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EAACI Hybrid Congress 2021

EDITOR'S PICK

High-Sensitivity Assays for C-reactive Protein as a Systemic Inflammatory Marker in Assessing Asthma

INTERVIEWS

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"I am delighted to welcome you to the latest issue of EMJ Allergy & Immunology, comprised of the most up-to-date developments in this discipline."

Spencer Gore, CEO

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EMJ Allergy & Immunology 6.1

Welcome

Dear Readers,

I am delighted to welcome you to the latest issue of *EMJ Allergy & Immunology*, comprised of the most up-to-date developments in this discipline. Due to the ongoing COVID-19 pandemic, we were pleased to be able to attend the European Academy of Allergy and Clinical Immunology (EAACI) 2021 Hybrid congress virtually. This eJournal shares the most recent advancements in allergy and immunology in the form of peer-reviewed articles, interviews with experts in the field, and a selection of congress review highlights from EAACI 2021.

Following the success of last year's digital congress, the EAACI 2021 took on a hybrid format. Held physically in Kraków and virtually in Madrid, this year's congress delivered hundreds of expert-led sessions, as well as hands-on interactive workshops. Key abstract summaries are included in this issue, written by the presenters themselves and covering innovative topics such as the importance of telemedicine in COVID-19, the potential of screening tests for severe bronchial asthma, and many more.

This issue of *EMJ Allergy & Immunology* includes a range of exciting peer-reviewed articles presenting the latest advancements in the field. Our Editor's Pick by Ong discusses the use of high-sensitivity C-reactive protein as a marker of systemic inflammation in the assessment of asthma. Gupta and Kulkarni explore the possibility of repurposing antiviral drugs to combat symptoms of COVID-19, and Mandanas presents current research on the immunologic features of lymphatic filarial worms.

We were thrilled to interview Marek Jutel, President of EAACI, and Maria Jose Torres, a valued EAACI board member. The interviewees spoke about their careers, the EAACI congress and their respective roles, and how COVID-19 has impacted the field of allergy and immunology.

I would like to take this moment to share my gratitude for the Editorial Board, contributors, peer reviewers, interviewees, and the Editorial team. Without their continued efforts it would not be possible to publish such high-quality content. Finally, I would like to thank the readers; we, as a team, hope that you enjoy this latest issue of *EMJ Allergy & Immunology*.

Spencer Gore

Chief Executive Officer, EMG-Health

Foreword

Dear Readers,

Here you will find the 2021 issue of *EMJ Allergy and Immunology*. The European Academy of Allergy and Clinical Immunology (EAACI) Congress 2021 was held 10th–12th July and, for the first time, presented in a hybrid format! Thanks to all the organisers and, naturally, a special thanks is devoted to the scientific committee for their choice of the topics and also to the presenters. You could follow the Congress at home, but the exchanges with colleagues were a great miss and I am looking forward to meeting everyone next year! This hybrid format will probably be around in the future, so welcome to this new world of meetings!

Again this year, the presentations were mixed and comprised symposia, educational sessions, thematic poster sessions, sister society symposia, ePosters, and more. I also like the exchanges, live with the speaker and with the help of a Question and Answer session in the same chat making for an exciting interactive workshop. The option to return to the virtual congress a few weeks later to watch the presentations again is a very good initiative!

There was a large variety of topics, and the majority were closely related to the new challenges emerging from new concepts related to T cells. The information showing the important role played by the microbiome and nasal polyposis in regard to the physiopathology and its relation to other diseases was widely discussed, with a lot of questions about the new ways of treatments. Novel insights to allergic diseases with a focus of immunotherapy for aero-allergens and foods were widely presented with great interest. The phenotyping of diseases with new biomarkers stimulates our thoughts and makes us look forward to a new way of treating allergic diseases! Naturally, the acquired skills to treat patients with COVID-19 was mainly explored.

Again, this year brought some high-quality papers for this journal issue. My Editor's Pick is dedicated to the paper 'High-Sensitivity Assays for C-Reactive Protein as a Systemic Inflammatory Marker in Assessing Asthma' by Ong. As there is a real interest to establish early diagnosis of asthma, the C-reactive protein could play the role of a very simple and relatively low-cost marker. This review aims to explore the potential of this marker, especially with the severity of the disease.

So, I would really like to thank you for your interest in *EMJ Allergy and Immunology*. I hope that you will like the 2021 issue and I am looking forward to seeing you in person next year at the EAACI Congress.

Enjoy reading!

Jacques Bouchard

Associate Professor of Clinical Medicine, Université Laval; Head of the Medicine Department, La Malbaie Hospital, Quebec City, Canada

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EMJ 6.2

Symposium Review

Pioneering Best Practices in Atopic Dermatitis: Results from the Quality-of-Care Initiative

Poster Review

Late-Breaking Abstracts: Health Status Benefits of Mavacamten in Obstructive Hypertrophic Cardiomyopathy and the Modifying Effect of Ejection Fraction on the Therapeutic Benefit of Omecamtiv Mecarbil in Heart Failure

Articles

Editor's Pick: Relating Ventilatory Support and Drug Treatment Strategies to the Fundamental Pathophysiology in COVID-19 Illness

Understanding the Impact of Non-Dystrophic Myotonia on Patients and Caregivers: Results from a Burden of Disease Healthcare Survey

Oxy-hydrogen Gas: The Rationale Behind Its Use as a Novel and Sustainable Treatment for COVID-19 and Other Respiratory Diseases

Prevalence of Scoliosis in Hypermobile Ehlers-Danlos Syndrome

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Congress Review

Review of European Academy of Allergy and Clinical Immunology (EAACI) Hybrid Congress 2021

Location: Kraków, Poland and Madrid, Spain

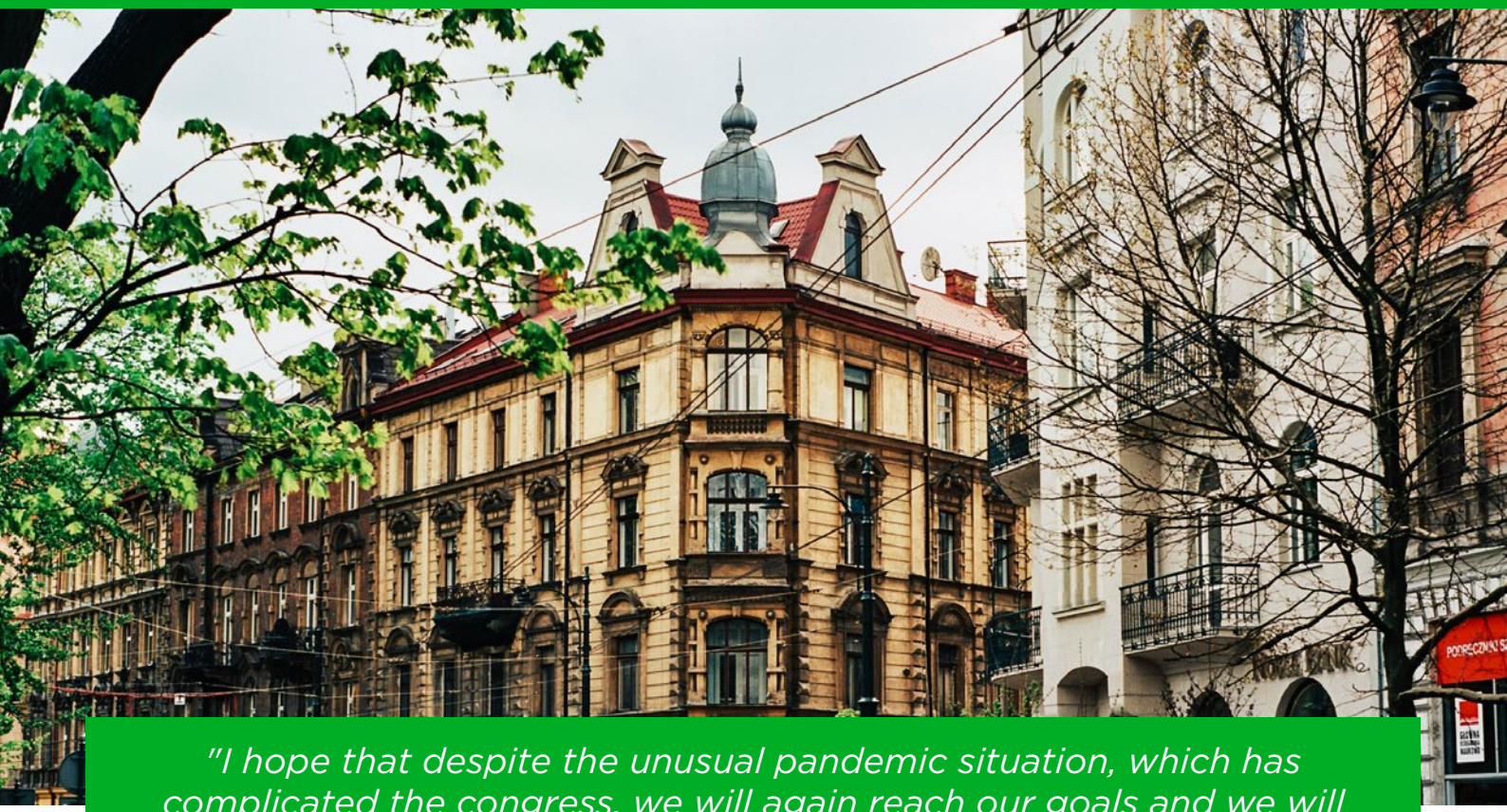
Date: 10th–12th July 2021

Citation: EMJ Allergy Immunol. 2021;6[1]:13-21. Congress Review.

A PIONEERING format was adopted for this year's European Academy of Allergy and Clinical Immunology (EAACI) Congress, as a hybrid model was adopted for the congress to enable both digital and in-person attendance for delegates. The congress, which was attended by more than 1,000 delegates on-site and 6,500 delegates online, was co-organised by the Spanish Local Organising Committee and the Polish Society of Allergology, with the in-person sessions taking place in the historical city of Kraków, Poland, 10th–12th July 2021, under special precautions imposed by COVID-19. In his introductory remarks from Krakow, Marek Jutel, President of EAACI, hailed this new format as a great success. Joakim Sastre, Chair of the Spanish Local Organising Committee, highlighted: "This type of hybrid congress breaks into a new era of how to celebrate congresses, at least in the near future."

With close to 1,300 research abstracts submitted to the congress, the abstract themes centred around the patient, allergy, and COVID-19. This year's congress saw a total of six plenary symposia, nine further symposia, and 23 hybrid interactive workshops, in which the speakers addressed some frequently asked questions on relevant topics. For the first time, the congress also hosted a total of three World Leadership Dialogues, which are high-level sessions aiming to present the high-calibre science by top researchers on key topics that aim to complement the plenary themes and sessions. The overarching theme for this year's congress centred around harmonising research with patient care in allergy, asthma, and clinical immunology. The patient was at the forefront of research presented in the congress, with COVID-19 and food allergy being dominating themes.

In his introductory comments, Marek Kulus, Chair of the Polish Organising Committee, commemorated Marek Kowalski, a former President of the Polish Society of Allergiology and Treasurer of EAACI, who sadly passed away 3 weeks prior to the congress. "I hope that despite the unusual



"I hope that despite the unusual pandemic situation, which has complicated the congress, we will again reach our goals and we will not allow this to slow the development of allergology"

pandemic situation, which has complicated the congress, we will again reach our goals and we will not allow this to slow the development of allergology," Kulus commented.

In his speech, Santiago Quirce, a Chair of the Congress, elaborated on the motto of this year's congress stating that "it is particularly important to harmonise and integrate clinical practice with research focussed on the field, with the aim of achieving a solid scientific purpose and developing a practical and generalised framework that allows to implement allergy practice under optimal conditions." Quirce stated that it is important to adapt to the great challenges that are faced in the current world, and to better understand the role of allergy and immunology in the future that we are going to build together.

The awards ceremony saw a number of researchers receiving prestigious awards: Isabella Annesi-Maesano, INSERM Research Director, Université de Montpellier, France, was the recipient of the Clemens von Pirquet Award for Clinical Research 2021. Ewa Nizankowska, Jagiellonian University Medical College, Kraków, Poland, received the Daniel Bovet Award for Treatment and Prevention 2021. The Paul Ehrlich Award for Experimental Research went to Heim Breiteneder, University of Vienna, Austria, for his research in the field of Molecular Allergology. Stefan Vieths, Johann Wolfgang Goethe-Universität Frankfurt am Main, Germany, was the winner of the Charles Blackley Award for the Promotion of the Specialty in Europe. Oscar Palomares, Complutense University of Madrid, Spain, was the recipient of the PhARF Award, and María del Mar del Pinos Yanes was the recipient of Allergopharma Award. Jean Bousquiet, Charité– Universitätsmedizin Berlin, Germany, and Mübeccel Akdis, the Swiss Institute of Allergy and Asthma Research, Davos, Switzerland, were the recipients of the EAACI Fellow Award. Finally, Graham Roberts, University of Southampton, UK, and Stephen Durham, Imperial College London, UK, were awarded the Clinical and Research Fellow awards, respectively.

