (PL), particularly phosphatidylcholines (PC), increasing unsaturation levels and, hence, its fluidity.

The effects of dietary delivery of rumen-protected supplementation of CLA was also assessed; the results indicate that CLA may be used to prevent aberrant accumulation of saturated FA, frequently observed in *in vitro* production,[61] and confirms the influence of dietary supplementation on lipid profile. Nevertheless, recent data showed that, although CLA was able to reduce intracytoplasmic lipid

content, CLA addition to IVP media had no effect on post-thaw viability of embryos.[62] Similarly, there was no beneficial effect of supplementing IVM medium with both CLA and L-carnitine on embryo development or post-thaw cryosurvival.[63] Therefore, further studies are necessary to confirm the possible effects on the oocyte and the precise mechanism of CLA action.

Cholesterol

Ideally, a viable cryopreservation process should spare structural lipids present in the bilayer of organelles and plasma membranes. However, it is known to affect all membrane systems within the cell, with full recovery after post-thaw rehydration being an exception.[64] Membrane systems mostly suffer with decreased fluidity; therefore, the most desirable change in cell membranes exposed to low temperatures is to avoid an increase in the viscosity of the membrane lipid bilayer.

Apart from the CLA and other FA supplementations mentioned previously, another strategy used to enhance membrane fluidity to protect the oocyte from cryoinjuries is the addition of cholesterol. The increase in cholesterol content of sperm and oocyte membranes seems to improve cryotolerance by making them more fluid at low temperatures.[65] Arcarons et al.[66] observed that pre-treatment with cholesterol-loaded methyl-β-cyclodextrin, which transfers the cholesterol into cellular membranes, before the vitrification of bovine oocytes did not affect cleavage and embryo development rates. However, it did improve the quality of embryos derived from vitrified oocytes and altered the gene expression related to lipid metabolism.

LIPID PROFILE: ASSESSING THE CHANGES IN LIPID CONTENT

Knowledge of what is really changing on the lipid profile of oocytes is just as important as knowing the impact of metabolism regulation and lipid supplementation on the outcomes of cryopreservation and development.

Mass spectrometry has revolutionised the field of lipidomics; current advances allow for precise and quick analyses with little or no preparation of gamete cells.[67] Examination of the lipid profile of individual and/or pools of up to five oocytes have been performed by distinct mass spectrometry ionisation methods, including MALDI-MS,[13] electrospray-ionisation mass spectrometry,[68] and desorption electrospray-ionisation mass spectrometry.[66] These innovative tools confirmed that the lipid profile is indeed dynamic and showed great potential of such techniques for detailed lipidomic studies in the reproductive field. Using a variety of techniques, studies have successfully quantified lipids in oocytes and determined distinct lipid profiles due to the physiologic or *in vitro* environment that oocytes were exposed to.[8,11,39,69]

Impact of Lipid Profile on Oocyte Cryopreservation

While most studies in the field had mainly observed changes in zygotes and embryos, a few studies presented important findings regarding the alterations on oocyte lipid profile. The aforementioned study by Xu et al.[23] used mass spectrometry to show that phospholipids m/z 728.7 (phosphatidylcholine [PC] 32:3), 746.9 (PC 32:5), 760.6 (PC 34:1), 768.8 (PC P-36:3), and 782.6 (PC 36:4) were more abundant in fresh oocytes than in vitrified oocytes. Lapa et al.[70] tested the effect of CLA on bovine oocyte competence and FA composition. Total lipids and FA profiles were determined by gas chromatography after CLA supplementation to IVM medium. Besides CLA presence, lower levels of arachidonic acid (C20:4n-6) in FA profile of mature oocytes was observed, suggesting oocyte consumption and improving bovine oocyte competence to develop into higher quality embryos. Despite lower levels of arachidonic acid, the total level of polyunsaturated FA was maintained. Cryosurvivability was not assessed, but these results indicate a possible mechanism in which CLA compensates for the polyunsaturated FA used during maturation process, maintaining membrane fluidity.

Supplementation with acetyl-L-carnitine altered the composition of three phospholipids (m/z 734.6 [PC 32:0], 760.6 [PC 34:1], and 782.6 [PC 36:4]) of vitrified buffalo oocytes, all of which were more abundant in the supplemented group.[23]

Further confirming the influence of short-term exposure of lipid supplementation on

the composition of the lipid profile of oocytes, Pitangui-Molina et al.[10] showed that the addition of soybean PC to maturation media resulted in greater relative abundances of PL PC (32:1), PC (34:2), PC (36:6), PC (36:4), and PC (38:6) in oocyte membrane, and did so without compromising development rates.

The incorporation of so many polyunsaturated PL is a remarkable finding. Recently, a report suggested that preserving PL during cryostorage is of utmost importance and that PL monitored by MALDI-MS could even be used as possible biomarkers for healthy oocytes after cryopreservation procedure.[68]

Another major step for lipid modulation and its influence on cryopreservation is the possibility of using non-invasive techniques. Besides mass spectrometry, quantification of oocyte lipid is also being explored by Raman microspectroscopy.[71] Interestingly, this technique has been used to non-invasively examine the actin cytoskeleton of sheep oocytes following vitrification and and the structural changes of lipids within lipid droplets during the process.[22,70] Coherent anti-Raman stokes microscopy has been successfully used to quantify lipid content of oocytes from species with known differences, including mouse, human, and pig.[69,71] Differences in the chemical composition of lipid droplets in living oocytes matured in media supplemented with different saturated and unsaturated FA can also be detected using coherent anti-Raman stokes microscopy.[72]

CONCLUSION

Knowledge about the precise changes in lipid profile induced by the addition of different biomolecules, such as FA, PL, and lipid modulators, in the culture medium is of great importance to assess improvements in the quality of oocytes and have direct application on the understanding of oocytes' metabolism and membranes response to freezing and, consequently, to the possible refinement of cryopreservation techniques. Mass spectrometry has been an undeniably valuable tool in this process. The advances of non-invasive techniques for lipid profile assessment and the refinement of equipment and CPA used in oocyte vitrification to better monitor and manage metabolism and cell membranes composition are seen as promising steps to improve overall success rates of female gamete storage. However, it might not be enough since there are many gaps in the knowledge of oocyte lipid dynamics and oocyte metabolism whether applied to cryoresistance or not.

For instance, the lack of studies using human oocytes is strikingly evident. Ethical and procedural barriers are responsible, in part, for this shortage. However, the use of discarded immature oocytes after IVF cycles is a clear viable alternative that is not being explored. While there are plenty of other species being studied, there is a need to define the best animal model for translational studies of oocyte lipidomics and oxidative metabolism.

In addition, accumulated data shows that few groups have directed their attention to the basis of oocyte metabolism and lipid and/or protein dynamics, while many studies focus on finding substances that improve individual aspects of gametes storage and development *in vitro* with disregard to their precise mechanism of action in culture medium and/or freezing solutions.

The identification of classes of lipids of interest is a good start. Important work has been done to single out possible candidates for further research, but now that the influence of lipids on oocyte development has been well-established, the authors believe that forthcoming studies should help to contextualise the results obtained so far. More co-ordinated studies that integrate genetic, metabolomic, and morphologic aspects are needed to deepen our understanding of oocyte lipid metabolism and cryobiology.

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GZEMEA.AST.19.02.0019 | Date of approval: February 2019

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Partial Clinical Remission of Type 1 Diabetes Mellitus in Children: Clinical Applications and Challenges with its Definitions

Author: *Benjamin Udoka Nwosu

Division of Endocrinology, Department of Pediatrics, University of Massachusetts Medical School, Worcester, Massachusetts, USA
*Correspondence to Benjamin.Nwosu@umassmemorial.org

Disclosure: The author has declared no conflicts of interest.

Support: This research was funded by the United States National Institutes of Health (NIH).

Received: 10.06.18

Accepted: 21.11.18

Keywords: Children, honeymoon phase, insulin, paediatrics, partial clinical remission (PCR), Type 1 diabetes mellitus (T1DM).

Citation: EMJ. 2019;4[1]:89-98.

Abstract

The honeymoon phase, or partial clinical remission (PCR) phase, of Type 1 diabetes mellitus (T1DM) is a transitory period that is marked by endogenous insulin production by surviving β cells following a diabetes diagnosis and the introduction of insulin therapy. It is a critical window in the course of the disease that has short and long-term implications for the patient, such as a significant reduction in the risk of long-term complications of T1DM. To promote long-term cardiovascular health in children with newly diagnosed T1DM, three key steps are necessary: the generation of a predictive model for non-remission, the adoption of a user-friendly monitoring tool for remission and non-remission, and the establishment of the magnitude of the early-phase cardiovascular disease risk in these children in objective terms through changes in lipid profile. However, only about 50% of children diagnosed with T1DM experience the honeymoon phase. Accurate and prompt detection of the honeymoon phase has been hampered by the lack of an objective and easily applicable predictive model for its detection at the time of T1DM diagnosis, the complex formulas needed to confirm and monitor PCR, and the absence of a straightforward, user-friendly tool for monitoring PCR. This literature review discusses the most up-to-date information in this field by describing an objective predictive model for non-remission, an easy tool for monitoring remission or non-remission, and objective evidence for the cardiovascular protective effect of PCR in the early phase of the disease. The goal is to present non-remission as an independent clinical entity with significantly poorer long-term prognosis than partial remission.

INTRODUCTION

A literature search was conducted to identify publications addressing the honeymoon phase in children with Type 1 diabetes mellitus (T1DM). Medline, EMBASE, and Ovid were searched using the following search terms: 'clinical remission', 'partial remission', 'partial clinical remission', 'honeymoon phase', 'C-peptide', 'type 1 diabetes', 'children', 'pediatric type 1 diabetes',

and‘paediatrics type 1 diabetes’. Nine papers were excluded because they featured an unclear definition of partial clinical remission.