Looking to the year ahead in preparation for EAACI 2022, the organising committee are yet to finalise their plans on the location and the format of next year's congress. In light of the everchanging situation with COVID-19, this is not surprising. If anything, the EAACI Hybrid 2021 pioneered a highly successful format that can be implemented again if necessary. ■

EAACI 2021 REVIEWED →

Flare-up Phenomenon of the SARS-CoV-2 Vaccine Following Epicutaneous Testing with Polyethylene Glycol

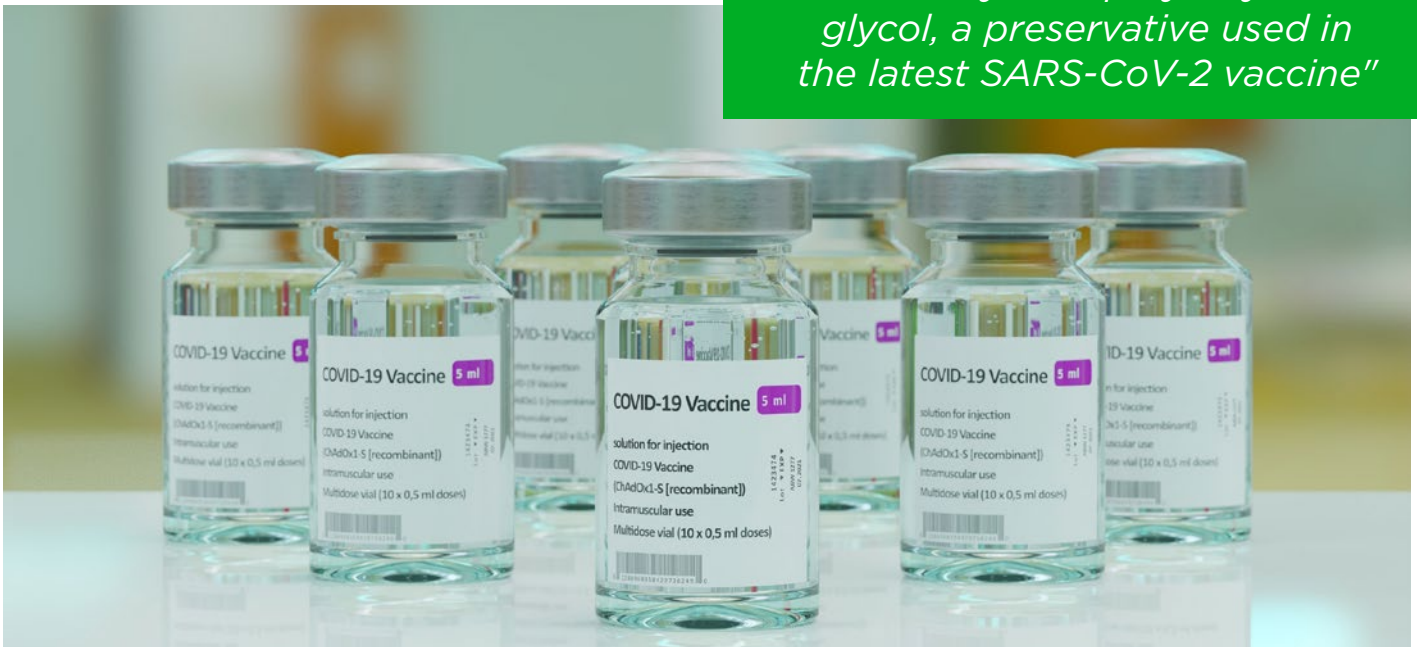
AT THIS YEAR'S EAACI Hybrid Congress, 10th-12th July 2021, Inés Torrado Español, Guadalajara University Hospital, Spain, and collaborators presented the case of a 41-year-old female diagnosed with β-lactam hypersensitivity who was referred to their allergy department in 2005 because a large local reaction (from elbow to shoulder) developed within several hours of receiving the influenza vaccine. The reaction disappeared after 1 week, following treatment with oral and topical corticosteroids. As a result of the current COVID-19 pandemic, the patient enquired about the possibility of having the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine administered.

The authors performed skin prick and intradermal (ID) tests with the available influenza vaccine as well as prick and ID (using dilutions of 1:100, 1:10, and 1:1) tests with the available SARS-CoV-2 vaccine. Tests with the influenza vaccine were negative. A positive 1:10 ID test was recorded with the SARS-CoV-2 vaccine. In addition, the 1:1 ID test was also found to be positive; however, later studies indicated that this concentration seemed to be irritating and therefore it was not taken into account.

Several influenza vaccines commercialised in Spain are known to contain polysorbate 80, which has shown cross-reactivity with polyethylene glycol, a preservative used in the latest SARS-CoV-2 vaccine. For this reason, the researchers conducted epicutaneous tests with both polysorbate 80 and polyethylene glycol 400 7 days after neutralisation of the first tests. Although polysorbate 80 was negative, polyethylene glycol 400 was shown to be positive 96 hours later. Moreover, it reactivated both the 1:10 and 1:1 ID tests of the SARS-CoV-2 vaccine.

In summary, the authors highlighted a flare-up phenomenon of ID tests to the SARS-CoV-2 vaccine after an epicutaneous test with a low-molecular-weight grade of polyethylene glycol. This is notable for potentially being the first documented case report of its type, involving polyethylene glycol and the SARS-CoV-2 vaccine. ■

"Several influenza vaccines commercialised in Spain are known to contain polysorbate 80, which has shown cross-reactivity with polyethylene glycol, a preservative used in the latest SARS-CoV-2 vaccine"



Reduced Allergenic Potentialof Birch Pollen with Vitamin D3

BIRCH trees produce birch pollen, which is responsible for hay fever in 25% of sufferers. Birch pollen can travel long distances in the wind, and people can experience allergic reactions even if they are nowhere near this silver-barked tree. Bet v 1 is a major type of birch pollen that can bind small ligands such as lipocalin to its intramolecular pocket. In a previous study, researchers from the University of Vienna, Austria discovered Vitamin D3 (VD3) could bind to this pocket. This discovery led the scientists to investigate whether the binding of VD3 affects the ability of Bet v 1 to cause an allergic reaction.

"The results showed a significant decrease in the cytokines levels of IFNγ and IL-10 in VD3 loaded allergen compared with the empty allergen."

To begin with, *in silico* docking analysis predicted the high binding affinity of VD3 to Bet v 1. This result was confirmed by *in vitro* ANS binding assay. Researchers used donated peripheral blood mononuclear cells from individuals allergic to birch pollen to determine various immune-related cellular processes such as mast cell release, production of cytokines, IgE binding, and CD-marker expression with empty allergen without VD3 and VD3 loaded allergen.

The results showed a significant decrease in the cytokines levels of IFNγ and IL-10 in VD3 loaded allergen compared with the empty allergen. The allergen loaded with VD3 decreased the expression of co-stimulatory molecules HLA-DR+ and CD68+ on CD14+ monocytes. Further to this, VD3 loaded allergen showed a significant decrease of T-cells expressing the Th-2 marker, specifically in the CD3+CD4+ cells. However, regulatory T-cells were the same in empty allergen and VC3 loaded allergen.

The researchers also observed a reduction in other cells such as CD19+ and mediator release from primary mast cells in VC3 loaded allergen. Interestingly, ELISA showed IgE binding was significantly reduced in VD3 loaded allergen compared to the empty allergen. From the *in silico* structural analyses, the scientists inferred that the binding of VD3 to the allergen could be interfering with the IgE binding site in Bet v 1.

Overall, the scientists concluded that the allergen loaded with VD3 had immunomodulatory properties. Additionally, from the decrease of cytokine levels and other immune cells, the data suggests that VD3 binding reduces the ability of Bet v 1 to cause an allergic reaction. Future studies could involve testing the efficacy of VD3 in treating birch pollen allergies in human clinical trials. ■

Pronounced Type 2 Immune Response in Nasal Polyps Cells

CHRONIC rhinosinusitis with nasal polyps (CRSwNP) is a painful condition characterised by inflammation and large non-cancerous growths inside the nasal cavity or sinuses. One out of 10 patients with CRSwNP have asthma and intolerance to non-steroidal anti-inflammatory (NSAID) drugs; this sub-type is called NSAID-exacerbated respiratory disease (N-ERD). Although there are similarities between the nasal polyps in N-ERD and CRSwNP, a detailed phenotypic characterisation of the cells involved is yet to differentiate the two conditions.

"Intriguingly, the expression of genes linked to a type 2 response, TSLP and POSTN, was distinctly elevated in N-ERD. "

To discriminate between N-ERD and CRSwNP, the authors of a new study conducted a transcriptomic analysis of epithelial cells and leukocyte subsets found in both conditions. Firstly, the scientists performed flow cytometric sorting followed by single-cell RNA sequencing of the nasal polyp tissue in N-ERD and CRSwNP. Finally, the scientists assessed the cytokines from the nasal secretions and serum of patients using a multiplex ELISA-based approach.

The results from the flow cytometric sorting showed that in N-ERD and CRSwNP, CD8+ and CD4+ T-cells formed the largest cell clusters. Other large cell clusters included NK cells, mast cells and macrophages. When analysing gene expression, scientists discovered that there were only minor differences in T-cell clusters between the two conditions. On the other hand, there was higher gene expression of most cell types in N-ERD, namely EGRF-ligand AREG and cytokines TGFB and NEAT1. Intriguingly, the expression of genes linked to a type 2 response, TSLP and POSTN, was distinctly elevated in N-ERD. Further to this, there was also an increase in IL13, IL17RB and HPGD expression in most mast cell clusters in N-ERD. Lastly, cytokine analysis showed significantly higher 1L-5 and IL-13 in N-ERD compared to CRSwNP.

In conclusion, the analysis of epithelial and mast cells showed there was a higher expression of genes involved in Type 2 immune response in patients with N-ERD. The findings suggest that mast cells are a key player in the development of N-ERD. Moving forward, scientists could explore the possibility of targeting the elevated genes or mast cells found in N-ERD to treat this painful condition. ■

Cytokine IL-33 as a Potential Therapeutic Target in Asthma

DEFINING the relationship between serum IL-33 and the severity of asthma symptoms could reveal IL-33 as a potential therapeutic target in asthma treatment. This relationship was explored in research carried out at Nuevo Hospital San Rogue in Córdoba, Argentina, shared at EAACI 2021 and in a press release dated 13th July 2021. IL-33 is an inflammatory cytokine which acts upon several immune cells, including eosinophils, through its receptor sST2. For this reason, IL-33 has recently become a focus for research in asthma and allergic disease.

The study recruited 129 patients with asthma and divided into three severity groups, mild (n=25), moderate (n=69), and severe (n=35) along with 59 healthy controls. All patients had their blood levels of IL-33, sST2, IgE, and Eosinophils measured. A skin prick test was also carried out to establish responses to air antigens relevant to the region (Argentina). A multivariant analysis was used to compare results from the serum tests to asthma severity and prick tests. Significance was considered ($p<0.05$). This multivariant analysis research was presented at the EAACI Hybrid Congress which took place on the 10th–12th July 2021.

When serum levels were compared between patients with asthma and controls, a significant increase for all the measured elements was demonstrated in the asthmatic group: IL-33 ($p=0.0021$), sST2 ($p<0.0001$), IgE ($p<0.0001$), and eosinophils ($p<0.0001$). The prick test was positive for 92 cases of the asthmatic group and negative for 32. The multivariant analysis showed a strong correlation of 92% between the assessed components.

The results demonstrated a dramatic increase in levels of IL-33 and its receptor sST2 in patients with asthma over the control group. The multivariant analysis further suggests that increasing levels of IL-33 in the serum are linked to worsening severity of symptoms. This supports the hypothesis that IL-33 is a strong contender for a therapeutic target in asthma research and treatment. ■

"The results demonstrated a dramatic increase in levels of IL-33 and its receptor sST2 in patients with asthma over the control group"

Could IL-3 Treatment Improve Allergic Asthma through ILC2 Regulation?

PROMISING developments have emerged linking recombinant IL-3 (rIL-3) treatment and improved lung function in allergic asthma. This research was prompted following recent reports of IL-3 downregulation being associated with severe allergic asthma in children. The experiment carried out by scientists in the Department of Molecular Pneumology at Friedrich-Alexander-Universität (FAU) used an Ovalbumin (OVA)-induced murine model and offers important data regarding the role of IL-3 in allergic asthma treatment through innate lymphoid cell (ILC) regulation. These findings were presented at EAACI, which took place from 10th–12th July 2021.

Cases of allergic asthma see symptoms including induced airway inflammation, airway hyperresponsiveness, mucus production and airway remodelling, all of which have been associated with abnormal differentiation of cytokine-producing Th2 cells. ST2+-ILC2 cells, which produce a variety of Th2 cytokines, have been identified to play a part in allergic asthma. The IL-3 cytokine is known to facilitate specific immune cell differentiation and survival; however, its role on ILC2 in asthma is lacking, further warranting the research carried out at FAU.

The FAU experimental murine model treated the asthmatic mice both with and without low and high intranasal doses of rIL-3 *in vivo* and expanded lung ILC2s using *in vitro* methods. This took place over a period of 5 days and results were analysed using flow cytometry. Increased lung inflammation was observed which correlated with the presence of IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-13 cytokines in lung cells of IL-3-deficient mice. The results also demonstrated an increase in ST2hi memory-type Th2+, killer cell lectin-like receptor-G+, Thy-1+-gated (ST2hi+KLRG+Thy1+gated) on lineage-marked (Lin)-ILC2s in OVA-induced asthmatic mice, however these observations lacked in OVA-mice treated with rIL-3 *in vivo*. Mice treated with rIL-3 also saw reduced airway hyper-responsiveness and serum IgE. These data suggest that IL-3 plays an important role in the resolution of allergic asthma by regulating ILC2 and therefore opens new strategies for immunotherapy of this disease. ■

"These data suggest that IL-3 plays an important role in the resolution of allergic asthma by regulating ILC2 and therefore opens new strategies for immunotherapy of this disease."