OVERVIEW AND DEFINITION OF PARTIAL CLINICAL REMISSION IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

A key limitation of the early management of T1DM is the lack of a uniform strategy to prevent early dysglycaemia in non-remitters; these are children and adolescents who fail to experience the honeymoon phase or the partial clinical remission (PCR) phase of the disease.[1-4] T1DM is a disorder of persistent hyperglycaemia resulting from autoimmune destruction of the pancreatic β cells.[5,6] PCR often follows the diagnosis of T1DM and this phase is marked by an increased functionality of the surviving β cells with increased endogenous insulin production.[4,7] PCR typically lasts 3–12 months;[8] however, recent studies have shown evidence for C-peptide production and thus residual β cell function at >5 years following the diagnosis of T1DM.[9] C-peptide is cosecreted with insulin from pancreatic β cells and represents a surrogate marker of residual β cell function. In physiologic concentrations, C-peptide acts to improve both microvascular blood flow and microvascular endothelial function through the release of endothelial nitric oxide.[10] Following the diagnosis of T1DM, serum C-peptide concentration undergoes an initial exponential fall followed by a stable phase of decline that can last for several years.[9] The presence of residual endogenous insulin secretion in patients with T1DM has been linked to a reduced risk of severe hypoglycaemia,[11,12] development of diabetic retinopathy,[13] increases in statural growth in prepubescent children,[14] and improvement in long-term glycaemic control.[2,15] These findings have been recently corroborated by a longitudinal study that reported a significantly reduced risk for chronic microvascular complications at 7-year follow-up in young adults who experienced PCR.[2]

Though remitters have an overall long-term prognostic advantage over non-remitters, this dichotomy is rarely taken into consideration during the early phase of diabetes management, as there is no specified strategy to prevent early dysglycaemia in non-remitters, which represents a key limitation of the early management T1DM in children.[1-4] Research in this field has primarily focussed on prolonging the duration of PCR in remitters. A review of these studies showed differing conclusions because of the severe side effect profile of some of the agents,[16-20] insufficient doses of other experimental agents,[21,22] and, more importantly, the non-standardisation of insulin regimens[16,17,21,22] during these interventions, which confounded the effect of the experimental agents. The immunomodulatory approach is promising, but the regimens tested lack sufficient benefit to justify the risk.[16,17] Autologous haematopoietic stem cell transplantation,[19] an approach that halts autoimmune destruction of targeted tissue and re-establishes tolerance, has huge potential in carefully selected patients, in whom it has resulted in remission for >3.5 years in the best cases. However, its effect is predicated on the existence of functional β cells and so would not be effective in non-remitters.[23] More importantly, its numerous side effects, including alopecia, febrile neutropenia, nausea, *de novo* autoimmunity, infections, and death,[20] have limited its widespread acceptance. Vitamin D supplementation, on the other hand, is safe and may slow T1DM progression,[21,22] but existing studies have caveats that prevent widespread implementation of this recommendation. There is an ongoing randomised control trial evaluating the effect of moderately high-dose vitamin D supplementation on the duration of PCR; its results will be published in 2021.[24] It is therefore crucial to understand the unique disadvantages of non-remitters to ensure that they are protected from early-phase metabolic derangements and the attendant long-term complications of T1DM.

Prevalence of Non-Remission in Children with Type 1 Diabetes Mellitus

The introduction of the gold-standard definition for PCR, the insulin dose-adjusted HbA1c (IDAA1c), in 2009 allowed for a consensus on the estimation of PCR.[8] Based on recent studies, the prevalence of PCR in children and adolescents is approximately 50%.[2,4,25,26] This means that a significant proportion of children and adolescents diagnosed with T1DM will not experience PCR.[1,27-29]

Mechanisms of Non-Remission

The molecular mechanisms underlying remission or non-remission are not fully characterised; however, certain factors play key roles, including increased β cell strain,[30] unfavourable cytokine profile,[31] increased glucagon concentration,[32] and the role of immune mediators and genetic markers. Increased β cell strain is marked by poor processing of proinsulin because it has been shown that overweight male children who are more likely to undergo remission have improved processing of proinsulin.[30] In addition, remitters are reported to possess a distinctive cytokine profile with a less damaging effect on the β cells.[31] Remitters also have a lower glucagon concentration, a finding that is consistent with the premise that glucagon production is suppressed by intra-islet insulin production and release.[32] A study suggesting a key role of immune mechanisms in PCR[33] reported significantly lower concentrations of IFN-γ in remitters compared to non-remitters and controls. A higher frequency of $CD4^+$ $CD25^+$ $CD127^{hi}$ cells, a non-regulatory T cell subset of memory T cells, correlated with a slower rate of T1DM progression,[34,35] supporting the hypothesis of a protective role for immune mediators for PCR. Moya et al.[35] reported that a combination of the frequency of the $CD4^+$ $CD25^+$ $CD127^{hi}$ cells with glycaemic markers at the time of diagnosis of T1DM could serve as a predictor of the duration of PCR. A similar study reported that the highest levels of apoptosis of $CD4^+$ $CD25^{+hi}$ T cells are seen in subjects with either new-onset T1DM or those with an increased number of diabetes-associated autoantibodies.[36] Another study found that an increase in islet antigen-specific IL-10-producing cells in subjects with new-onset T1DM correlated with improved glycaemic control, while increased FoxP3 expression in similar subjects predicted a worse outcome.[37] A genetic study[38] reported that the level of circulating microRNA, has-miR-197-3p, at 3 months following the diagnosis of T1DM was a strong predictor of residual β cell function 1 year after the diagnosis of the disease. Thus, a constellation of genetic, immune, hormonal, and inflammatory factors predicts the occurrence and the duration of remission or non-remission.

The Shortcomings of the Singular Focus on the Determinants of Remission at the Expense of Non-Remission

The decision to focus this review on the determinants of clinical non-remission was predicated on the urgent need for a paradigm shift in the approach to the management of children with new-onset T1DM. A clear characterisation of non-remission as an entity would enable clinicians to institute measures to ensure optimal glycaemic control very early in the course of the disease in non-remitters,[3] thus preventing early dysglycaemia. This new approach, which is based on the predictive model for non-remission, will have a significant impact on diabetes complications given the high prevalence of non-remission (>50%) in both paediatric and adult patients.[1,27,29,39] A predictive model would also enhance candidate selection for β cell preservation trials, as well as in trials focussed on the prodromal phase of T1DM, such as the Type 1 Diabetes TrialNet Study.[40]

A recent study of 204 children and adolescents <18 years of age with new-onset T1DM examined the prevalence and key indicators of non-remission and reported a prevalence rate of non-remission of 57.8%.[41] This study further demonstrated that the principal predictors of non-remission in this population were serum bicarbonate <15 mg/dL, younger age at diagnosis, increasing number of diabetes-associated autoantibodies, and female sex.[41] In contrast, male sex and older age were associated with decreased risk of non-remission. One study reported that remission occurred more frequently in younger patients;[42] however, other published studies reported otherwise.[4,8,25,41,43-45] Serum 25-hydroxyvitamin D did not affect the risk of non-remission. This study further quantified the glycaemic cost of non-remission by demonstrating a prolonged period of a significantly elevated HbA1c level of 3–18 months post-diagnosis in non-remitters compared to remitters[25] (Figure 1).

MONITORING OF REMISSION AND NON-REMISSION

Despite the adoption of IDAA1c as the gold-standard marker for PCR, there is no consensus

on a simple and user-friendly tool for the detection and monitoring of PCR in children and adolescents.[4,26,41] This has limited the application of IDAA1c as a universal tool for the monitoring of PCR in children with new-onset T1DM. Therefore, the next challenge in preventing early-phase dysglycaemia in children with T1DM was to develop a simple, user-friendly tool for the monitoring of both remission or non-remission to ensure the institution and maintenance of intensive glycaemic control in these patients, especially the non-remitters who lack the honeymoon-associated endogenous protection from early dysglycaemia and the attendant long-term complications.[1,11-13,27-29,46]

The Drawbacks of the Gold Standard Marker for the Detection of Partial Clinical Remission: Insulin Dose-Adjusted HbA1c

The IDAA1c formula is expressed as HbA1c (%) + (4 x total daily dose of insulin [TDDI] [units/kg/24 hours]). This formula, which integrates both HbA1c and TDDI, has been validated in multiple cohort studies;[4,7] however, this surrogate marker of serum C-peptide concentration has been criticised for its numerous shortcomings.[4] The first major drawback is that age, which is a principal determinant of PCR, is not included in the formula.[8] Secondly, IDAA1c underestimates PCR in younger children who often have lower serum C-peptide concentrations given their smaller pancreatic β cell mass, as the formula for IDAA1c was derived using a higher C-peptide cut-off value of 300 pmol/L, instead of the 200 pmol/L validated by the Diabetes Control and Complications Trial.[3,8] Thirdly, IDAA1c underestimates PCR frequency in older, overweight European females with insulin resistance,[4] as IDAA1c is unable to discriminate between insulin sensitivity and insulin secretion.[4] IDAA1c also has poor sensitivity for the risk of hypoglycaemia in patients with newly diagnosed T1DM[3] and the multistep approach to the calculation of IDAA1c represents a major barrier to its adoption by busy clinicians, which limits its widespread use in endocrine clinics. The final shortcoming of this formula is that IDAA1c may not be generalised to all children with T1DM because it was derived from a cohort of European and Japanese subjects, who have markedly different diabetes characteristics from the general population of the USA.[3,47] Poor generalisation of the IDAA1c was recently demonstrated in the USA in a retrospective study that used IDAA1c to compare the glycaemic and cardiovascular parameters of 80 African-American, 216 Hispanic, and 631 non-Hispanic white (NHW) youths <19 years old with T1DM.[48] The authors reported that African-American and Hispanic youths had higher mean HbA1c and BMI, and a lower frequency of remission, compared to NHW youths.[48] Surprisingly, this study found no statistically or clinically significant differences in lipid parameters between the groups in the first 3 years of disease, despite the persistently higher HbA1c in the ethnic minority youths, suggesting that IDDA1c underestimated PCR in these youths in the USA, similar to the underestimation of PCR in obese European females.[4] In other words, most of the African-American and Hispanic youths classified as non-remitters were false-negatives.[47] This underestimation of PCR is exacerbated by the fact that USA ethnic minority youths with T1DM have a mean HbA1c of 0.4% higher than NHW youths for the same mean glucose concentration.[49,50] They also have a more rapid rise in HbA1c trajectory from the time of diagnosis onwards.[51] As a result of these shortcomings, there is now a call to design an ethnic group-specific IDAA1c value for ethnic minorities in the USA,[47] and for additional research to clarify the usefulness and performance of IDAA1c in routine clinical practice.[4] Specifically, there is a need to determine age, sex, and demographic-specific IDAA1c limits for the definition of PCR based on the serial determination of serum C-peptide concentration to ensure that the false-negative rate from the current criterion is reduced and that all remitters are accurately identified.[4,47,52,53]

The Comparison of Insulin Dose-Adjusted HbA1c to Other Markers of Partial Clinical Remission

A comparison of the IDAA1c to earlier definitions of PCR,[26-30,47,54,55] such as HbA1c ≤7.5%, TDDI ≤0.5 units/kg/day,[54] or combinations of the parameters, such as HbA1c ≤7.5% and TDDI ≤0.5 units/kg/day,[55] showed that while IDAA1c has a stronger correlation with stimulated C-peptide concentration than previous definitions,[8] IDAA1c is less sensitive than TDDI <0.5 units/kg/day for

early detection of PCR, but is more specific for the detection of PCR between 6 and 12 months.[8] Furthermore, the use of either TDDI ≤0.5 units/kg/day or HbA1c <7.5% in isolation was found to overestimate the prevalence of PCR, although the use of a combination of TDDI ≤0.5 units/kg/day and HbA1c <7.5% underestimated PCR.[8] Recently, a Belgian group, citing the above shortcomings of the IDAA1c, developed a new tool for the detection of PCR called the glycaemic target-adjusted HbA1c,[26] which has a sensitivity of 72% to detect PCR based on the IDAA1c definition.

Given the drawbacks of the IDAA1c and the shortcomings of the other definitions, a recent study compared IDAA1c to a new, straightforward, user-friendly definition of PCR: TDDI <0.3 units/kg/day.[25,56] This comparison was based on the hypothesis that a TDDI <0.3 units/kg/day could fall in an intermediate position between the detection potential of TDDI ≤0.5 units/kg/day[54] and the combination of HbA1c <7.5% and TDDI ≤0.5 units/kg/day.[55] The rationale for this investigation was that endocrinologists, who routinely calculate TDDI during regular clinic visits, would find the application of TDDI <0.3 units/kg/day more practical and user-friendly for early detection and monitoring of either remission or non-remission than the IDAA1c, which would ensure a greater acceptance by clinicians. The results of this study, which was conducted in a USA population, showed that TDDI <0.3 units/kg/day and IDAA1c ≤9 identified a similar proportion of patients entering PCR: 40.2% versus 42.2%, respectively, with both criteria showing peak prevalence of remission at 6–12 months and a similar longitudinal HbA1c pattern in the first 3 years of disease (Figure 1). Specifically, Figure 1 shows that when PCR was defined by IDAA1c ≤9 criterion, mean HbA1c was similar at diagnosis between the remitters and non-remitters; thereafter, HbA1c became significantly lower in the remitters from 3–18 months and remained non-significantly lower in the remitters thereafter. When PCR was defined by TDDI <0.3 units/kg/day, HbA1c level appeared significantly lower in the remitters at diagnosis and at 3 months, and then became non-significantly lower in the remitters until Month 15, when the mean HbA1c values became similar between the groups. This study concluded that, in a head-to-head comparison, the criterion TDDI <0.3 units/kg/day was noninferior to IDAA1c, as both criteria showed similar sensitivity and specificity for the detection of PCR in children. However, TDDI <0.3 units/kg/day had the advantage of being a readily accessible tool for prompt detection and monitoring of PCR in a busy clinic setting. Therefore, the adoption of the routine application of TDDI <0.3 units/kg/day in clinical practice should improve the surveillance for both remission and non-remission to ensure the maintenance of euglycaemia in children requiring insulin doses >0.3 units/kg/day.[25]

CLINICAL CONSEQUENCE OF NON-REMISSION

The next challenge in the characterisation of PCR in children and adolescents is to provide objective proof of the deleterious cardiovascular effects of non-remission in the early phase of T1DM, specifically within the first 5 years. Such a demonstration could provide the basis for recommendations for early detection and monitoring of PCR in children to prevent the long-term microvascular and macrovascular complications that begin in the first few years of disease.[57] This is particularly important because early changes in lipid profile between remitters and non-remitters have not been previously reported.[52]

Pathobiology of Early-Phase Dyslipidaemia in Children with Type 1 Diabetes Mellitus: The Need to Stratify Patients by Partial Clinical Remission History in the Investigation of Differences in Lipid Profile

Despite the report by the Diabetes Control and Complications Trial of a protective role of C-peptide on vasculature in remitters,[15] there have been no data on the characterisation of early-phase dyslipidaemia in remitters and non-remitters until recently.[52] A review of the current literature on dyslipidaemia in children and adolescents with T1DM showed no consensus on lipid pattern and it is believed that a lack of stratification of subjects by PCR history may have confounded these results.[58-61] While one longitudinal study reported that 25% of youths with T1DM have progressive and persistent dyslipidaemia and increased arterial stiffness,[58]

another study in children and adolescents with poorly controlled T1DM found a positive association between increased arterial stiffness and total cholesterol, low-density lipoprotein (LDL) cholesterol, and HbA1c.[59] In contrast, a longitudinal retrospective cohort study in a similar population with T1DM found that changes in HbA1c and BMI z scores had minimal impact on LDL cholesterol and non-high-density lipoprotein cholesterol.[60] Furthermore, whereas some studies reported a significant relationship between poor glycaemic control and dyslipidaemia in T1DM,[58,60] others reported an inconsistent pattern of correlation of lipids and HbA1c,[62] or no correlation at all.[63]

However, none of the aforementioned studies explored the differences in lipid profiles based on patients' remission statuses, except Redondo et al.,[48] whose findings were confounded by the underestimation of PCR by IDAA1c in ethnic minority youths.[47] The stratification of children and adolescents with new-onset T1DM by PCR status is of fundamental importance to ensure meaningful comparisons between the groups to reduce the inconsistencies in lipid outcomes, especially LDL cholesterol concentration, in previous studies.[58-61] For instance, it is possible that the study that reported progressive and persistent dyslipidaemia[58] could have contained a higher proportion of non-remitters, while the study that reported only a modest effect of HbA1c and BMI on lipid parameters[60] might have had a higher proportion of remitters. The fact that non-remitters make up >50% of children and adolescents with new-onset T1DM[27,29] makes it crucial to stratify subjects based on PCR history in all investigations in circulating lipid concentrations in patients with T1DM.

Furthermore, the characterisation of early-phase dyslipidaemia in T1DM, specifically the changes in LDL cholesterol, based on stratification by PCR is imperative because atherosclerosis originates in childhood and early adolescence,[64,65] and dyslipidaemia is a primary contributor to the increased risk of cardiovascular disease in patients with T1DM.[64,65] Such an emphasis may clarify the possible role of non-remission as a non-modifiable risk factor for dyslipidaemia in T1DM.

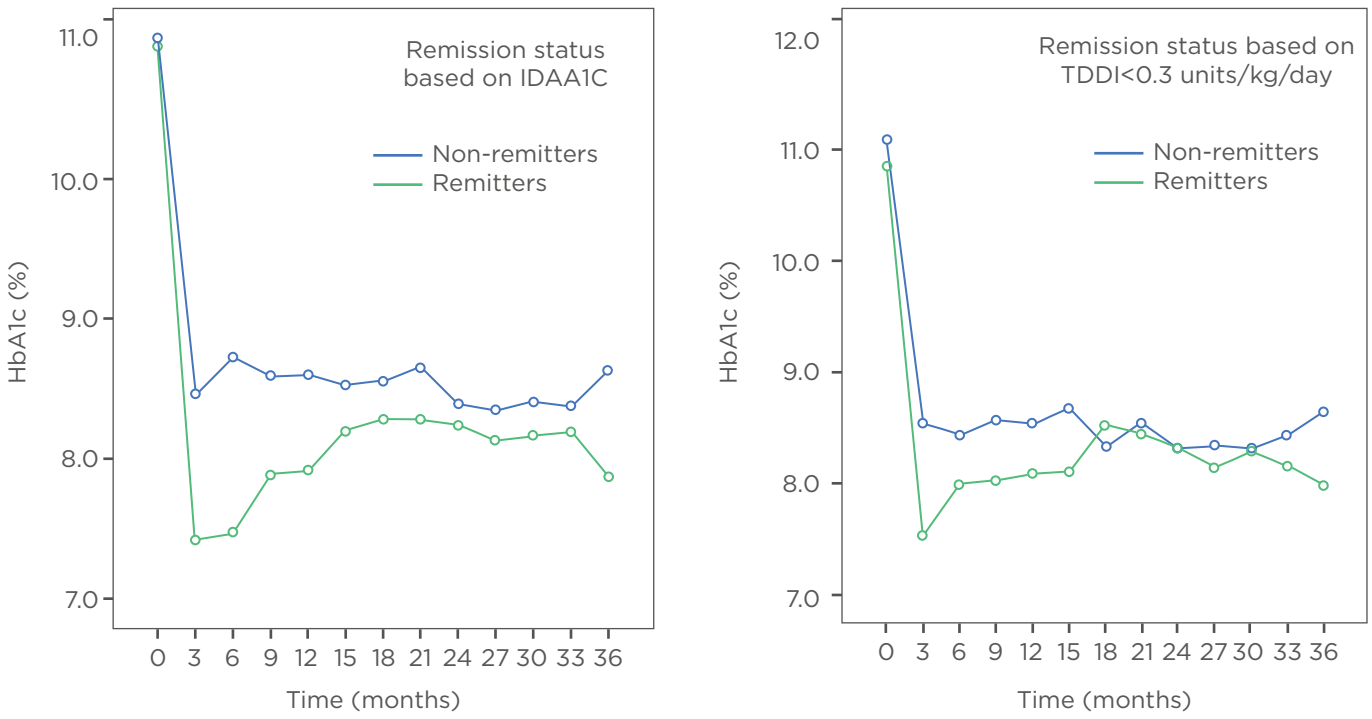

Figure 1: A longitudinal representation of the patterns of haemoglobin A1c (HbA1c) excursions in remitters and non-remitters in the first 3 years of diagnosis of Type 1 diabetes mellitus using either insulin dose-adjusted HbA1c ≤9 or total daily dose of insulin <0.3 units/kg/day.[7,8]

IDAA1c: insulin dose-adjusted HbA1c; TDDI: total daily dose of insulin.