Endothelial Cells Pattern Profile Altered by the *in vitro* Contact of Acute Anaphylaxis Serum

ANAPHYLACTIC reaction has a rapid onset and is life-threatening especially due its pathophysiology within the cardiovascular system. Previous studies have shown that patients who have undergone an anaphylactic reaction have differential extracellular vesicle (EV) protein pattern profile, however additional molecular research is required to understand the association of endothelial cells (EC) and EV release during anaphylactic reactions. A new study presented at the EAACI Congress 2021 that took place on 10th–12th July by Sergio Fernandez-Bravo, Fundación Jiménez Díaz University Hospital Health Research Institute, Madrid, Spain, aimed to look into the EC metabolic profile.

"The results of the immunoblotting assays, after 2 hours, showed an increase of EVs syntenin-1 of A-EVs-ECs supernatants compared to B-EVs-ECs supernatants"

The researchers collected plasma samples from patients during acute phase (A) anaphylaxis reaction and at the baseline (B) following recovery and purified via ultracentrifugation. ECs incubated with A and B-EVs-free plasma samples were collected for 2 and 24 hours, using an *in vitro* system. An immunoblotting technique was used to identify EVs' syntenin-1 protein expression from the EC supernatants. Platelet-activating factors and histamine were utilised as positive controls. Additionally, the EC lysates that had been stimulated for 2 hours by the collected A and B samples were quantified using liquid chromatography coupled to mass spectrometry. A multivariate model of partial least squares discriminant analysis was used to assess the differences between A and B samples.

The results of the immunoblotting assays, after 2 hours, showed an increase of EVs syntenin-1 of A-EVs-ECs supernatants compared to B-EVs-ECs supernatants. The stimulation of platelet-activating factors and histamine also elevated the expression syntenin-1 EVs-ECs for the initial 2 hours, however after 24 hours no further substantial changes were observed. Interestingly, the metabolic profile for the studied samples was only from ECs which consisted of 687 compounds. Further statistical analysis noted that 77 metabolites were identified between ECs incubated with both A and B samples. In conclusion, there is a clear increase of the EVs release and change in metabolic pattern profile in endothelial cells A samples compared to B samples. ■

New Research Could Revolutionise Immunotherapy in Canines

IMMUNOTHERAPY has gained momentum as an option for cancer treatment in humans. Previous studies have demonstrated that monoclonal antibodies (mAbs) are effective in blocking checkpoint inhibitors like PD-1 and PD-L1 which are anti-cancer therapeutics. Although the advancements in applications for immunotherapy in humans are evolving, its is unfortunate that dog anti-immunotherapy is lagging. A study presented at the EAACI Congress that took place on 10th–12th July 2021 by Rodolfo Bianchini, University of Vienna, Austria aimed to address this limitation.

A pairwise sequence alignment with EMBOSS Needle webtool was used to identify the similarities between human and dog PD-1 and PD-L1 protein sequences. Additionally, the binding effectiveness of the human antigen presenting cells therapeutic mAbs targeting both PD-1; pembrolizumab, nivolumab, and cemiplimab, and PD-L1; atezolizumab, avelumab, and durvalumab, was evaluated on the on the canine macrophage-like cell line DH82 by flow cytometry. THP1 and U937 the human monocytic cell lines were included as controls. A ligation-independent cloning technique was the used to produce canine models, with the highest binding efficiency, of the human anti-PD-1 and anti-PD-L1 mAbs.

"This study discloses a novel approach in the production of anti-PD-1 and anti-PD-L1 mAbs in canines and further research may lead to dog anti-cancer therapies using the checkpoint inhibitors."

The results from the pairwise sequence alignment revealed a similarity of 66.2% between human-PD-1; Uniprot Q15116 and canine-PD-1; A0A024FCJ9. In addition, a similarity percentage of 75.7% between human-PD-L1; Uniprot Q9NZQ7 and canine-PD-L1; EZRKZ5. The human therapeutic mAbs pembrolizumab and atezolizumab also revealed a high binding capability to the DH82 canine macrophage cell line. Using the ligation-independent cloning the researchers generated canine mAbs to create a pVitro1 cassette for fast genetic recombination of various human heavy and light chain sequences with canine IgG1 or IgG4 heavy and k light chain sequences, acquired from the international Immunogenetics information system (IMGT) database. This study discloses a novel approach in the production of anti-PD-1 and anti-PD-L1 mAbs in canines and further research may lead to dog anti-cancer therapies using the checkpoint inhibitors. ■

Moving -omics and Machine Learning into Clinical Practice in Asthma and Allergy Medicine

Evan Kimber
Editorial Assistant

Citation: EMJ Allergy Immunol. 2021;6[1]:22-24.

COMPELLING discussions took place at the European Academy of Allergy and Clinical Immunology (EAACI) Hybrid Congress 2021, exploring the emergence of machine learning (ML) in current practice and potential avenues within allergy research. Keeping to an underpinning focus on asthma but touching on eczema and other allergic conditions such as α-Gal syndrome, conversations began with a consideration of metabolomics and biomarkers, progressing to discuss phenotype characterisation, with an overall clinical focus. Applications of ML upon early-life microbe exposure and the development of the immune system were tackled, before concluding with the basophil activation test (BAT) and its use in studying allergen sensitisation.

The session opened with Maria Escribese of the University of San Pablo, Madrid, Spain, who summarised current standing in metabolomics and focussed on the challenges and next steps for utilising biomarkers. Next, Adnan Custovic, Imperial College London, UK, discussed the implementation of ML in understanding asthma heterogeneity, followed by Jenni Lehtimäki of the University of Helsinki, Finland, who provided insight on ML analytic studies that investigate the development of the immune system and risk of allergies based on early exposure to microbes. Concluding with the use of BAT centred on α-Gal allergy was Bernedette Eberlein of the Technical University of Munich, Germany.

LESSONS LEARNED FROM METABOLOMICS

Reminding us that asthma is a multifactorial, chronic syndrome involving genetic and environmental interaction, Escribese began by emphasising the complexity of the disorder. Her presentation gave an update on the unmet needs in asthma and challenges facing biomarker-based treatment in allergic disease, highlighting the idea that clinical phenotypes are governed by underlying endotypes. Current practice uses IgE and eosinophilia as biomarkers, but adipokine and periostin are showing promise and are expected to be widely adopted soon.

"Metabolomics has provided unique and novel insights into asthma profiling at the molecular level," stated Escribese, addressing targeted or untargeted classifications and mentioning

'breathomics' as the first approach incorporating metabolic biomarkers into clinical practice. Returning to the overall ML focus in this session, this breath-based initiative captures, identifies, and quantifies volatile organic compound patterns in human breath, which is useful for diagnosis of a wide spectrum of medical problems. Escribese stressed that this method allows identification of new biomarkers for monitoring intervention, patient classification, and biological drug management, promising that it will prove useful for explaining the underlying mechanisms associated with diseases and could help identify novel therapeutic interventions.

EXPLANATORY AND PREDICTIVE MACHINE LEARNING APPLIED TO ASTHMA

Energising proceedings, Custovic brought an enormous passion to his presentation, which examined a shift from diagnosis-based to mechanism-based treatment. He tied together ML for asthma diagnosis, assessing severity and future risk, and patient stratification, stating: "In the last decade or so, the development of high-throughput technology has really transformed the way we do research, and offers tremendous opportunity to unravel the complexities of asthma."

"In the last decade or so, the development of high-throughput technology has really transformed the way we do research, and offers tremendous opportunity to unravel the complexities of asthma."

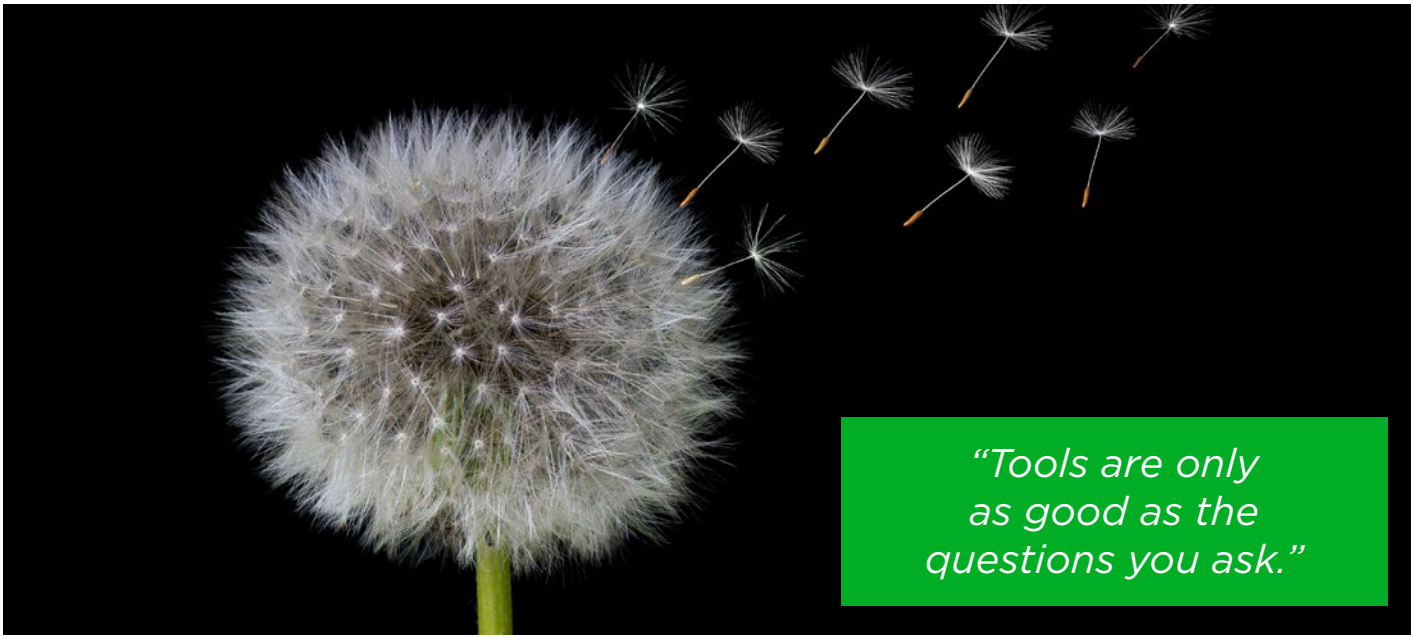
Custovic spotlighted the vast amount of data ML can analyse and interpret where traditional methods would find processing challenging, driving the movement away from hypothesis-driven study towards data-driven approaches. Splitting ML into two categories, he outlined that explanatory ML aims to understand patterns in data, meanwhile predictive ML makes predictions without attempting to explain the data. Predictive ML applied to children and adults to forecast the diagnosis of asthma, using algorithms applied to complex data, has produced a noteworthy correlation network, examining structural homology and IgE dynamics. Patients with severe asthma express a higher connectivity among these components, but with weak interaction; in moderate asthma there are fewer connections but stronger bonds between components.

Looking at ML applications for longitudinal data, Custovic touched on latent variable modelling and discussed future action for asthma: "ML and data-driven methodology allow us to understand the underlying pathophysiology and offer us pointers as to where to go next." He concluded by underlining that no single data source or method can uncover the complex mechanisms involved in asthma heterogeneity, and that without understanding mechanisms we cannot improve patient outcomes. He closed by highlighting the requirement for a shift towards a more integrated cross-disciplinary and cutting-edge analytic approach, incorporating ML-based science in large datasets and facilitated by collaboration from all parties involved in study.

RURAL VERSUS URBAN UPBRINGING AFFECTING LATER ALLERGY

Lehtimäki elaborated on analysis conducted in Denmark comparing the allergy profiles of children born in, and spending critical periods of their development in, rural and urban areas. Including observation of asthma, eczema, and allergic rhinitis, the differences in microbe exposure and health outcomes were recorded at 1 week, 1 month, 6 months, and 1 year, only including those who remained for the full year.

The ML aspect of this study was seen through the use of a single-variable simplification of data, describing markers of rural or urban development by creating a sliding gradient scale and outlining composition of a participant's microbiota of the airways and gut. Describing a sparse partial least-squares model, Lehtimäki noted higher asthma, eczema, and aero-allergen sensitisation levels in children raised in an urban environment, and observed a superior immune response in rural-born children.



Speaking about an innovative study that involved vacuuming infant beds to collect dust, Lehtimäki expanded on investigation of the composition of the samples collected, implementing a similar ML method to create a single-variable scale of the microbiota found in the beds. Children with an urban background and no siblings had less-diverse microbes in the sample and an increased likelihood of developing asthma and allergic rhinitis at 6 years.

"It seems both the microbes you are exposed to, and the microbes which are colonising your airways and gut, are important in defining later health," was the summary delivered by Lehtimäki, going on to state: "And it might seem the rural ones are protecting." This presentation of ML findings has sparked debate into how cities must be changed to allow more access to diverse green space to mimic a rural environment upbringing and potentially reduce later allergy.

USING BASOPHIL ACTIVATION TESTS TO DIFFERENTIATE ALLERGY AND SENSITISATION

Eberlein began by contextualising the discovery of α-Gal syndrome and the delayed anaphylaxis it causes with consumption of red meat. The ML aspect here involved exploiting the known association with tick bites and IgE, using BAT to identify triggers. This method is a cellular *in vitro* test for IgE-mediated reactions, involving basophil identification and measuring activation flow cytometrically.

The diagnostic workflow followed was initiated with selection of patients with a history of urticaria and anaphylaxis, progressing to stratify based on IgE diagnostics and skin prick-to-prick testing with products such as raw beef and pork. Cellular BAT was then applied in a provocation test, producing evidence of an association between α-Gal serum IgE positivity with IgE levels and recent tick bites. Findings showed that patients can be sensitised to α-Gal without having a clinically manifested allergy; the diagnostic workflow is an example of ML being used to confirm diagnosis and determine individual allergen sensitivity.

The usefulness of BAT was emphasised by Eberlein, who discussed potential for use as a generalised clinical test and the possibility of creating an algorithm used in a mobile application to calculate risk, using co-factors like alcohol consumption and exercise. Strengths lie with the ability of the test to identify triggers and sources of α-Gal-containing substances, differentiate sensitisation and allergy, as well as elucidate mechanisms of the syndrome.

CONCLUSION

A host of advanced machine-driven initiatives were brought forward in this session, and will no doubt change the way allergy research is conducted in the near future. But a human element will always remain present in investigations, according to Custovic, who assured: "Tools are only as good as the questions you ask."

Advancing the Management of Cow's Milk Protein Allergy with Human Milk Oligosaccharides: Priming the Immune System

This digital satellite symposium took place on 12th July 2021, as part of the European Academy of Allergy and Clinical Immunology (EAACI) hybrid congress

Chairperson: Liam O'Mahony[1]

Speakers: Yvan Vandenplas,[2] Ralf Heine,[3] Sophie Nutten[3]

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Meeting Summary

During this symposium, leading experts in paediatric allergy and immunology discussed the importance of the early-life microbiome in driving immune maturation and preventing allergy. They explored key evidence supporting the benefits of human milk oligosaccharides (HMOs) in priming the immune system and promoting infant health, particularly in cow's milk protein allergy (CMPA). Vandenplas summarised clinical trials of HMOs in CMPA to date, highlighting data on growth, tolerance, and reduction in infection risk achieved with HMO-supplemented specialty formula. The major role that HMOs play in shaping the gut microbiome in early infancy was discussed by Heine. He presented recent data showing how supplementation of standard or extensively hydrolysed infant formula with two HMOs, 2'-fucosyllactose (2'FL) and lacto-N-neotetraose (LNnT), can shift the gut microbiome in CMPA closer to the profile of breastfed infants. Nutten outlined how 2'FL and LNnT can beneficially modulate Type 2 immune responses, which may have important implications for both allergy prevention and treatment strategies.

The Clinical Relevance of Human Milk Oligosaccharides in Infants with Cow's Milk Protein Allergy

Yvan Vandenplas

Risk factors for CMPA in infants include genetic background, mode of delivery, feeding, and medication, all of which are intrinsically linked to the gut microbiome. Two components of breast milk play a major role in delivering immune support: HMOs, which are virtually absent in cow's milk and unsupplemented infant formula milk, and lactose.[1-4] Lactose acts by upregulating the immune defences, while HMOs act as a major substrate for *Bifidobacterium*, enhancing levels of short-chain fatty acids (SCFAs), in particular, butyrate.[4-7] Lactose and HMOs therefore work synergistically to stimulate development of a well-balanced gut microbiome in breast-fed infants, explained Vandenplas.

Of the more than 200 different HMOs identified so far in human breast milk, Vandenplas described non-fucosylated HMOs (e.g., LNnT) and fucosylated HMOs (e.g., 2'FL) as the two key categories.[8-10] HMOs confer a protective effect in allergy via modulation of mucosal immunity. In a study of infants born by caesarean section, those fed breast milk containing higher levels of 2'FL had a lower incidence of IgE-associated atopic eczema compared to those receiving milk from 'non-secretor' mothers with lower 2'FL levels (14% versus 43%; p=0.095).[11]

Observational studies have revealed differences in the composition of the gut microbiota between allergic and non-allergic infants, which are present before any manifestation of disease. This clearly shows that dysbiosis is primary to CMPA and not secondary, stressed Vandenplas.[12,13] Breast milk contains HMOs, which drive development of a healthy gut microbiota. Alterations during this critical development period increase the risk of the infant developing allergic disease. Infants with CMPA also show a higher propensity for developing infections.[14]

Currently, the recommended first-choice option in the management of CMPA is an extensively hydrolysed formula (EHF).[15] An amino-acid based formula (AAF) may also be indicated in certain cases.[16] Vandenplas reviewed clinical data that support a key role for HMOs added to these specialty formulas in the treatment of CMPA. Studies have shown that whey-based extensively hydrolysed formulas (w-EHF) supplemented with HMOs (Althéra® HMO, Nestlé, Vevey, Switzerland) are well tolerated and support normal growth in infants with CMPA. The hypoallergenicity of w-EHF containing additional 2'FL and LNnT has also been confirmed in infants and children with CMPA, meeting guidelines laid down by the American Academy of Pediatrics (AAP).[17,18] The test formula supplemented with HMOs was well tolerated and levels of 2'FL and LNnT in w-EHF proved comparable to those found in breast milk.[17,19,20] In the CINNAMON European study of nearly 200 infants with CMPA, confirmed growth was also achieved in infants with CMPA being fed w-EHF with added 2'FL and LNnT.[21-23]

HMO-supplemented formula may confer additional clinical benefits in infants with CMPA and Vandenplas highlighted further recent evidence from the CINNAMON study. Secondary analysis of the CINNAMON study revealed a reduction in the risk of infections, including respiratory tract infections and otitis media, in infants who received HMO-supplemented formula (n=94) versus control (n=96).[21-23] Infants with CMPA fed w-EHF formula with additional 2'FL and LNnT showed a clinically significant reduction of 23% in lower respiratory tract infection episodes per month, as well as a statistically significant reduction of 42% in monthly upper respiratory tract infections.[21-22] For upper respiratory tract infections in particular, w-EHF with 2'FL and LNnT was associated with a clear reduction in the rate of respiratory infections per month of study formula use, with a hazard ratio (HR) of 0.58 (p=0.003).[21-22] Similar results have been seen in healthy infants fed with 2'FL and LNnT supplemented formula, with a significant relative risk (RR) reduction of 44% for reported lower respiratory tract infections.[23] Collectively, these data show that the addition of HMOs to w-EHF results in a clinically significant reduction in the risk of respiratory tract infections, Vandenplas concluded.

This reduction in infection risk also led to a clinically significant trend towards decreased use of antibiotic (RR: -20.3%) and antipyretic (RR: -24.9%) medications in infants with CMPA fed w-EHF supplemented with 2'FL and LNnT.[21-22] Lower rates of infection-related

medication use with HMOs may be linked to gut microbiota community types, suggested Vandenplas. Notably, healthy infants with a high bifidobacteria-dominated gut at 3 months have been shown to be less likely to require antibiotics during the first year of life.[24] These clinical benefits may be extended to formula-fed infants by using HMO supplementation to achieve a microbiotic community which mirrors that of breastfed infants.

Data from the new multicentre PLATYPUS study have also highlighted the tolerance, safety, and growth efficacy of AAFs supplemented with HMOs in infants with moderate-to-severe CMPA.[25] This multicentre, open-label, non-randomised, single-arm study evaluated AAF containing 2'FL and LNnT (Alfamino® HMO, Nestlé) in 32 infants aged 1–8 months over a 4-month period. Normal growth, as measured by for-age Z-scores for weight, length, and head circumference and compared with World Health Organization (WHO) reference values, was achieved in infants fed the study formula with two HMOs. There was even some evidence of catch-up growth, noted Vandenplas. The study formula had an excellent safety profile and was tolerated well by infants with CMPA.[25]

Vandenplas concluded that, while breast milk remains the best option, the addition of two HMOs (2'FL and LNnT) to infant formula brings the second choice for infant feeding one step closer to this gold standard. Evidence supports the efficacy of HMOs added to w-EHF containing lactose with the additional clinical benefits of decreased respiratory tract infections and antibiotic use. Supplementation with these two HMOs also achieves normal growth in infants with moderate to severe CMPA fed with an AAF formula.

Human Milk Oligosaccharides Shaping the Gut Microbiome in Infants with Cow's Milk Protein Allergy

Ralf Heine

The early development of the gut microbiome in infancy can be divided into three stages that take place during the first 1,000 days of life: developmental, transitional, and stable. Determinants of early microbiome development were evaluated in the TEDDY study, which analysed 12,500 sequential stool samples from 903 children from birth to 26 months.[26] Several significant dietary and environmental factors were identified but breast milk had "the stand-out influence in the early years of life," said Heine, explaining that the reason for this is the strong effect of HMOs on shaping the gut microbiome.[26]

2'FL in human milk promotes healthy bacteria such as bifidobacteria and lowers the growth of pathogenic strains like streptococci, as evidenced by a study of relative abundance in infants from secretor versus non-secretor mothers.[27] This HMO is therefore a major modifier in bacterial composition in early infancy, stressed Heine. Overall, HMOs have a range of effects that help support the immune system of infants and young children with CMPA, including promoting the growth of beneficial bacteria. A study of cow's milk-based infant formula in healthy infants supplemented with 2'FL and LNnT showed that the faecal microbial composition of infants fed the HMO-supplemented formula was significantly different to control formula and shifted closer to that of breastfed infants, particularly regarding *Bifidobacteriaceae* abundance.[24]

Heine went on to review microbiome data from the multicentre CINNAMON study that evaluated the growth, tolerance, and safety of a w-EHF containing 2'FL and LNnT versus a virtually identical control formula with no HMOs in infants with CMPA.[21-22,28] The per-protocol set for the microbiome analysis included 132 infants. Each group contained similar numbers of infants (68 control and 64 HMO), and the male:female ratio was similar. The HMO group was slightly younger than the control group (mean age at enrolment 98.6 days versus 107.9 days, respectively) but still in a comparable range.[28] Stool collections were performed during the CINNAMON study at four timepoints: baseline (Visit [V] 0, with infants aged between 14 days and 6 months at enrolment), after 1 and 3 months follow-up (V1 and V3), and at 12 months of age (V6). Heine explained that, although there was a significant age gradient during early visits, at V6 all infants were synchronised at 12 months of age, allowing for a clearer assessment and comparison of microbiome outcomes at that timepoint. Gut

microbiome composition analysis was carried out via metagenomic sequencing, analysing the microbial genetic information, and profiling the bacterial species accordingly. Microbial richness and diversity were compared between groups at each timepoint using the Shannon index. There was also an enrichment analysis at the genus, family, and phylum level, focusing mainly on bifidobacteria. The study tracked faecal community types (FCTs) over time in order to develop a transition model and analyse the temporal development of the microbiome.

Data from the CINNAMON study looking at the evolution of faecal microbial diversity over time from V0 to V6 showed that at 12 months of age (V6), the gut microbiome of infants receiving HMO-supplemented test formula had lower diversity, with significant differences in richness (p=0.003) and Shannon index (p=0.006).[28] The effect of HMO supplementation on the abundance of certain bacterial species was also evaluated after 1 month at V1. When grouped by genus, there was a significant enrichment of *Bifidobacterium* species, with these key bacteria being more abundant in HMO-treated infants (p=0.0002).[28]

A post hoc subanalysis was also carried out in infants aged <3 months at V0 (i.e., before starting a complementary diet, as the introduction of fibre is itself is an important modifier of bacterial composition) to assess whether the change in *Bifidobacterium* abundance between V0 and V1 was affected by mode of delivery. The enrichment of *Bifidobacterium* in young infants fed HMO-supplemented formula was much more pronounced in those born via Caesarean section, although a non-significant trend for increased *Bifidobacterium* was also seen in vaginally delivered infants receiving HMO-supplemented formula. These results highlight that the beneficial effect of supplemental HMOs on *Bifidobacterium* was greatest in infants born via Caesarean section, suggested Heine, but is still found across the spectrum.[28]

Faecal communities within the gut microbiome were shown to evolve with age during the first year of life in both study groups in the CINNAMON study, reflecting changes in diet and environmental exposures. In infants fed either HMO-supplemented or control formula, transition models showed a temporal development from 'early' to 'late' FCT clusters, using all 481 study samples, reflecting a gradual change to an 'older' microbiome. Importantly, the positive effect of HMO supplementation on microbiome age was confirmed by comparing differences in FCT distribution between treatment groups, stratified by visit from enrolment to V6 (Figure 1). At 12 months, the microbiome of HMO-treated infants was shown to be significantly enriched in early-type FCT as compared with control (p=0.014).[28] The progression to more advanced microbiome FCTs was more advanced in the control group, which, according to Heine, "represents an acceleration of the microbiome age towards an adult-type gut microbiome in non-breastfed infants." Overall, the evidence demonstrates that HMOs play a central role in shaping the developing gut microbiome of infants with CMPA. Supplementation with 2'FL and LNnT in both standard and w-EHF formula is associated with an enrichment in bifidobacteria and lower microbial diversity, as well as a lower gut microbiota age at 12 months because of the effects of HMOs. HMO supplementation therefore appears to slow the premature shift towards an adult-type gut microbiome, which was previously described in infants receiving no/limited breast milk, Heine concluded. The long-term clinical benefits of HMOs on early immune development and the resolution of CMPA require further study.