The first step to address this came from a recent longitudinal retrospective cohort study of 123 children and adolescents with T1DM of 5-year duration.[66] The subjects' mean age was 11.9±2.9 years and the cohort consisted of 55 male subjects and 68 female subjects. There were 44 remitters and 79 non-remitters. A timeline of 4–5 years after diagnosis was chosen in concert with the American Diabetes Association (ADA) recommendation to initiate screening for diabetes complications in children either at the inception of puberty or 4–5 years after diagnosis,[67] because it was previously believed that there was minimal risk of dyslipidaemia during the prepubescent years. This study excluded children with dyslipidaemia or a family history of lipid abnormalities. The results showed that children and adolescents who experienced PCR had significantly lower mean LDL cholesterol 4–5 years after the diagnosis of T1DM compared to their peers who did not experience PCR[52] after controlling for age, puberty, glycaemic control, and adiposity. The significantly lower LDL in remitters was rather striking because a greater proportion of the remitters were in puberty 4–5 years after the diagnosis of T1DM compared to the non-remitters. This is because a previous report indicated that pubescent youths with T1DM had elevated LDL cholesterol compared to their healthy peers,[63] and this was attributed to the fact that children with T1DM do not show the usual pattern of decreasing LDL cholesterol during puberty.[63] Hence, this study clarified the previous report by demonstrating that remitters in puberty had similar mean LDL cholesterol concentrations as children and adolescents without diabetes, a finding that has not been previously reported. This report advances the field by providing critical and objective evidence of early cardiovascular protection by PCR. Larger studies are needed to confirm these differences in lipid fractions between remitters and non-remitters.

CONCLUSIONS AND SUGGESTED CHANGES TO THE LIPID MONITORING GUIDELINES OF THE INTERNATIONAL SOCIETY FOR PEDIATRIC AND ADOLESCENT DIABETES (ISPAD) AND THE AMERICAN DIABETES ASSOCIATION (ADA)

This literature review details new developments in the clinical application of the honeymoon period of T1DM by focussing on non-remission as a specific clinical entity with poorer long-term prognosis than partial remission. The author hopes to advance the field by presenting strategies to predict and monitor non-remission, as well as a justification for the initiation of lipid monitoring at the time of diagnosis of T1DM in children and adolescents. This review has summarised the genetic, immune, inflammatory, and biochemical markers that could predict remission and non-remission. It has also characterised the first clinical predictive model dedicated to non-remission in children with new-onset T1DM that is based on easily obtainable clinical parameters at the time of T1DM diagnosis, such as age, sex, BMI, body pH, number of diabetes-associated autoantibodies, and serum bicarbonate. It further reviewed a study showing that a TDDI <0.3 units/kg/day is noninferior to the gold standard marker of PCR, IDAA1c ≤9. Finally, it reviewed the results of a 5-year longitudinal study showing evidence for early-phase dyslipidaemia in non-remitters who had a significantly elevated LDL cholesterol level compared to remitters after adjusting for confounding variables. Though larger studies are needed to confirm these early findings, the distinct pattern of the early changes in LDL cholesterol in this recent study[52] could explain the dichotomy in the prevalence of long-term complications of T1DM between remitters and non-remitters.[15] Significantly elevated LDL cholesterol in non-remitters makes a strong case for targeted lipid monitoring in T1DM because it suggests that non-remission could be a non-modifiable risk factor for cardiovascular disease in T1DM. Equally, the early divergence in serum LDL cholesterol concentration in these paediatric subjects with T1DM supports a modification of the current guidelines by the International Society for Pediatric and Adolescent Diabetes (ISPAD)[68] and the ADA[67]

to recommend the initiation of lipid monitoring at the time of diagnosis of T1DM regardless of the age of the child, in a similar approach to the current guidelines for Type 2 diabetes mellitus.[68] This will ensure an early detection of hypercholesterolaemia in non-remitters that could be amenable to the institution of early therapeutic and dietary interventions coupled with ongoing lipid monitoring.

In conclusion, the adoption of the following measures may serve to improve the long-term cardiovascular health of diabetic children and adolescents well into adulthood: the implementation of a predictive model to detect non-remission; using TDDI <0.3 units/kg/day to more easily monitor remission or non-remission; and the introduction of targeted lipid monitoring at the time of diagnosis in all children with newly diagnosed T1DM. Larger studies including patients of different nationalities are needed to confirm the generalisability of these measures.

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MET Inhibition in Non-Small Cell Lung Cancer

Authors: *Shabnam Rehman, *Grace K. Dy

Department of Medicine, Roswell Park Cancer Institute, Buffalo, New York, USA
*Correspondence to shabnam.rehman@ttuhsc.edu or grace.dy@roswellpark.org

Disclosure: The authors have declared no conflicts of interest.

Received: 16.10.17

Accepted: 13.07.18

Keywords: Hepatocyte growth factor (HGF), mesenchymal–epithelial transition (MET), MET inhibitors, non-small cell lung cancer (NSCLC).

Citation: EMJ Respir. 2018;6[1]:100-111.

Abstract

Cancer treatment paradigms have evolved over recent years with an emphasis on personalised medicine. Targeted agents are being used to improve treatment outcomes and quality of life. For the treatment of non-small cell lung cancer, several agents with unique genetic and epigenetic targets are available. To this extent, mesenchymal–epithelial transition (MET), a heterodimer receptor tyrosine kinase involved in embryogenesis and organogenesis, has been investigated as a potential target for biological agents. MET dysregulation can occur via different mechanisms and trigger tumourigenesis and disease spread. Besides driving the oncogenic dependence of cells, MET is also involved in acquired resistance to epidermal growth factor receptor inhibitors. As such, many small molecule kinase inhibitors and antibodies have been developed or are currently in different phases of clinical trials to counteract the MET-induced neoplastic activity. Some of these agents are selective while others are nonselective with multiple other potential targets. This article aims to present an overview of biological functioning of MET, its role in oncogenesis and resistance to treatment, and clinical studies evaluating MET inhibitors for treatment of non-small cell lung cancer.

INTRODUCTION

There has been a decrease in the number of incidences of lung cancer recorded among men over the past few decades as well as among women in the last decade,[1] which has been attributed to the advances in screening methods, surgical and radiation techniques, and new therapeutic modalities. In non-small cell lung cancer (NSCLC), many targetable receptors or protein kinases linked to complex cascades of signalling pathways have been identified to be oncogenic and/or dysregulated by oncogenic processes. The *c-MET* proto-oncogene, along with its protein product, MET (mesenchymal–epithelial transition; also known as hepatocyte growth factor [HGF] receptor) and cognate ligand is one such example of the potential drug targets that are being investigated in NSCLC and will be the focus of this review.

The *MET* proto-oncogene is located on chromosome 7 at locus 7q31. Its protein product is a 170 kD receptor tyrosine kinase, with highly glycosylated extracellular α-subunit and a transmembrane β-subunit, linked together by a disulfide bond (Figure 1).[2] The extracellular subunit contains the semaphorin domain, cysteine-rich MET-related sequence domain, and four immunoglobulin-plexin transcription domains. The β-chain contains a juxtamembrane domain, an intracellular tyrosine kinase domain that mediates MET

biological activity, and a tail on the C-terminal, which is vital for the docking of substrates and downstream signalling.[3] The juxtamembrane domain contains a serine residue (Ser 985), which inhibits the receptor kinase activity upon phosphorylation of a tyrosine molecule (Tyr 1003), which is responsible for MET polyubiquitination, endocytosis, and degradation upon interaction with the ubiquitin ligase Casitas B-lineage lymphoma (CBL).[4] Two ligands of MET have been identified, mammalian HGF and scatter factor, along with their splicing isoforms and a bacterial leucine-rich surface protein named Internalin B.[5] *MET* transcription is activated by HGF and other transcription factors, including hypoxia inducible factor-1 (HIF-1), leading to dimerisation and transphosphorylation of tyrosine residues in the intracellular domain that engages signal transducer proteins leading to downstream signalling. Some transducers, such as Grb2, Shc, Src, and the p85 regulatory subunit of phosphatidylinositol-3 kinase (PI3K), interact with the docking site of MET either directly or indirectly through the scaffolding protein Gab1.[6,7] Phosphorylation of Gab-1 by MET interaction results in a sustained signal that mediates most of the downstream signalling pathways culminating in a range of biological activities, including mitogenesis, angiogenesis, morphogenesis, scattering, motility, migration, survival, and invasion.[4,8]