Immunological and Microbial Effects of Human Milk Oligosaccharides in Experimental Models of Food Allergy

Sophie Nutten

Breastfeeding is currently recommended to prevent the development of allergic diseases in high-risk infants; however, the data are conflicting and underlying mechanisms of prevention remain unclear.[29] Breast milk consists of essential nutrients plus numerous bioactive compounds that could theoretically impact immune development in the neonatal gut.[29] Nutten explained that HMOs have been "heavily studied over the last decade" and are now recognised as a major bioactive component of breast milk that could modulate the development of allergic disease.

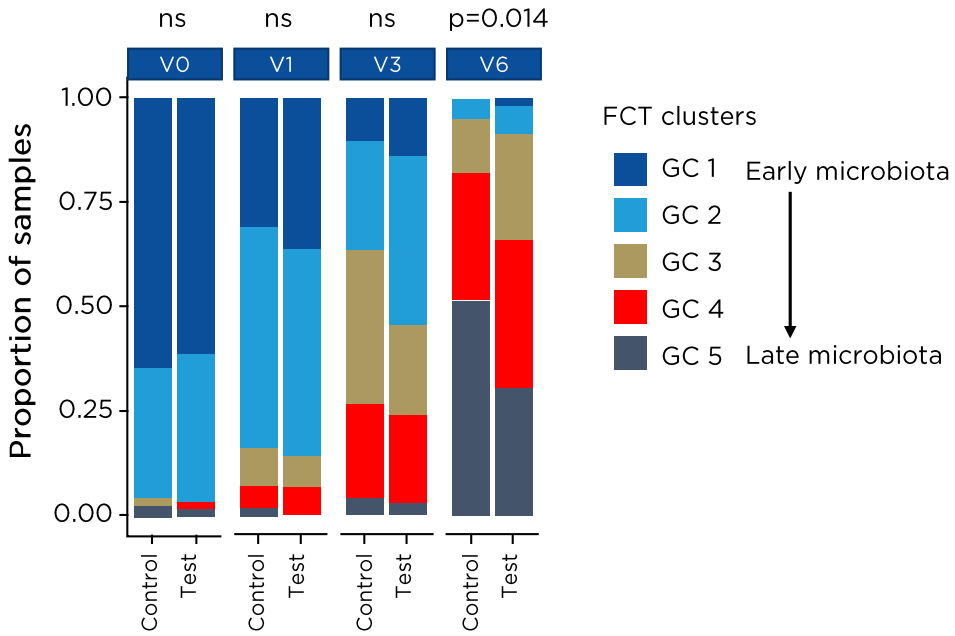

Figure 1: Effect of human milk oligosaccharide supplementation on temporal changes in faecal community types in infants with cow's milk protein allergy.[28]

FCT: faecal community type; GC: group cluster; ns: not significant; V: visit.

HMOs comprise the third largest solid component in breast milk after lipids and lactose. Every mother synthesises and secretes a distinct and individualised composition of HMOs influenced by various factors including maternal genetics, lactation stage, and environmental conditions.

HMOs are undigested so have no nutritive function but support the infant immune system in four main ways.[30] They promote a bifidobacteria-dominated microbiome, deflect pathogens, and strengthen gut barrier function.[31] Approximately 1% of HMOs are also absorbed into the systemic circulation and interact directly with immune cells to educate the developing immune system.[30] For all these reasons, HMOs have been proposed to influence the development of allergic disease. This hypothesis has been supported by several observational studies in humans showing that HMO composition is associated with the development of food sensitisation and other types of allergies.[6,11,31,32] No interventional study has yet been published, so the causality of these associations remains to be determined; however, pre-clinical data suggest a benefit of HMO supplementation in both allergy prevention and amelioration of allergic symptoms.

In the first pre-clinical study to demonstrate the protective effect of HMOs, addition of 2'FL or 6'-sialyllactose (6'SL) was shown to attenuate food allergy symptoms such as diarrhoea in a mouse model induced by oral ovalbumin challenge.[33] The addition of HMOs was also associated with a reduction in mouse mast cell proteinase 1 in the serum, indicating less mast cell degranulation, and an increase in IL-10+ CD4+CD25+ cell populations in Peyer's patches and mesenteric lymph nodes.[33] In a passive cutaneous anaphylaxis model, oral administration of 2'FL and 6'SL induced suppression of antigen-induced mast cell degranulation by CD4+CD25+ cells isolated from the mesenteric lymph nodes.[33] Collectively, these data suggest that HMOs can reduce the symptoms of food allergy through induction of IL-10 regulatory cells and indirect stabilisation of mast cells, Nutten explained.

The second pre-clinical study looked at the oral administration of 3'-sialyllactose (3'-SL) in two different mouse models of atopic dermatitis (AD).[34] 3'SL reduced ear thickness (a proxy for AD symptoms) and produced dose-dependent decreases in mast cell number and IgE production in the serum, while simultaneously reducing levels of inflammatory and AD-related cytokines.[34] Additionally, *in vitro* assessment revealed that

3'SL was able to prevent skin inflammation by directly inducing transforming TGF-β mediated regulatory T-cell (T-reg) differentiation. This study also looked at the effects of 3'SL on the mouse microbiome and showed *Bifidobacterium* levels, in particular *B. bifidium*, increased in a dose-dependent manner with oral administration. Rising levels of *Bifidobacterium* were associated with the 'rescue' of AD phenotypes, noted Nutten. Taken together, these findings indicate that 3'SL has therapeutic effects against AD by inducing T-reg differentiation, downregulating AD-related cytokines and increasing the *Bifidobacterium* population.

Nutten went on to describe unpublished pre-clinical results obtained at Nestlé Research looking at the immunological and microbiome effects of HMOs. In the first experimental model, mice were sensitised with a mix of ovalbumin and cholera toxin and dietary intervention was performed with a mixture of 2'FL and LNnT in a 2:1 ratio, administered at increasing doses (0.2%, 1%, 5%, 10% of total diet). Results showed that oral administration of the blend of two HMOs decreased allergic sensitisation induced via the gut as shown by a dose-dependent decrease in anti-ovalbumin IgG. Consistent with other studies, a decrease in mast cell numbers in the gut and mouse mast cell proteinase 1 in the serum was also seen, together with an increase in T-reg count in the mesenteric lymph nodes. Analysis of the microbiome effect of the HMO mix showed modulation of the gut microbiota composition with increasing doses of HMO, together with a decrease in the richness of the microbiota. Looking at specific species, Nutten noted that there was an increase in *Akkermansia*, which is known for its role in strengthening the gut barrier, and *Parabacteroides*, which is one of the predominant species in the intestinal tract.

In a separate model of allergy, mice underwent epicutaneous sensitisation with *Aspergillus fumigatus* applied to the skin and then received the same nutritional supplementation of HMOs as previously described. This model confirmed that oral administration of the blend of 2'FL and LNnT decreased allergic sensitisation induced via the skin, as shown by decreases in specific IgG1 and cytokines involved in Type 2 immune responses, such as IL-4, in the serum. Modulation of the murine gut microbiota was also observed with oral administration of the 2'FL and LNnT mix. HMOs promoted the relative abundance of Bacteroides and led to decreasing levels of Bilophila, a potentially deleterious genus linked to various diseases. Finally, the HMO mix was also shown to induce an increase in propionate production in the caecum. This SCFA has been associated with a decreased risk of allergy in children.

In summary, human observational studies suggest a key role of HMOs in allergy. Notably, HMO composition is associated with the development of food sensitisation in the first year(s) of life. However, human interventional studies are still needed to confirm the role of HMO supplementation in allergy management. Pre-clinical models have shown that supplementation of mice with HMOs has an impact on both allergy sensitisation and symptoms, and this research has also helped to decipher the underlying mechanisms of action (Figure 2). Data show that HMOs modulate the Type 2 immune response via interaction with immune cells such as basophils and dendritic cells. They also play a vital role in reinforcing the gut barrier and modulating the microbiota towards increased production of SCFAs, which promotes T-reg differentiation. These mechanisms could work together synergistically to explain the beneficial effects of HMOs in allergy, Nutten concluded.

Questions and Answers

As part of the interactive Question and Answer session that followed the main symposium, Vandenplas explained that HMOs protect against infection via several different mechanisms. HMOs stimulate the development of a healthy microbiome by increasing bifidobacteria and lactobacilli and decreasing pathogenic strains; act as decoy receptors to block pathogen adherence to the gastrointestinal (GI) mucosa; and exert positive modulatory effects on the immune system. Asked what is known about risks in infants breastfed by non-secretor versus secretor mothers, Heine noted that the risk of infections (both GI and respiratory) and AD is reduced in secretors, suggesting that 2'-FL acts as an important immune modifier in this group to confer protective effects.

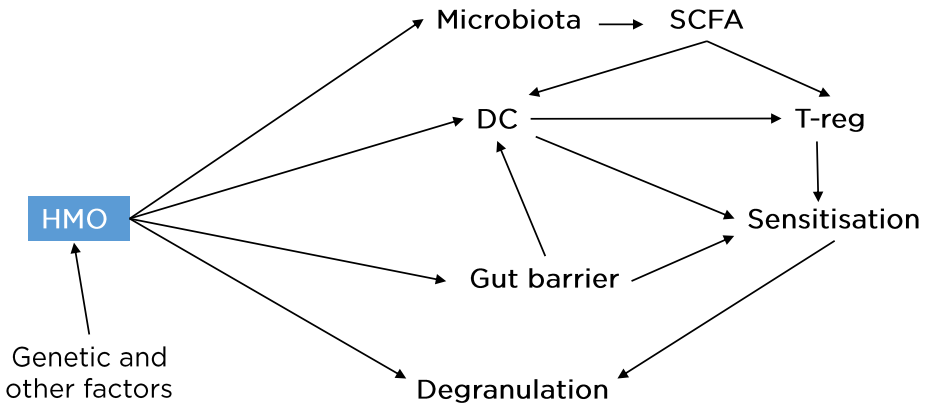

Figure 2: Proposed mechanisms for human milk oligosaccharide effects on allergy sensitisation and symptoms.

DC: dendritic cells; HMO: human milk oligosaccharide; SCFA: short-chain fatty acid; T-reg: regulatory T-cell.

On the selection of particular HMOs for study in mouse models, Nutten explained that choice is driven by availability and the accumulated evidence from published association studies, which confirms an important role for 2'-FL. Nutten was also asked, from a mechanistic point of view, whether HMO effects in mice models are microbiome-dependent. She replied that the health benefits of HMOs are likely to arise from a combination of both microbiome-mediated effects and direct effects of HMOs on immune cells such as dendritic cells or basophils/mast cells.

In response to a question on the risks of microbiome deficiency in infants born by caesarean section, Heine noted that pure elective populations undergo inoculation with the 'wrong' bacteria (predominantly skin and environmental flora) and also tend to have lower rates of breastfeeding and increased antibiotic usage. All of these factors compound the risk of a bifidobacterial-deficient microbiome in the perinatal period, which could be corrected by using HMO-supplemented formulas if breastfeeding is not possible.

Looking at potential future areas of research, Vandenplas highlighted the important role of metabolites and the need for further study into specific HMOs of therapeutic relevance. There is some literature to suggest differences in HMO profiles between allergic versus non-allergic children, he noted, but whether that is consequential or causal remains to be clarified.

A question from the audience queried the lack of dose-dependency in the final mouse study showcased by Nutten. She suggested this could indicate a plateau effect with 5% HMO, with the microbiota incapable of digesting higher concentrations. Another question from the audience raised the possibility of a role for HMOs in neural cross-talk in the developing neonatal gut nervous system. Vandenplas said this is currently unknown but it is clear that the microbiome is important in stimulating GI motility, with HMOs likely to play a role in this. There is also some data in healthy infants to show that probiotics result in more rapid gastric emptying.

The final question focused on the biological rationale for the natural diversity of HMOs seen in human breast milk. Heine explained that HMOs possess an incredibly complex array of functions and targeted effects, including a role in brain neurodevelopment. Moving forward, this space will continue to evolve, fuelled by improvements in the technical feasibility of making breast milk identical HMOs and increased understanding of their important immune and health benefits.

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Abstract Reviews

Shared in this issue are a wide variety of interesting abstracts from the EAACI 2021, from the importance of telemedicine in COVID-19 in the paediatric allergology unit to the clinical efficacy of complex therapy for mixed allergic and non-allergic rhinitis in immunocompromised patients.

Phadiatop as a Screening Method for Prescribing Immunobiological Therapy

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Asthma phenotypes, biologicals, immunobiological therapy, Phadiatop, severe bronchial asthma, target therapy.

Citation: EMJ Allergy Immunol. 2021;6[1]:33-35. Abstract Review No. AR1.

BACKGROUND

Over the past two decades, new opportunities have emerged in the treatment of severe bronchial asthma due to the development of immunobiological therapy. The effectiveness of biologics depends on correct phenotyping of asthma in patients.[1] The Phadiatop test has been known since the 1980s and has established itself as a screening test for the detection of atopy, allergic rhinitis, and allergic asthma.[2-9] When selecting patients with severe bronchial asthma for targeted therapy in the Sverdlovsk region, Russia, the Phadiatop™ (Phadia AB, Uppsala, Sweden) screening test was used for phenotyping asthma in this group of patients.

AIM

To assess the informative value of the Phadiatop test when selecting patients with severe bronchial asthma for target therapy.