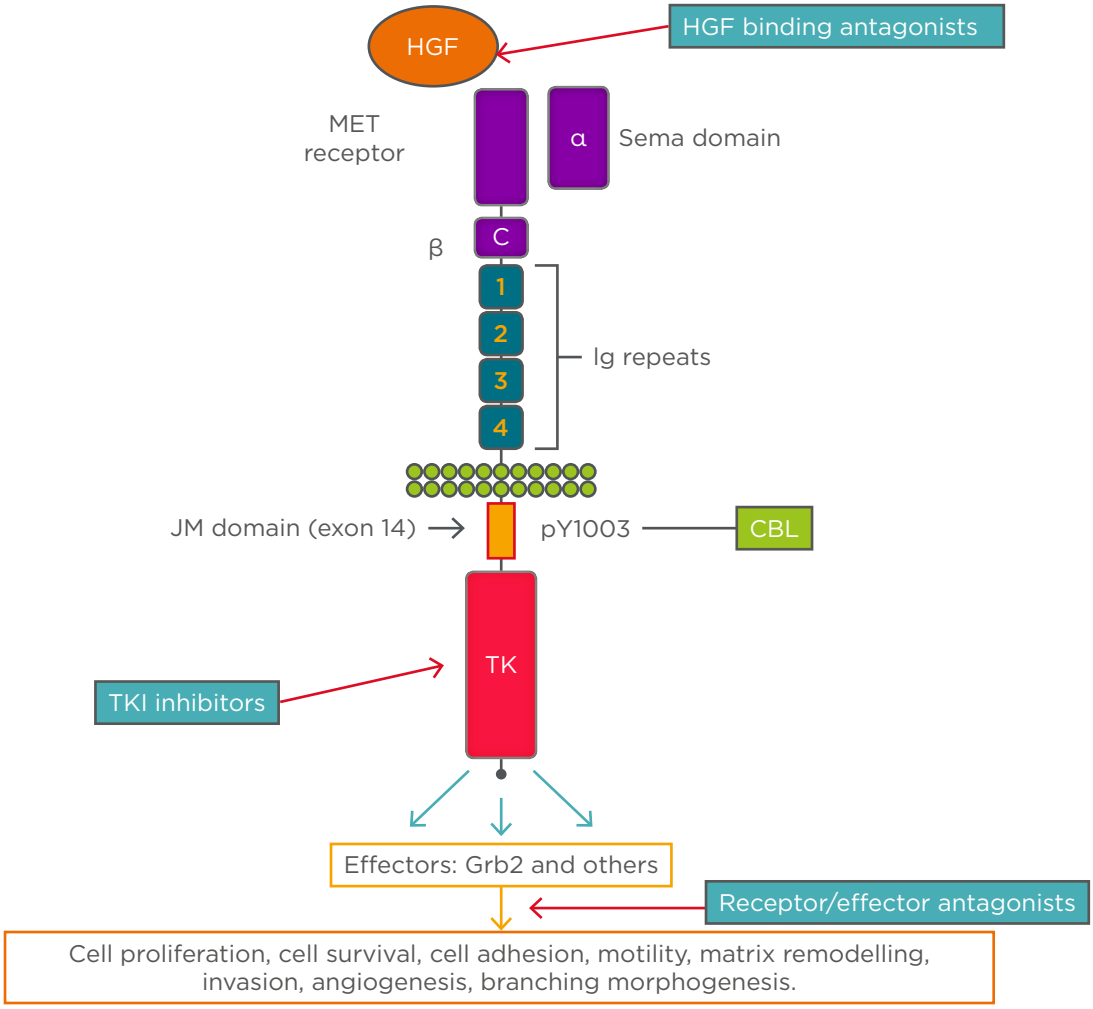

Figure 1: A schematic representation of cMET receptor and an overview of signalling and cellular effects. The figure also shows the sites of action of various cMET directed targeted agents along the receptor-signaling pathway.

C: cysteine-rich region; CBL: Casitas B-lineage lymphoma; HGF: hepatocyte growth factor; JM: juxtamembrane; MET: mesenchymal–epithelial transition; TK: tyrosine kinase domain; TKI: tyrosine kinase inhibitor.

Adapted from Ma.[2]

Different signal transduction pathways activated via MET engagement mediate different biological activities.

Ligand-independent activation of MET also occurs, which involves either the cell adhesion complex α5β1 integrin and fibronectin, or transactivation by other cell surface receptors, such as ErbB/HER family receptors or the interaction of Semaphorin 4D with its receptor Plexin B1.[9,10]

Cross talk between MET with other signalling pathways and the potential development of resistance to their receptor inhibitors is also well known. This is particularly seen in NSCLC where increased expression of epidermal growth factor receptor (EGFR)/HER family receptors directly leads to the transactivation of MET or vice-versa, and MET activation of Src indirectly leads to EGFR phosphorylation and creation of docking sites for EGFR transducers.[9] MET has also been found to co-regulate oncogenic signalling pathways in collaboration with tumour suppressor genes (*PTEN*,[11] *VHL*[12]) and promote angiogenesis and invasiveness of vascular endothelial growth factor (VEGF) via transcriptional upregulation of hypoxia-inducible factors.[13]

ROLE OF MET INHIBITION IN NON-SMALL CELL LUNG CANCER

MET Gene Mutations

A few gene mutations in the *MET* coding sequence have been found with varying degrees of relevance to oncogenesis. Somatic intronic mutation in the juxtamembrane domain, resulting in loss of CBL-E3 ligase binding, was characterised to demonstrate elevated MET expression and prolonged ligand-dependent MET activation.[14] Normally, introns flanking *MET* exon 14 are spliced out, resulting in an mRNA transcript with an intact exon 14 coding for CBL-E3 ligase binding site. The mutation disrupts the splicing sites, resulting in aberrant splicing and skipping of exon 14 in the transcript. With the loss of the CBL-E3 ligase binding site, there is decreased polyubiquitination, delayed downregulation by the receptor, and sustained MET activation. Various exon 14 splice site alterations exhibiting diverse sequence composition that trigger constitutive activation of MET have been identified. More than half of these are indel mutations, many of which are novel. This diversity poses a diagnostic challenge and highlights the need for appropriate laboratory and analytical methods that can detect these alterations with high sensitivity and specificity. Approximately 3.5% of *MET* exon 14 alterations act as the driver mutation in lung adenocarcinomas, portending poor overall survival.[15] *MET* exon 14 alterations are found in about 20.0% of pulmonary sarcomatoid carcinomas, a rare histologic subtype that is relatively refractory to conventional cytotoxic therapies.[15,16] In a study of 933 patients with non-squamous NSCLC, patients with *MET* exon 14 alterations did not harbour activating mutations in *KRAS*, *EGFR*, or *ERBB2*, or rearrangements involving *ALK*, *ROS1*, or *RET* proto-oncogenes and as such were mutually exclusive to other oncogenic drivers.[17] However, they can overlap with other alterations such as MET amplification or copy number gain and *MDM2* proto-oncogene amplification.[15] The incidence of this overlap is dependent on the definition of amplification used. The presence of *MET* exon 14 mutations was associated with advanced age and stage-dependent MET amplification, with a higher disease stage indicating a more likely presence of concurrent MET amplification. The presence of concurrent genomic alterations as potential modifiers of response to MET inhibition in *MET* exon 14–altered lung cancers will require exploration. Mutations in the MET tyrosine kinase domain have rarely been found in NSCLC as a secondary event resulting from exposure to prior therapies, such as tyrosine kinase inhibitors (TKI).[18]

In vitro, both small molecule TKI and MET-directed monoclonal antibodies have been found to be active in cell lines harbouring *MET* exon 14 alterations.[14,17] These results have been replicated in the clinic, with a number of case reports of patients with lung adenocarcinoma harbouring exon 14 mutations with a clinical response to MET inhibition with TKI. In the majority of reports, the most frequent exon 14 alteration was splice donor mutation and partial responses to either selective or multi-targeted kinase inhibitors.[14,16,17,19-21] This has further increased the impetus in drug development for these alterations and clinical

trials investigating the therapeutic efficacy of both multi-targeted agents or selective MET kinase inhibitors are ongoing. No literature is yet available documenting clinical responses in these patients with the use of anti-MET or anti-HGF antibodies.

MET Gene Amplification

Amplification of the *MET* gene has been reported as the primary oncogenic event in 2–4% of TKI-naïve NSCLC and the secondary event in 5–20% in EGFR mutant NSCLC with acquired resistance to EGFR TKI.[22] An increased copy number of the *MET* gene can be detected by fluorescence *in situ* hybridisation (FISH) or reverse transcriptase-PCR. *MET* gene amplification is expressed as the level of gene copy number gain (Capuzzo scoring system) and MET:CEP7 ratio. More than five signals per cell for copy number gain and a MET:CEP7 ratio >2 are both considered positive results for gene amplification. Setting the cut-off point for positivity too low may dilute the apparent drug benefit and fail to distinguish the true driver state.[23] In the absence of oncogenic overlap with other drivers, lower ratios may signify a MET-associated phenotype. Nonetheless, *MET* amplification has been associated with a poor prognosis in patients with NSCLC[24] and offers a targetable alteration.

Using *MET* gene amplification as a biomarker to predict response to MET inhibitors has been explored. In a Phase I expansion cohort, 13 *MET*-amplified NSCLC patients were treated with crizotinib.[25] *MET* amplification status was determined by FISH and expressed as MET:CEP7 ratio. Patients were categorised into three groups based on the ratio: low with a MET:CEP7 ratio of ≥1.8–≤2.2 (n=1), intermediate for a ratio of >2.2–<5.0 (n=6), and high for a ratio of ≥5.0 (n=6). A response rate of 33% was reported (low n=0, intermediate n=1, and high n=3). It should be noted that *MET* exon 14 alterations harbour concurrent high-level *MET* copy number gain in approximately 20% of cases.[15,17,19] In patients with *EGFR* mutations, secondary *MET* amplification leads to acquired EGFR TKI resistance by transactivating ErbB3 signalling.[26] This has provided the rationale for various further clinical trials exploring the combination of MET and EGFR TKI in patients with mutant EGFR (Phase II expansion of TATTON trial,[27] Phase II expansion of INSIGHT trial[28]).

MET Gene Fusion and Rearrangement

MET was first identified when the oncogenic chromosomal rearrangement *Tpr-Met* was induced in a sarcoma cell line.[29] Though *MET* fusion gene products are not frequently found, they have recently been documented in lung adenocarcinoma. Stransky et al.[30] demonstrated translocation events involving *MET* across different tumour types. Specifically in lung adenocarcinoma, fusion of the dimerisation motif to intact kinase domain led to generation of a chimeric fusion protein, KIF5B–MET. This novel fusion could explain part of the *MET* oncogenic activation and represent a potential therapeutic target.

Hepatocyte Growth Factor and Hepatocyte Growth Factor Receptor Overexpression and MET Activation

Studies have shown that higher levels of the HGF ligand can lead to aberrant MET activation and signalling independent of the changes at the receptor level.[31] Increased expression of stromal-derived HGF was found to mediate VEGF-receptor (VEGFR) inhibitor-resistance and vascular remodelling in NSCLC by altering hypoxia-regulated molecules.[32] Overexpression of the receptor itself without gene amplification, for example, by promoter demethylation, can result in MET upregulation.[33] Similarly, modifications at the translational level can result in MET overexpression.[34]

Overexpression of HGF and activation of MET has been shown to confer resistance to chemotherapy agents. Specifically in NSCLC, in a study by Chen et al.,[35] HGF was shown to induce cisplatin resistance via MET by activating focal adhesion kinase (FAK) and down regulating the expression of apoptosis-inducing factor. MET upregulation and increased secretion of HGF has been shown to occur in response to ionising radiation. In a preclinical study by Gao et al.,[36] inhibition of MET led to radio sensitisation of cancer cell lines. Whether these processes occur in NSCLC post-radiation and mediate resistance and distant spread is currently unanswered.

Table 1: Summary of IC50 data from available literature for oral MET TKI.

Drug	Study	Type of inhibitor	Targets with IC50 in nM (except otherwise indicated)													
			MET	ALK	VEGFR1	VEGFR2	VEGFR3	ROS	KIT	RET	AXL	RON	PAK3	FLT3	FLT4	TIE-2
Selective MET inhibitors																
Tivantinib	Srivastava et al.,[37] 2013	ATP non-competitive	327									>10 µM	6.6 µM		16 µM	
Bozitinib	Shih et al.,[38] 2017	I	8													
Capmatinib	Liu et al.,[39] 2011	Ib	0.13													
Savolitinib	Jia et al.,[40] 2014	Ib	5													
Tepotinib	Bladt et al.,[41] 2013	Ib	3								1,566	>1,000				
Multikinase inhibitors																
Crizotinib	Zou et al.,[42] 2007; Zou et al.,[43] 2015	Ia	11	24				0.6								
Cabozantinib	Srivastava et al.,[37] 2013; Feneyrolles et al.,[44] 2014	II	1.3			0.035		<25	4.6	5.2	7	124		11.3		14.3
Merestinib	Yan et al.,[45] 2013; Feneyrolles et al.,[44] 2014	II	4.7					23		11	2	0.8		7		
Glesatinib	Bonfils et al.,[46] 2012	II	1		3	3	4					2				7
Sitravatinib	Bauer et al.,[47] 2016		10–25			<10				10–25	<10					

The table shows both selective and multikinase inhibitors along with the half maximal concentration of a particular inhibitor required for inhibition of its target molecule, expressed in nanomoles.

MET: mesenchymal–epithelial transition; TKI: tyrosine kinase inhibitor.

MET INHIBITORS

Small Molecule Inhibitors

Capmatinib

Capmatinib is an ATP-competitive Type Ib selective inhibitor of MET that has shown anti-tumour activity by blocking c-MET dependent signalling and cross talk with EGFR and HER-3 in mouse xenograft models (Table 1). In a Phase I study in patients with advanced solid tumours, including a cohort of EGFR-wild-type MET-mutated, amplified, or rearranged NSCLC patients, strong preliminary activity was observed in pre-treated patients with a high *MET* gene copy number (≥6) or MET overexpression of >3 as measured by immunohistochemistry (IHC). The objective response rate (ORR) was 47% and 24%, respectively. Significant tumour volume reduction of >45% was also observed in four patients with *MET* exon 14 alteration via molecular profiling.[48] Capmatinib is being further investigated in a Phase II trial in NSCLC patients with *MET* exon 14 mutation (GEOMETRY mono-1)[49] and in combination with erlotinib in patients with EGFR TKI-resistant NSCLC and acquired *MET* amplification (GEOMETRY duo-1).[50]

Tepotinib

Tepotinib is a Type Ib ATP-competitive selective MET inhibitor that has shown anti-tumour activity in patients with MET overexpressed or amplified NSCLC (Table 1). In a Phase Ib trial,[51] combination of tepotinib and gefitinib

was investigated in Asian patients with MET+/EGFR-mutant NSCLC. MET positivity was determined by IHC.[51] Patients who had received a median of two prior lines of therapy, including an EGFR TKI, were enrolled (n=18). The combination was well tolerated with no dose-limiting toxicities reported and tepotinib 500 mg/day was confirmed as the recommended Phase II dose. Treatment-related adverse effects included increased blood sugar, amylase, and lipase levels, and neutropenia. Partial response was noted in 5 of the 18 patients (n=4 IHC 3+ and n=1 IHC 2+ tumours) and 4 of the 18 patients (IHC 2+, n=3) had stable disease. A Phase II trial of the drug is currently underway in patients with *MET* exon 14-altered NSCLC.[52] Another Phase II trial of tepotinib plus gefitinib or cisplatin/pemetrexed in a 2:1 randomisation is also currently ongoing in patients with Thr790Met-negative and MET+ tumours who have failed first-line gefitinib.[28]

Savolitinib

Savolitinib is another Type Ib potent selective MET inhibitor that has shown anti-tumour activity in preclinical studies and entered Phase I and II studies (Table 1). In a Phase I study in patients with NSCLC, preliminary anti-tumour activity was observed in patients with increased *MET* gene copy number, gene amplification, or high MET protein expression.[53] A Phase II trial of savolitinib is currently ongoing in patients with *MET* exon 14-positive pulmonary sarcomatoid carcinoma.[54] It is also currently being investigated in a single-arm global Phase II study in combination with osimertinib based on encouraging data seen in the Phase I component.[55]

SAR125844 and Bozitinib (CBT101, PLB-1001, CBI-3103) are additional selective MET inhibitors in clinical development. Unlike the other kinase inhibitors discussed previously that are administered orally, SAR125844 is administered intravenously.[56] It demonstrated modest activity, with low partial response rate of approximately 11% in patients with MET-amplified solid tumours. As a result, further development is currently halted. Bozitinib has demonstrated superior efficacy compared to crizotinib and capmatinib in preclinical lung cancer models.[38] Several clinical trials are ongoing for bozitinib, including a Phase I study in patients with MET+ NSCLC.[57]

Crizotinib

Crizotinib is a multi-targeted kinase that was initially developed as a Type I MET inhibitor (Table 1) targeting resistance pathways to EGFR TKI; however, subsequent clinical development shifted upon the discovery of oncogenic ALK translocations in NSCLC, hence its first U.S. Food and Drug Administration (FDA) approval for this NSCLC genotype. Crizotinib was tested in patients harbouring *MET*-amplified metastatic NSCLC, with *MET* amplification by FISH used as the selection criteria for patients in a Phase I trial.[25] Patients with intermediate and high levels of MET amplification showed significant tumour shrinkage with response rates of 17% and 67%, respectively. Further study of crizotinib in advanced *MET*-amplified NSCLC to optimise biomarker selection criteria is ongoing. More recently, the anti-tumour activity in patients with *MET* exon 14-altered NSCLC were enrolled into an expansion cohort of the Phase I PROFILE 1001 study. Partial responses were observed in 8 out of 18 patients, with an ORR of 44%, and 9 patients had stable disease.[58] Enrolment of patients in this trial continues and further analyses are awaited. It is also being tested in the UK as part of the National Lung Matrix trial for patients with *MET* exon 14-altered lung cancer.[59] Crizotinib in combination with erlotinib was also investigated as part of a Phase I trial.[60] At a maximum tolerated dose of 150 mg of crizotinib, 1 of the 18 patients had partial response and 6 had stable disease. Reports of response to crizotinib in EGFR-mutant NSCLC patients are also emerging in the literature for those who have *de novo MET* mutations or acquire these during treatment with EGFR TKI, highlighting the role of MET alterations as mechanism for resistance to EGFR TKI and providing rationale for combination treatment. A significant clinical and radiological response was seen to the combination of erlotinib and crizotinib in a 70-year-old patient with EGFR-mutant NSCLC with *de novo*, high-level MET amplification who experienced primary resistance to erlotinib.[61] Likewise, response to the combination of erlotinib and crizotinib was seen in a patient with primary EGFR-mutant and secondary MET-amplified NSCLC, though a novel crizotinib-resistant mutation MET Gly1108Cys eventually emerged.[62]

Cabozantinib

Cabozantinib is a multikinase Type II inhibitor of MET, ROS1, VEGFR, RET, KIT, and FLT3 (Table 1). It has been studied in a Phase II combination trial with erlotinib in patients with pretreated EGFR-mutant NSCLC patients who were found to have clinical activity, though *MET* amplification was not detected in any of the patients.[63] Complete response to cabozantinib was reported in a case series of patients with concurrent *MET* exon 14-altered and MET-amplified NSCLC.[19] In another case report, response to a combination of cabozantinib and erlotinib was reported in an EGFR and MET inhibitor pretreated patient who developed a new mutation in MET (Asp1228Val).[64] Currently, this is being further investigated in a Phase II trial in NSCLC patients with RET fusion positive/ROS1/NTRK fusion/increased MET or AXL activity.[65]

Multiple additional multikinase MET inhibitors are undergoing Phase I/II studies in NSCLC patients. Glesatinib, a Type II inhibitor, has recently shown clinical activity in a NSCLC patient with *MET* exon 14 skip mutation upon development of resistance to crizotinib, with tumour response demonstrated in a Tyr1230His mutated metastasis.[66] Sitravatinib, an inhibitor of MET, VEGFR2, PDGFRA, TAM family (AXL and MERTK), RET, NTRK1, and DDR2 has demonstrated favourable pharmacokinetic features, with plasma exposure exceeding the projected required levels for antitumour efficacy observed in preclinical studies.[47] Merestinib targets ROS1, AXL, RON, MERTK, FLT3, DDR1/2, MST1R, and MKNK-1/2 in the nanomolar range, in addition to MET. In preclinical studies, it has shown several-fold more potent anti-proliferative activity against tumour cell lines with high *MET* gene amplification than the cell lines without *MET* amplification.[45] Similarly, the study demonstrated a significant anti-tumour effect in two MET autocrine xenograft mouse models (U-87MG, KP4), a model with very high *MET* gene amplification (MKN45), and a model with MET overexpression and low *MET* amplification. Given its impressive multi-phenotypic anti-MET profile, it is being tested further in a Phase I study.[67]

Tivantinib

Tivantinib, thought to be a selective, non-ATP competitive inhibitor of MET, has been tested in many clinical trials (Table 1); however, subsequent studies have indicated that its efficacy is independent of MET signalling, for example through its ability to inhibit polymerisation of microtubules and induce cytotoxic effects.[68-70] In a randomised Phase II trial evaluating activity of tivantinib in combination with erlotinib compared to erlotinib plus placebo in advanced NSCLC patients at progression, a trend was identified with regard to median progression-free survival (PFS) at 3.8 months in the experimental arm versus 2.3 months (p=0.24) in the placebo group.[71] Pre-planned subgroup analyses revealed improvements for patients with non-squamous NSCLC, both in terms of PFS and overall survival (OS) with the combination treatment. Molecular exploratory analysis identified a trend with increasing magnitude of benefit that was directly correlated with level of *MET* gene copy number gain, although this did not reach statistical significance.