Asthma phenotypes	Phadiatop, PAU/L Me (Q1—Q3)	p	Total IgE, IU/ml Me (Q1—Q3)	p
J45.0 Allergic severe asthma (n=43)	3.86 (1.21—7.29)	<0,001 (J45.0—J45.1)	159.6 (91—370.8)	0.249
J45.1 Non-Allergic severe asthma (n=29)	0.1 (0.04—0.24)		132.6 (49.1—477.5)	
J45.8 mixed severe asthma (n=5)	2.36 (0.38—6.24)		277.8 (152—1350)	

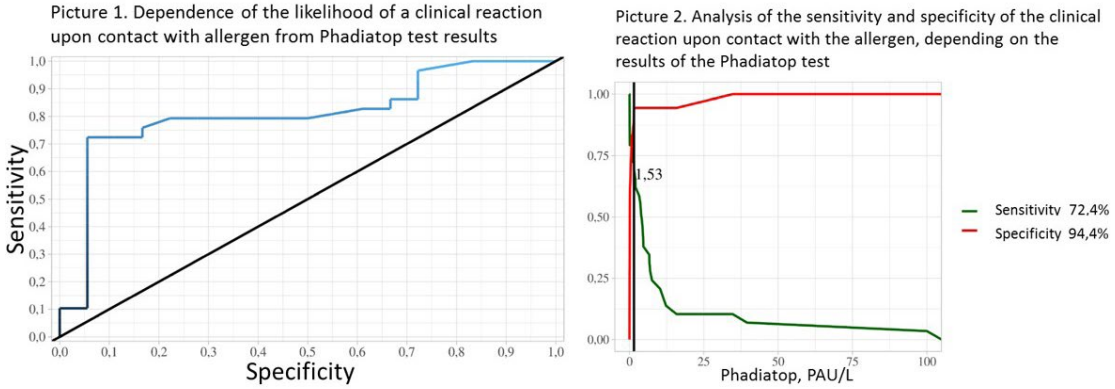

Figure 1: Phadiatop and total IgE results in different asthma phenotypes groups in register of severe asthma of Sverdlovsk region, 2016-2020.

MATERIALS AND METHODS

In the course of work on a regional register of patients with severe asthma (SA), patients were selected and asthma was phenotyped. Allergic anamnesis, skin tests with allergens, specific IgE to allergens, and blood eosinophil level were used as standard methods for asthma phenotyping. The Phadiatop test has been used since 2016 to screen for allergic phenotypes of SA when selecting patients for target therapy. The registry included 77 patients at the time of the cross-sectional study (January 2021). The study analysed data from all patients in the registry. The diagnosis of allergic SA was established with a positive allergic anamnesis (there is a connection between the clinical symptoms and exposure to allergens) and at least one positive test (skin tests, specific IgE) confirming sensitisation.

RESULTS

According to phenotyping, the patients were divided into three groups: atopic SA J45.0 (n=43); non-allergic SA J45.1 (n=29); and mixed SA J45.8 (n=5). The result of Phadiatop testing in patients with atopic (3.86 PAU/L; Q1–Q3: 1.21–7.29) and mixed SA (2.36 PAU/L; Q1–Q3: 0.38–6.24) exceeded the results of this test in patients with non-allergic asthma (0.1 PAU/L; Q1–Q3: 0.04-0.24) 38.6 times (p=0.001) and 23.6 times (p=0.001), respectively (Table 1). Total IgE level analysis in phenotypic groups of asthma did not show significant differences (p=0.2).

The results of Phadiatop testing were compared with the results of standard diagnostic methods for atopy. In the group of patients with a positive allergic anamnesis, the result of the Phadiatop test was statistically higher than in the group of patients without an allergic anamnesis: 3.86 PAU/L (Q1–Q3: 0.89–7.00) and 0.12 PAU/L (Q1–Q3: 0.03–0.46), respectively (p<0.001). In the group of patients with positive skin tests, the result of the Phadiatop test was higher than the result in the group of patients with negative skin tests: 3.56 PAU/L (Q1–Q3: 1.27–6.79) and 0.07 PAU/L (Q1–Q3: 0.03–0.13), respectively (p=0.005). When comparing

the results of Phadiatop testing in groups of patients with positive and negative specific IgE to allergens, statistically significant differences were also obtained: 2.64 PAU/L (Q1–Q3: 1.43–6.76) and 0.04 PAU/L (Q1–Q3: 0.02–0.07), respectively (p=0.022). The total IgE levels in the atopy and non-atopy groups did not differ significantly.

In the receiver operating characteristic analysis, threshold values were obtained at the cut-off point: with a Phadiatop test value >1.53 PAU/L, the patient is predicted to have a clinical reaction to the allergen (sensitivity: 72.4%; specificity: 94.4%) (Figure 1, Pictures 1 and 2) and positive skin tests (sensitivity: 75%; specificity: 100%); with a Phadiatop result >0.64 PAU/L, a positive result of specific IgE was predicted (sensitivity: 100%; specificity: 100%).

CONCLUSION

It is advisable to use Phadiatop testing as a screening method for detecting the atopic phenotype of SA and selecting patients for immunobiological therapy. ■

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The Importance of Telemedicine in the COVID-19 Era in the Paediatric Allergology Unit

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Keywords: Asthma control, COVID-19, telemedicine.

Citation: EMJ Allergy Immunol. 2021;6[1]:35-37. Abstract Review No. AR2.

BACKROUND AND AIMS

Asthma is the most common chronic disease of childhood, with a prevalence of 4.4–30.6% among school-age children in European birth cohorts.[1] The COVID-19 pandemic has affected healthcare services worldwide, and many clinics have ceased office appointments and stopped taking new patients.[2] However, only few clinics reported more negative asthma disease control during the COVID-19 era.[2]

The objective was to evaluate the paediatric allergology unit and whether COVID-19 has affected physician–patient contacts or feedback of care. The hypotheses were that there was no significant reduction to patient contacts

and the patients seemed to be pleased with telemedical visits.

MATERIALS AND METHODS

This was a register-based study from the electronic medical records at the Helsinki University Hospital, Skin and Allergy Hospital Paediatric Allergy Unit, Helsinki, Finland, from 1st April 2019 to 30th October 2020.

The proportion of telemedical visits via Terveyskylä internetplatform[3] and telephone contacts was analysed. The patient feedback was documented using Net Promoter Score value (NPS),[4] which was collected via short message service of the hospital.

Statistical analysis was performed with SPSS version 22 (IBM, Armonk, New York, USA). The Mann–Whitney U test was used for continuous variables and chi-squared for categorical data.

The monthly number of patients at the intensive care units in the Hospital District of Helsinki and Uusimaa was calculated as the mean number of patients on Wednesdays of each week according to the Finnish Institute for Health and Welfare (THL) database.[5]

RESULTS

In the paediatric allergology unit, the decrease of physician visits in April–May 2020 compared to 2019 was 9.7% (1,188 versus 1,315 visits). In April–October, the decrease was only 5.4% (4,039 versus 4,270 visits).

None of the 4,270 visits from April to October 2019 were telemedical. In April–May 2020, during the first peak of COVID-19 incidence in Helsinki, 59% of visits (n=698/1,188), and 19% (n=543/2,851) between June–October 2020, were performed using telemedicine (p<0.001) (Figure 1).

NPS feedback was improved significantly during the COVID-19 era from April to October 2020 compared to the period from November 2019 to March 2020 (median values of 76.9, range: 62.4–82.8; and 63.2, range: 61.1–65.4, respectively; p=0.018). The mean number of feedback per month was 120 from November to March and 110 from April to October.

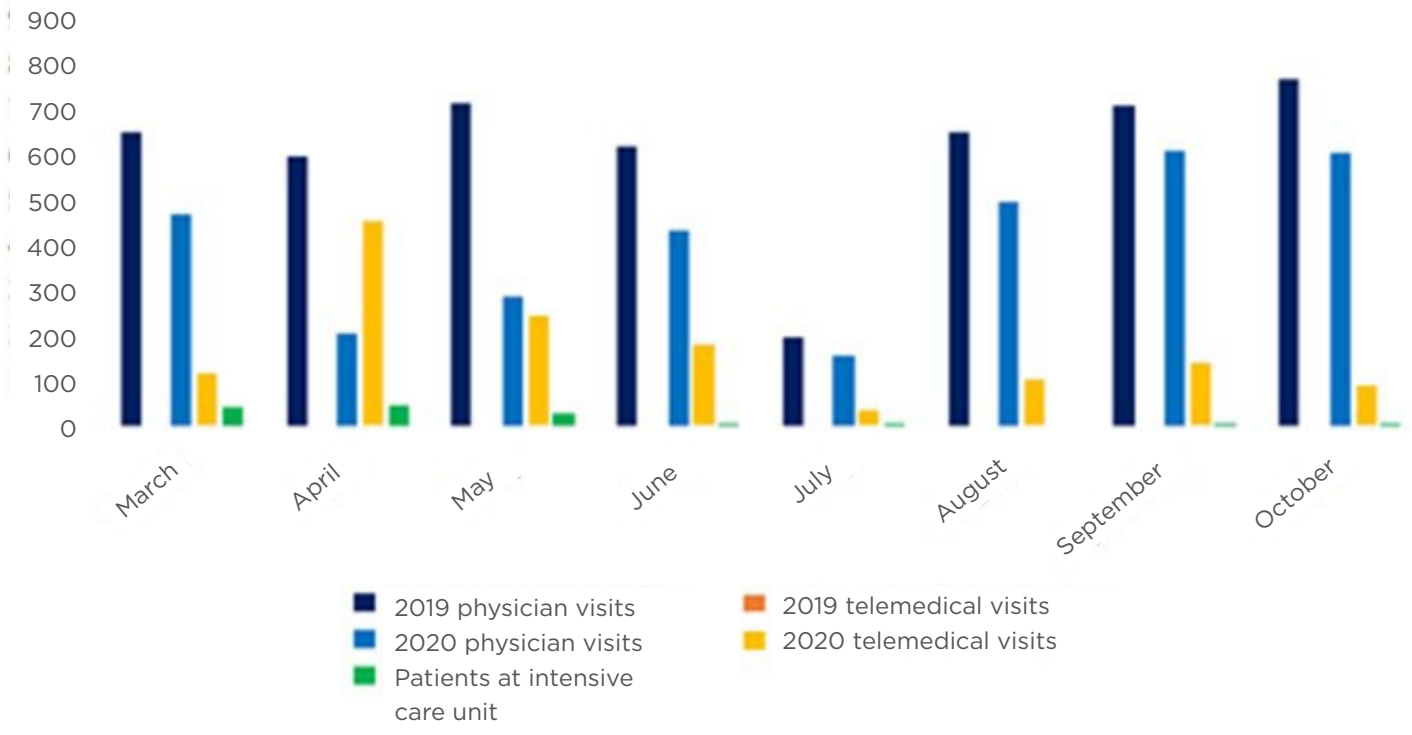

Figure 1: Physical and telemedical visits and number of patients in intensive care unit.

CONCLUSION

There was only a minor decrease of patient visits, contacts, and appointments during the COVID-19 period. The majority of contacts in spring 2020 were performed using telemedicine. Also, after May 2020, 20% of visits were performed via internet or telephone. Patients were satisfied: NPS feedback was improved to the COVID-19 era. ■

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Clinical Efficacy of Complex Therapy for Mixed Allergic and Non-allergic Rhinitis in Immunocompromised Patients with Recurrent Viral–Bacterial Infections

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Allergic rhinitis, immunocompromised, infection, non-allergic rhinitis.

Citation: EMJ Allergy Immunol. 2021;6[1]:37-38. Abstract Review No. AR3.

BACKGROUND

It is known that acute viral infections cause exacerbation of IgE-dependent allergic diseases. In this regard, the treatment of immunocompromised patients suffering from mixed allergic and non-allergic rhinitis (MANAR) with frequent exacerbations despite adequate therapy is difficult.[1,2] Episodes of repeated acute respiratory viral–bacterial (RAVBRI) and recurrent herpes virus infections (RHVI) caused by HSV1/2, which are immunocompromising clinical signs, have induced exacerbations of MANAR.[3]

MATERIALS AND METHODS

Twenty-one patients of both sexes were observed, aged 23–60 years, affected by MANAR associated with RAVBRI and RHVI caused by HSV1/2. The diagnostic assessment included complaints, anamnesis, physical examination, and assessment of MANAR symptoms on the visual analogue scale. Laboratory research included total IgE, specific IgE to exoallergens, and an evaluation of the IFN system examined by ELISA. HVI were tested by polymerase chain reaction and serodiagnostic testing; bacteria were tested by cultivation.

RESULTS

Symptoms of MANAR on the visual analogue scale were 3.19 points, which corresponded

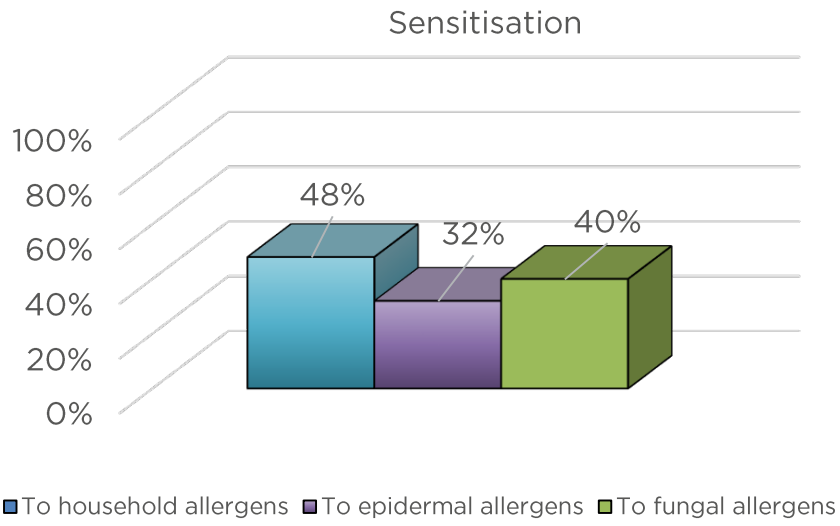

Figure 1: Sensitisation spectrum.

to an average–severe or severe stage. The level of total IgE was increased in all patients. Sensitisation to household allergens occurred in 48%, to epidermal in 32%, and to fungal in 40% (Figure 1). The frequency of episodes of RAVBRI was 7.45 (5.75–10.0) and of RHVI was 7.89 (4.75–11.0) per year.