This provided the rationale for the subsequent Phase III MARQUEE study, comparing tivantinib plus erlotinib versus erlotinib alone in non-squamous NSCLC patients.[72] However, the trial was terminated early due to negative results from a planned interim analysis of the primary end point of OS. During pre-planned subset analyses, PFS was higher with the combination treatment (3.6 months) compared to erlotinib alone (1.9 months; hazard ratio [HR]: 0.74; p<0.001) in the intention-to-treat population. Among patients with high MET expression tumours (as determined by a central laboratory using the Ventana SP44 rabbit monoclonal antibody), defined as membranous staining intensity of at least 2+ in at least 50% of tumour cells, a trend was identified that favoured OS in the combination treatment group (9.3 months) compared to the control group (5.9 months; p=0.03; HR: 0.70; 95% confidence interval: 0.49–1.01). Difference in PFS was similar to that observed for the whole intent-to-treat population. Tivantinib is currently being investigated in hepatocellular cancer but is not being further developed in NSCLC.

Anti-MET Receptor Antibodies

Onartuzumab

Onartuzumab is a humanised IgG1 monovalent anti-MET antibody with a single antigen-binding

fragment (Fab) fused to a complete constant domain fragment (Fc) that inhibits high-affinity HGF binding to MET. Its efficacy was investigated in a Phase II study in combination with erlotinib versus erlotinib alone as second-line therapy for NSCLC patients.[73] It was observed that 52% of patients who were MET positive by IHC analysis had a statistically significant reduction in the risk of disease progression by the addition of onartuzumab (median PFS: 1.5 versus 2.9 months; HR: 0.53; p=0.04). In the MET IHC-negative tumours, onartuzumab was detrimental. On further biomarker analysis, MET IHC was shown to be the most robust predictor of OS and PFS compared to other examined exploratory markers.[74] Investigation of the drug in the Phase III Met Lung study failed to duplicate the positive results of the Phase II study and the trial was closed prematurely after an interim analysis showed no difference in OS.[73] Additionally, patients with EGFR-mutant tumours in the experimental arm seemed to experience detrimental effects from the combination treatment on subgroup analysis. Onartuzumab is not currently being evaluated further as a treatment, though its use may potentially be repurposed through radiolabelling to assess the dynamic expression of MET in tumours as selection criteria for studies, such as clinical trials evaluating MET inhibitors in combination with EGFR TKI.[75]

Emibetuzumab

Emibetuzumab is a humanised anti-MET IgG4 monoclonal antibody that, despite its bivalency and thus potential to cause receptor dimerisation and activation, does not exhibit agonistic activity. It differs from onartuzumab because it also induces degradation of MET. It showed single agent clinical activity in a Phase I study of *MET*-amplified patients with advanced tumours, including NSCLC, with a disease control rate of 26% in patients with MET IHC >2.[76] In another recent Phase I study, emibetuzumab in combination with erlotinib in pre-treated patients with advanced or metastatic NSCLC showed an ORR of 14.3% and a disease control rate of 28.6%.[77] Emibetuzumab is currently undergoing Phase II studies in patients with NSCLC with EGFR mutations and in combination with ramucirumab in patients with advanced solid tumours including NSCLC.[78] Several anti-MET antibodies, including LY3164530, JNJ-61186372, SAIT301, and ARGX-111, have shown preliminary activity in preclinical models and are being actively investigated in Phase I studies.[79]

Anti-Hepatocyte Growth Factor Antibodies

Ficlatuzumab

Ficlatuzumab is an anti-HGF antibody that binds to HGF and inhibits its binding to MET. In a Phase I trial of ficlatuzumab in patients with advanced solid tumours including NSCLC, stable disease was observed in 44% of patients who received the drug as monotherapy or in combination with erlotinib.[80] In a Phase Ib study of ficlatuzumab combined with gefitinib in Asian patients with NSCLC, partial response and stable disease was noted in the 9 of the 15 trial participants.[81] In the Phase II study, the combination of ficlatuzumab with gefitinib versus gefitinib alone was investigated in Asian patients with NSCLC. No statistically significant difference was observed regarding response rate or PFS between the two arms. However, on subgroup analysis, patients with EGFR mutations and low MET expression receiving the combination treatment showed an ORR of 41% versus 22% of the patients in single arm and median PFS of 11.0 months versus 5.5 months, respectively.[82]

In another Phase II study involving patients with NSCLC and EGFR activating mutations, the combination of ficlatuzumab and gefitinib did not improve clinical outcomes compared to the single agent gefitinib.[83] Subgroup molecular analyses using VeriStrat, a proteomic platform, indicated that patients classified as 'VeriStrat poor' showed significant benefit from combination treatment in terms of PFS and OS, in both the intent-to-treat population and in the EGFR-mutant patients. However, a follow-up Phase II FOCAL study of ficlatuzumab in combination with erlotinib in NSCLC patients with a Veristrat-poor signature and EGFR mutations was terminated after interim analyses showed higher discontinuation rates than observed with the previous study.[84]

Rilotumumab

Rilotumumab is a fully human anti-HGF IgG2 antibody that has been investigated for the treatment of a variety of tumours

due to its antineoplastic activity.[85] Based on encouraging Phase II study results in patients with MET-positive gastric or gastroesophageal adenocarcinoma, two Phase III trials (RILOMET-1 and 2) were initiated. However, both trials were terminated after an interim safety review found rilotumumab had a lack of efficacy, including an increase in the number of deaths (128 deaths in rilotumumab arm versus 104 deaths in placebo arm) and significantly shorter median OS in the treatment arm (9.6 months versus 11.5 months; HR 1.37; p=0.016),[86] irrespective of the level of MET expression. All subsequent trials, including a combination treatment arm with erlotinib for squamous cell NSCLC in the LungMap in NSCLC, have been terminated since late 2014.

RESISTANCE TO SMALL MOLECULE KINASE INHIBITORS

Several predominant mutations have been identified conferring resistance to small molecule kinase inhibitors in MET-dependent tumours. Acquisition or emergence of pre-existing clones with mutations in the MET activation loop Y1230 (also known as Y1248) or D1228 (also known as Y1246) have recently been shown to mediate resistance to type I kinase inhibitors such as crizotinib in NSCLC with *MET* exon 14 skip mutation.[87] But sensitivity to type II kinase inhibitors such as cabozantinib is maintained,[88] providing rationale for a clinical strategy of sequential therapy. Another cause of resistance is activation of the EGFR pathway due to increased expression of transforming growth factor α[89] and inactivation of *NF2* concomitantly with *NRG1* overexpression.[90]

FUTURE DIRECTIONS

Researchers' understanding of the biology and treatment of NSCLC with MET alterations continues to evolve. A multitude of agents utilising MET as a potential target have been tested or are under investigation at present. So far, the trials investigating these targeted agents have had mixed results, some promising and others negative. Over time, we have learnt that different MET alterations do not share the same clinical or functional equivalence and relevance. The selection of an appropriate patient population and optimal biomarker has been challenging, compounded by the myriad of methodologies and assays in the market that make analyses more complex. This has in part led to the variability in treatment outcomes among patients with MET alterations. To utilise the wealth of research that has gone into the understanding and development of MET inhibition and inhibitors, biomarker development should be validated more rigorously alongside efficacy evaluation. Rational drug combinations need to be developed to overcome resistance mechanisms. The pharmacological properties of novel agents have to be understood fully to gain insight into the toxicity profile and potential resistance mechanisms. Lastly, with the number of agents in development, a bottleneck has surfaced as a result of the limited number of biomarker-selected NSCLC patients who can qualify for the expanding number of trials testing these agents individually. Utilisation of multi-arm, multi-institutional umbrella trials permitting rapid inclusion of agents for clinical testing should be encouraged to accelerate and facilitate clinical evaluation and expand drug access for patients.

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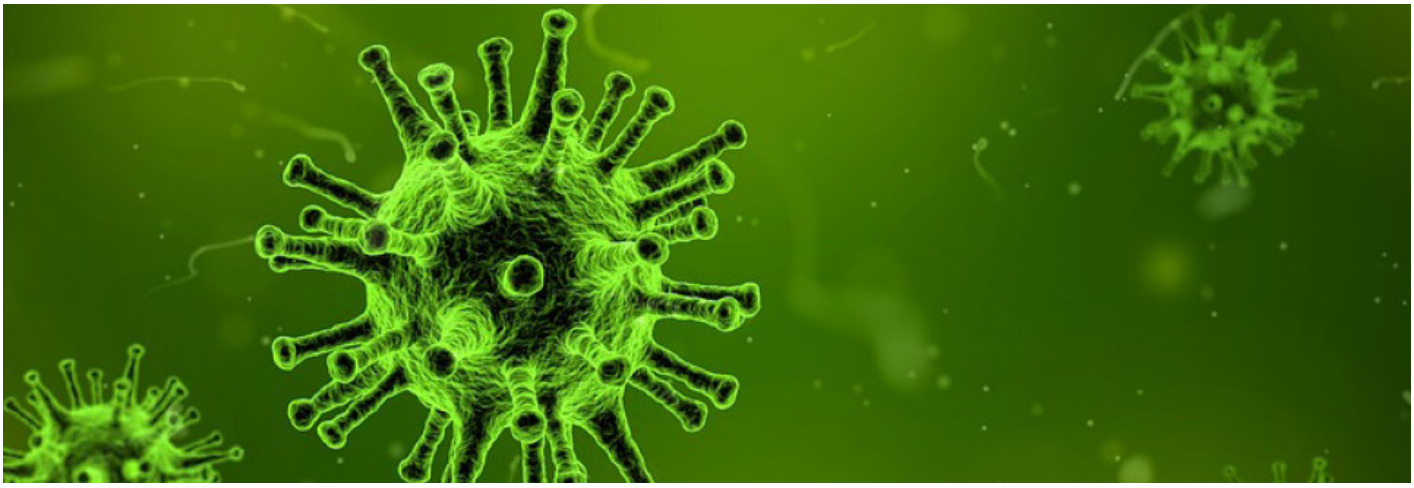
What's New



HIV Undetectable in a UK Patient Following Stem Cell Transplant

A STEM CELL transplant from an HIV resistant donor has left a patient 'free' of HIV, becoming the second person to achieve remission from HIV via this treatment. The London-based patient, who was being treated for Hodgkin's lymphoma, received chemotherapy and stem cell therapy that resulted in remission of his cancer and HIV. While this is a promising development, it is too soon to say whether the patient has been cured of HIV because a reservoir of the cells which carry the virus can remain in a dormant state for many years.