Disorders of induced IFNα production were detected in 100% of cases. The programme of complex therapy was developed, which included basic MANAR treatment using systemic antihistamines and intranasal glucocorticosteroids. Continuous local and systemic therapy with rIFNα2b, in combination with antioxidants and immunotherapy with glucosaminylmuramylpeptide alternated with arginyl-alpha-aspartyl-lysyl-valyl-tyrosyl-arginine, were used to restore anti-infection protection.[4,5]

After 3.5 months of therapy, the authors observed a decrease of the frequency of acute respiratory viral infections to 4.28 (3.0–4.25), after 6–8 months to 2.45 (2.0–3.0), and a reduction of RHVI frequency to 3.38 (1.38–5.0) and to 2.1 (1.0–3.0) episodes per year, respectively.

The restoration of induced IFNα production occurred in 42.8% of patients. The MANAR symptoms decreased to 1.62 points. ■

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Effect of Allergic Sensitisation to Eucapnic Voluntary Hyperventilation Test

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Keywords: Allergic sensitisation, asthma, atopy, bronchial provocation test, eucapnic voluntary hyperventilation (EVH) test, exercise-induced bronchoconstriction, pulmonary function.

Citation: EMJ Allergy Immunol. 2021;6[1]:39-41. Abstract Review No. AR4.

BACKROUND AND AIMS

Eucapnic voluntary hyperventilation (EVH) test is feasible among children aged 10–16 years in diagnosing asthma and dysfunctional breathing.[1] Aeroallergen sensitisation increases bronchial hyper-responsiveness (BHR).[2,3] The EVH test decreases exhaled nitric oxide among children.[4] There are no studies regarding allergic sensitisation compared to the EVH result. The authors evaluated the effect of aeroallergic sensitisation to fall of forced expiratory volume in 1 second (FEV1) after the EVH test.

MATERIALS AND METHODS

The authors recruited 134 patients aged 10–16 years with a history of exercise-induced dyspnoea and 100 healthy control children to undergo EVH testing for 6 minutes in University Hospitals of Turku and Kuopio, Finland, between 2013 and 2016. All children underwent a 6-minute EVH test according to European Respiratory Society (ERS) guidelines.[5]

Serum IgE levels to birch, timothy, mugwort, cat, dog, horse, house dust mite, and *Cladosporium herbarum* were measured by Phadiatop™ (Phadia AB, Uppsala, Sweden) in a total of 177 of 234 children (105 cases and 72 controls). Children with specific IgE ≥0.35 kU/L to any allergen were considered sensitised.

SPSS version 22 (IBM, Armonk, New York, USA) was used for the statistical analysis. For continuous data, the Mann–Whitney U test was used and for categorical data chi-squared tests were used. The Spearman correlation test was used for the correlation analysis. The Ethics Committee of the Hospital District of Southwest Finland approved the study.

RESULTS

The median age of all children was 13.4 years. In total, 82 of 177 children (46%) were sensitised by at least one aeroallergen. There were no differences among sensitised and non-sensitised children according to age (13.5 versus 13.4 years; p=0.79); being male (60% versus 55%; p=0.50); having atopic eczema (38% versus 24%; p=0.054); having a furry animal (48% versus 57%; p=0.25); parental asthma (28% versus 23%; p=0.48); or parental smoking (12% versus 21%; p=0.12). Sensitised children more often had a prior asthma diagnosis (29% versus 15%; p=0.020) and they more often had chronic rhinitis (59% versus 20%; p<0.001).

The median fall of FEV1 after the EVH test was similar in both sensitised and non-sensitised groups (median values of -4.1% and -4.1%; p=0.69 [Figure 1A]). Similar findings were observed among patients and controls (Figure 1B and 1C), including for the proportion of children having a minimum 10% fall of FEV1 after the EVH (13% versus 8.4%; p=0.29).

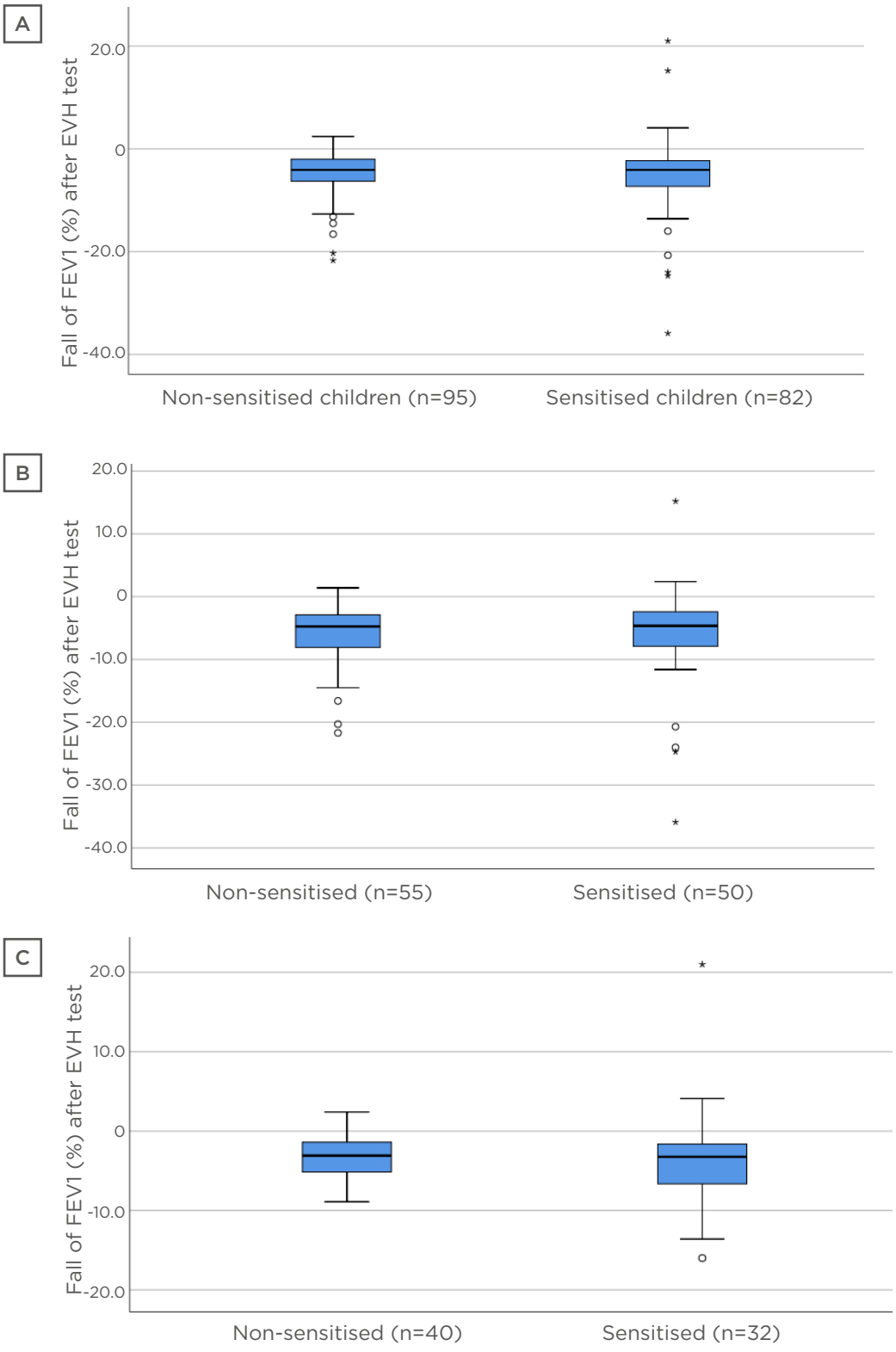

Figure 1: Fall in FEV1 after EVH test in A) all children (p=0.69); B) patients (p=0.85); and C) controls (p=0.53).

Mann–Whitey U was used for statistics for all analyses.

EVH: Eucapnic voluntary hyperventilation; FEV1: forced expiratory volume in 1 second.

In post-hoc analysis, similar results were observed among cases and controls. In addition, there were no differences between the groups during the pollen season between 15th April and 15th August.

In correlation analysis, there were no significant findings between fall of FEV1 and specific IgE level to birch, timothy, mugwort, cat, dog, or house dust mite.

CONCLUSION

Aeroallergen sensitisation did not have an effect on EVH results. ■

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The Nature of the Ratio of B1 to B2 Lymphocytes in Patients with Common Variable Immunodeficiency

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Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors would like to thank the enrolled patients for participating in this study.

Keywords: B1 and B2 lymphocytes, common variable immunodeficiency (CVID).

Citation: EMJ Allergy Immunol. 2021;6[1]:41-42. Abstract Review No. AR5.

BACKGROUND

Antibody production inhibition is a leading sign of common variable immunodeficiency (CVID), but the causes of this defect are not fully disclosed.[1,2] It is known that the basis of the violation of antibody synthesis results in a change in the functions of B lymphocytes. At the same time, the data on the quantitative and functional features of B lymphocyte subpopulations in CVID are very contradictory, which determines the interest in this area of research.[3,4]

AIM

To identify a characteristic of the B1 and B2 subpopulations of lymphocytes in patients with CVID.

MATERIALS AND METHODS

Ten patients aged 28–62 years (male: 4; female: 6) with a diagnosis of CVID were followed up. All the patients received intravenous Ig therapy (0.4 g/kg of body weight, monthly). The data of the examination before the next infusion in the conditions of clinical remission were analysed. The control group consisted of 10 blood donors. The phenotypic parameters of B lymphocytes were evaluated by flow cytofluorimetry using appropriate sets of monoclonal antibodies.

RESULTS

It is known that B lymphocytes of the peripheral blood are mainly represented by the

B2 subpopulation, and the increase in the content of B1 lymphocytes that are ontogenetically, phenotypically, and functionally different from them is a reflection of intra-immune dysregulation.[5] The results of the subpopulation characteristics of B cells of the control group showed that the proportion of B2 lymphocytes was 97.5% (95–99) with a ratio of B1:B2 of 1:40. In the cohort of patients with CVID, the B2 phenotype averaged 85.7% (77–92) of all circulating B lymphocytes. In this case, the ratio of B1:B2 in 100% of cases was lower than the data of the comparison group, but the spread of values varied very significantly, from 1:3 to 1:31. Comparison of the results of phenotyping of B cells with the data of the serum IgG revealed that the values of the ratio B1:B2 closest to the control (1:26; 1:22–1:31]), observed in patients with IgG pre-transfusion level of 8.1 g/L (7.9–8.3), whereas in the group of patients with CVID, where the ratio of B1:B2 in relation to control reduced significantly (1:7; 1:3–1:9), pre-transfusion values for IgG ranged from 6.8 (6.5–7.1) g/L.

CONCLUSION

Thus, a decrease in the proportion of B2 lymphocytes in the total pool of circulating B cells was common for all the presented observations of patients with CVID, and the variability in the degree of decrease correlated with the pre-transfusion level of IgG content. ■

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Congress Interviews

Marek Jutel, President of the European Academy of Allergy and Clinical Immunology (EAACI), and Maria Jose Torres, member of the Governance Committee and of the Advisory Board of the Research and Outreach Committee, EAACI, spoke with EMJ about their roles within the society, research interests, recent publications, and what the future of allergy medicine and immunology may hold.

Featuring: Marek Jutel and Maria Jose Torres.

Marek Jutel

Head of the Department of Clinical Immunology, Wrocław Medical University, Poland; and President of the European Academy of Allergy and Clinical Immunology (EAACI)

Q1 In 2019, you were elected as President of the European Academy of Allergy and Clinical Immunology (EAACI). What has been your proudest achievement in this role so far?

This is a difficult time because of COVID-19. So, I would rather say that my greatest achievement is maintaining current operations and the mission of the EAACI, which is providing education. And, also, the exchange platform and continuing education for specialists. So, we managed to organise the digital Congress last year, which was in June, and it was the first digital Congress of the EAACI. But, also, first in the field and we were the benchmark for the other societies dealing with similar problems in the same field similar diseases, and the next is actually the first hybrid meeting, in July 2021. So, this is for sure, the great achievement.

And the other one I would like to mention is that our *Allergy* journal increased its impact factor. It was announced a few days ago that now its impact factor is over 13, which is the highest journal in

the field. So, this is a very impressive impact. Actually, the journal is mostly the achievement of the Editor-in-Chief but actually the journal is supported by the EAACI and I, as the President, was also support, at least, and the EAACI provided financial support and the infrastructure for the for *Allergy* journal. This is a great achievement and also continuing this, the knowledge dissemination pathway.