HIV-1 most commonly enters cells using the receptor CCR5. A small number of people have two mutated copies of this receptor, called CCR5-delta32, and are resistant to HIV because the virus is unable to penetrate the cells it would normally infect. In this case, when the resistant donor's stem cells were administered to the patient, he similarly became resistant. The case was managed by a group of UK researchers from University College London, Imperial College London, Cambridge University, and Oxford University. The researchers believe it could be possible to use gene therapy in people with HIV by targeting the CCR5 receptors responsible for the virus.

This result follows a similar case from 10 years ago when a leukaemia patient in Berlin, Germany received a bone-marrow transplant from a naturally immune donor. Prof Ravindra Gupta from University College London, London, UK, who was the lead study author said: "By achieving remission in a second patient using a similar approach, we have shown that the Berlin patient was not an anomaly and that it really was the treatment approaches that eliminated HIV in these two people."

While the research is promising in terms of a potential future cure for HIV, it does not offer a viable treatment solution for the estimated 36.9 million people worldwide with HIV. The treatment is not practical for most patients due to the aggressive nature of the treatment and the toxicity of the chemotherapy.

Prof Graham Cooke, Imperial College London, London, United Kingdom, commented on this encouraging result: "If we can understand better why the procedure works in some patients and not others, we will be closer to our ultimate goal of curing HIV."

Painting a Detailed Picture of Acute Myeloid Leukaemia

ACUTE myeloid leukaemia (AML) often presents as a complex heterogenous mixture of both healthy and cancerous cell types in the blood and bone marrow. At the developmental stage, these cells often have distinct genomic and transcriptomic profiles, each of which, alongside the accumulation of mutations, shapes the way the cancer grows, spreads, and responds to treatment. Researchers at the Ludwig Center, Harvard Medical School, Boston, Massachusetts, USA have now developed a pipeline for distinguishing cancer cells from healthy leukocytes and categorising them accordingly.

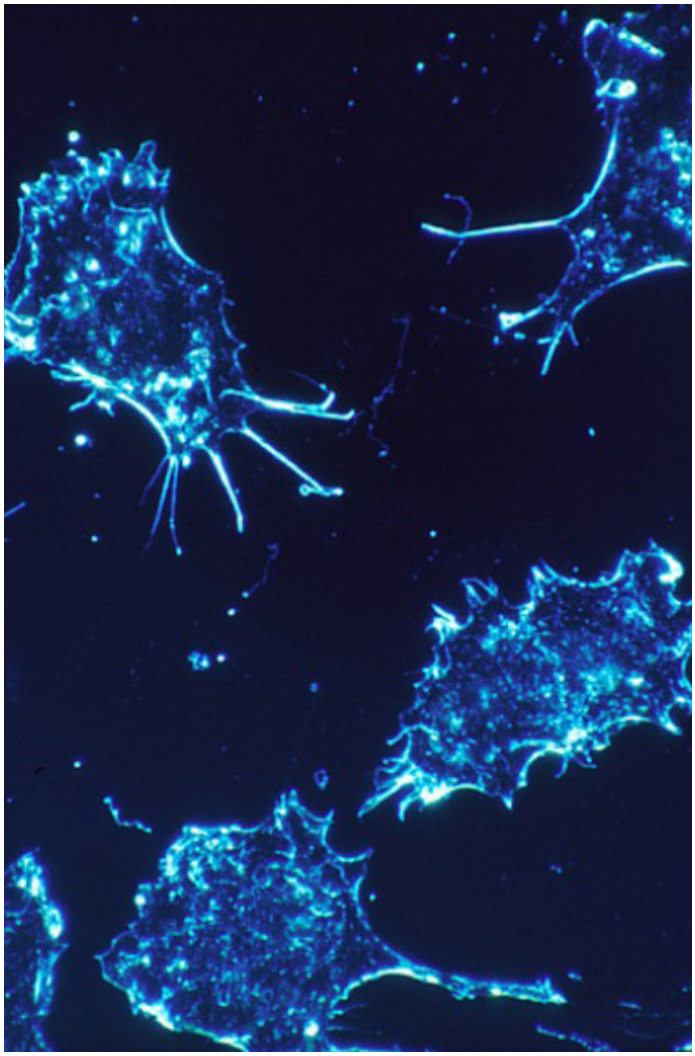
The process involved three primary steps:

1. scRNA sequencing: The cells' entire transcriptome was sequenced to monitor the evolution of cell lineages. In this study, 16 AML patients and 5 healthy controls contributed 40,000 bone marrow cells.
2. Long-read nanopore sequencing: Single cells were genotyped using known AML genetic markers to distinguish healthy and cancerous cells. The longer sequencing reads aim to capture as many mutations as possible in a single run.
3. Machine learning: A computational algorithm incorporates both sets of data to create an 'atlas' of different AML and healthy blood cell types.

The results allowed the Harvard researchers to not only determine which cells of the tumours were cancerous, but also within these cells what their differentiative states were and subsequently how far each tumour had developed.

Bradley Bernstein, leader of the study at the Ludwig Center, explained: "In normal cells, development progresses in stages[...] These leukemia cells co-express a mish-mash of genes from different stages."

An additional discovery by the team may also explain why certain immunotherapies are ineffective against AML. They found a sub-set of cells containing leukaemic mutations which actively inhibit anticancer immune responses, and are presumably an adaptive response by the tumour itself. This realisation should help researchers develop better immunotherapies, and combined with this new pipeline, pave the way for a more personalised approach to tackling AML.



What's New

Sexual Dysfunction: Quality of Life in Men with Prostate Cancer

SEXUAL DYSFUNCTION was one of the two major differences in quality of life (QoL) between men treated for advanced prostate cancer and those with early-stage disease. The other major difference related to the side effects of androgen deprivation therapy (ADT). This was according to the results of a UK-based study.

The study was questionnaire-based, with the researchers mailing a survey to 58,930 men who were alive 18–48 months after prostate cancer had been diagnosed. Of these men, 35,823 responded, with it being possible to determine disease stage for 30,733: 19,599 were at Stage I or II, 7,209 were at Stage III, and 3,925 were at Stage IV.

The authors noted that, based on their findings, men who were alive 18–42 months post prostate cancer diagnosis would have a similar health-related QoL to men of a similar age without prostate cancer. Such a finding makes for interesting consideration in the context of concern that prostate cancer is being overdiagnosed and then overtreated.

However, it was found that sexual dysfunction was commonly reported by men at all disease stages:

- 81.5% of respondents reported poor or very poor erections.
- 81.0% reported poor or very poor overall sexual function.
- 76.6% reported poor or very poor orgasm.

This was then compounded by the limited provision of sexual support in the UK, with 41.4% of study participants reporting that they were offered aids for sexual dysfunction. One of the study authors, Dr Amy Downing, University of Leeds, Leeds, UK, declared: "Our results suggest that there are subgroups of men who would benefit from service improvements around sexual rehabilitation and measures to reduce the effects of ADT."

Considering the impact of ADT, a sub-group analysis demonstrated substantial differences between the QoL outcomes of those who received ADT and those who did not:

- 30.7% of those treated with ADT reported hot flushes compared with 5.4% who were not treated with ADT.
- The respective figures for weight gain were 22.5% and 6.9%.
- The respective figures for low energy were 29.4% and 14.7%.

These results provide some insight for those responsible for the management of prostate cancer into some of the issues their patients may face.

The Double Life of p53, the Guardian of the Genome

WIDELY understood as a suppressor of cancer development, earning it the nickname 'the guardian of the genome', the protein p53 may, in fact, also promote some cancers. New research suggests that the cancer-protective effect of this broadly-studied protein may be only part of the story.

It has long been known that p53 responds to cellular stress by halting the cell cycle or inducing apoptosis; in this way, the protein regulates cell proliferation and can limit the development of cancer. With its central role in cancer prevention, it is perhaps unsurprising that studies have shown p53 to be frequently mutated and its unique pathways are inactivated in some human cancers. However, a 4-year study from the University of California, San Diego, California, USA, focussing on hepatocarcinoma in cell, murine, and human models, has shown that wild-type p53 can promote tumour growth by benefiting cancer metabolism.

Typically, mitochondria use the process of oxidative phosphorylation to make ATP molecules, which are paramount to intracellular energy transfer; however, in cancer cells, mitochondria use a more cumbersome process called glycolysis to generate energy. This change of mechanism from oxidative phosphorylation involves p53 and p53 upregulated modulator of apoptosis (PUMA), which induce apoptosis in damaged cells. The researchers' new study now shows that, under certain conditions, PUMA can trigger the switch to glycolysis, thus encouraging cancer development.

In this way, p53 can first reduce the generation of "genome toxins" but, once tumour growth does begin, p53 can support it. "It is actually the same function but playing exactly the opposite role in two different contexts," explained Prof Yang Xu, University of California, San Diego.

The authors describe their findings as "instrumental for cancer drug discovery" and hope that it will inspire caution in anti-cancer drug developers: drugs that operate by impacting the function of wild-type p53 could have the opposite effect in some cancers.