We also launched an e-learning platform with the Continuing Medical Education points, and we also initiated the involvement of this e-learning platform to the Knowledge Hub. So, in these difficult times where it is difficult to organise physical meetings, we intend to or actually we already have achieved quite a lot to provide a platform for education and knowledge, to exchange ideas, and exchange meetings in between the congresses, which is also very Innovative. And this is one of the greatest achievements.

Last but not least are our activities in the European Union (EU) that we have. We keep our interest group in the European Parliament. We have a number of European members and we are very active in this group and very much in line with what is going on in Europe. We align with the general policy of the EU as well as for the Green Planet.

There are a lot of achievements actually, not just the one, so that is great to hear.

Q2 What are the biggest challenges for the EAACI in their quest to tackle the burden of allergy in Europe and create the highest standard of practice in order to benefit patients?

So, the major challenges are, as it has been over the decades, that we have major problems with harmony, attention to patient management, and care between the different European countries. That is why we are so active at the level of the EU because this is how we can approach these countries, and through EU activities as well. And there are a number of initiatives we do that I mentioned.

And now, in more detail, there is the Europlanet Science Congress, which is within the EU's projects. Then, of course, the prevention of allergic diseases, and the role of mobile health and telemedicine, which is also our major focus because the development of the technology opens up enormous opportunities. By using this specialist technology in mobile health, we can improve the number of patients in debt due to their health. Then what we need is also to have enough evidence to convince the politicians and health authorities, as well as have real-world evidence for the health economics and registries. So, the real challenge is to have more economic health studies showing that we can achieve a lot, not only in terms of improvement but also in terms of rationalising the finances and the way that the money is being spent for treatment by the effectiveness.

Let's say that we organised treatment management of allergic patients. We can spend the money much better and more effectively, but we need to convince other stakeholders more than ourselves. I mean physicians and their patients, but we will have to reach out to the politicians. So, for this, we need a lot of data, and this is the real challenge, and we are going to focus very much on that. So again, mobile health, economics, registries, and real-world evidence.

However, this it is still very difficult because we have to tackle the different regulations in all the EU countries. It is not always easy to convince everyone, but we have achieved a lot. I think that we did a lot, especially by being very active in publishing guidelines, position papers, and statements that are really the benchmark, and are really important; they are being read and implemented virtually. This is mostly based on the elaboration of the international guidelines and position statements of people from different European countries and over for all world.

Key opinion leaders are involved and, by this involvement, of course, we can also disseminate the guidelines very effectively and gradually. They are being implemented, but the major problem is that some are not convinced. As I said, these stakeholders' are the major problem with this harmonisation as we need to convince the politicians and the people who make the laws. So, this is actually the most difficult part in this harmonisation; it is not really the scientific work and providing scientific evidence, which is, of course, important to convince these people. But then we really need to invest a lot of effort to

"But then we really need to invest a lot of effort to talk to them and to bring this knowledge to the people who are not specialists, but they have the good will to provide the best patient care"

talk to them and to bring this knowledge to the people who are not specialists, but they have the good will to provide the best patient care. But they need to be effective in talking for them.

Q3

You briefly touched upon the new EAACI Guidelines on the use of biologicals for severe asthma, which was published in early 2021. What would you say is the biggest change for clinical practice and how do you think they could improve the current practice for asthma?

These are very important guidelines because these biological treatments are rapidly developing and there is increasing number of products and relatively limited experience among physicians, because they are expensive. In many cases, they are only used in some specialised centres, especially in the countries with lower incomes, even within Europe, as it is the case, for example, in Poland. So, it is important to publish these guidelines again to convince the physicians and also the payers for the wider use of them. They would work, and the most important thing in our guidelines is a specialised approach, commanding a 'by patient' approach not by the patient groups. And this is very much in line with, as I said, a pretty perfect personalised and precision medicine approach. So, we paid a lot of attention to the mechanisms and the end of types, especially to the Type 2 response, which is very important as allergic type.

Let's say that the way we should decide on what kind of treatment to use with the patients is a new approach based on phenotype and other analysis that will provide, with very good guidance, how to apply biologics on the individual, as I said, on a personalised basis, with a background of precision medicine. Precision medicine is based on the profound in-depth knowledge of the mechanisms, which could be very individualised.

Even if the patients have similar symptoms, their mechanisms might be different and that is why biologicals are important. And the most effective way at that we are using them is with the stratified patients who would most benefit from this treatment.

Q4 One of your research interests includes using recombinant allergens in the treatment of allergic diseases. Could you tell us the current stage the research is that and how it has changed clinical practice for allergic diseases?

Common intelligence technology has been a milestone in the development of allergy in the first place with diagnostics and then treatment of the allergen sources. In other words, other than with extracts, they contain a number of allergens. However, there could be major, intermediate, or minor allergens and only by having this technology of recombinant allergens is it possible to develop the proper diagnostics. In terms of not only diagnosing, the sensitivity of the allergy to the whole extract like, for example, grass pollen, can be characterised the sensitisation spectrum.

We call for the sensitisation spectrum of the patients, based on the major allergens. We have a few major allergens (for example, grass pollen) in patients with allergies on the mycological scale. There is also this cross reactivity between these allergens. When we use this recombinant technology, we can better diagnose patients and better characterise the sensitisation spectrum. In this way, we can better use this precision medicine approach. The personalised approach is best because patients who have the same symptoms, even to the same allergens, can have a different sensitisation spectrum, and they could be and should be treated differently.

However, it is very difficult to go to the next step because the very logical approach would be to characterise the sensitisation spectrum and the patients, not going deeper into the individual allergens to extract and then match the vaccine because the way we should use it. The best thing to do is to design a vaccine, and ideally this should be an individual vaccine based on the sensitisation spectrum that would contain the main other components that are relevant to the patient's symptoms.

Also, based on this component is the result diagnostic. In detail, diagnostic is called the component or resolved diagnostics. This means we can diagnose the sensitivity to the component, which is this allergen's severity level. However, this is a very difficult step to do, as there is a problem, of course, with the of authorisation of this product. So, at the moment, we are at the stage of taking a little bit of a different approach, which is to design and develop a standardised vaccine that would contain these components that would match the majority of the patients, but not the individual patient. We are still at the stage of clinical trials and getting the authorisation for this product. At the moment, these products are not widely available on the market but it is our aim to finally replace the extracts with recombinant allergens.

Just to summarise: the problem with the extract is also that there are different sources and also different extract providers to companies. They try their best to standardise these extracts but they get these extracts from different sources, and they can differ in the content of these components of our major allergens. So, that's why there is a problem with extracts and the major challenge is to develop the real recombinant vaccines.

Q5 You currently have close to 150 international publications in your name for your research in allergic diseases and immunology. What do you believe are the current gaps in literature and what topics merit greater attention?

We need to better characterise the different endotypes of diseases. So, what is actually the mechanism, which is the background of developing of the symptoms in the patient? But the problem that we can have, for example, in a patient with asthma, is that we have to say to all patients with asthma with the same symptoms. However, it would show different endotypes of the mechanisms that lead to the symptoms are different and this is an extremely complex problem. There is a large number of endotypes. However, we will not move the field forward, especially using the biologics, if we will not further advance in the description of these endotypes.

"But then we really need to invest a lot of effort to talk to them and to bring this knowledge to the people who not specialists, but they have the good will to provide the best patient care"

So, this is one of the key points. And then, of course, we are going along these lines, which I believe that the new biologics are based on this and these endotypes. This should develop further and this is an enormous opportunity to try.

At the moment, we target a selected group of patients but, ideally, we should be using biologic therapies widely as they are, potentially, much more effective than the traditional pharmacotherapies. Now, we use them mostly in asthma but less in, for example, allergic rhinitis, which is well-served with pharmacotherapies, but this should also go this way.

However, as I mentioned in the very beginning, we need more data in terms of holistic approach to our health. I mean environmental science, I mean the exposome the epigenetic influences on people with allergies.

We would like to bring the awareness to the stakeholders, to the people. First of all, to the patients and the general public and also to the politicians, saying that allergies belong to this non-community of diseases that show a lot of common pathways, for example, with even cardiac disease.

We need more research that would allow this holistic approach and a more general view of it on a patient group with different diseases, where we will find a common ground, common pathway that would be best treated.

It is also with these genetic therapies and these biologics we would better manage the patient's condition.

Q6 Over the last year or so, you have published work investigating the effects of COVID-19. What impact has COVID-10 had on the clinical practice of allergic diseases?

Very large, I'm afraid. And it has raised a lot of questions.

Firstly, patients with allergies, especially patients with asthma, are at higher risk. With COVID-19, however, we are very lucky that it has not been shown to be more severe in individuals with allergies, especially in asthmatic patients. So, this is really good. This has not been the case with obesity metabolic diseases or diabetes. So, the good news is that patients with asthma to not show the most severe disease and do not die more frequently.

But in fact, there was an impact mostly because of the limited access to the allergy office and

we were struggling to keep going, especially if we had patients that receive an allergen specific therapy, which is a treatment that lasts for at least three years and, in some cases, even longer. The discontinuity was very important and it was not always possible to continue therapy. So, there was limited access. But I believe that after this first year that most of the allergy office is instantaneous, delivering the service at a very good level. So, I would say that the impact of COVID-19 was not extremely high in our speciality. We published a number of position papers again, where we put it clearly that, for example, the treatment with topical or a corticosteroid should be continued.

So, there was not a major problem that we had to modify the treatment of our patients, even with long-term treatments. The most important treatments were not modified or stopped except for allergen immunotherapy and biologics, which are an indication that the patient is actually sick. We have had to temporarily stop until these people recover and then we can continue.

So, we are very, very lucky to do this. The impact was not extremely high in our speciality.

Q7 With over 20 years of experience as an allergist, what was it that initially sparked your interest to pursue a career in this field and what advice would you give to an allergist starting their career now?

Now this is an extremely fascinating field because of this multidisciplinary and holistic approach to the patient. We are dealing with a large variety of diseases, starting from early diseases. So, bronchi and bronchial additives, upper respiratory tract diseases, and also dermatology and skin diseases, etc. But there is also a lot of other things like anaphylaxis, allergies to drugs, and others.

So, you need to have a large amount of knowledge. First of all, we need to combine your knowledge of different specialties, which is, in a way, very fascinating because it is good to be able to help the patients in this very holistic approach. But on the other hand, we combine this knowledge and put to the one pathway of hypersensitivity analogy and mechanisms that finally work together in the patient. So, this enables you to process this knowledge to best help the patients based on your profound knowledge of physiology and pathology, which involves the whole body. So, this is extremely important.

The other point is that in this speciality there is still a lot of space for your own initiative, for your own way on how you will treat the patients. While we might combine the knowledge of different specialities, we have our positions, and our guidelines, you also need to be you. You should invest a lot of your intellectual input to the individual patient who might have symptoms from different organs. As I said, from the lungs to the skin, etc.

Also, there is also food allergy and so forth, so this is really a fascinating speciality compared to what some others believe. Each medical speciality is interesting, but this one is very unique because in some others you just have some procedures, but we have some guidelines, and you follow these. And, in this speciality, you still have the need to improve.

You need your intellectual input, a lot of thinking is involved, of course, because the patients have this variety of symptoms of this very complicated part of physiology. So, this is a very fascinating, and is how I started my career at academic institutions. For me, it was extremely important that allergy gave me the opportunity for a very high level of research, which is not possible in many specialities.

Here, you have a lot of immunology research, for example, and you can publish in immunology journals with a high impact factor. So, this is also the perspective of performing high level science, not only clinical science, and not on the performance clinical trials, etc. But these complicated mechanisms of disease were very, very fascinating and I got interested.

And this also bearing in mind the enormous possibilities of using the immunological techniques and immunology terminology is, I would say, leading the field in medicine nowadays. We are just developing biologics and we are very working very quickly on it. We are part of this mainstream of medicine at the moment. So, there is, say, less space for surgery while it is still important. However, you cannot develop too many techniques in surgery; we have robotics, but I bet if you want to develop and learn a lot

and also input in science then the allergy clinic is also very interesting. You can immediately apply what your scientific knowledge and scientific approach to the clinic and to individual patients.

People are startled that I work in the lab for a few hours and then I work with the patients, and I can immediately apply my basic knowledge to put the patient's care, as I said, based on this very complicated patterns, very complicated background, the types of mechanisms, and pathophysiology of these allergic diseases. So, this is extremely fascinating.

When it comes to choosing a career, it always depends very much on the country and on the possibilities that the authorities give. So this must be very individualised, based on the country because in some countries allergies are very much supported and in some countries less so.

Their career is also based on the importance of the field would say in terms of the local health care system. So, this is one point. But on the other hand, I would advise that allergy is very interesting only to people who think out of the box, who have the ability to develop their own ideas, and they can benefit a lot in the allergy field. Finally, this field is still very much open if you consider what I explained; there are a lot of things to be done in the field. So, if the people are open-minded and want to achieve something, they have huge opportunities here because there is a lot of diagnostics and treatment options that could still be developed, and there is also the research part.

I think this is clear from the description of the mechanisms, the endotype structure is also extremely important and that there is a huge space for research and clinical development in allergy. So, for the people who want to do this, who are bright and intelligent, they can take a chance, especially also in view of very high impact journals like our *Allergy* journal, which was 13 with journals like this, they can immediately support their career with good publications if they manage to provide good research in the form of a manuscript. You can achieve a lot a lot because the field is still very much open for research and development.

Q8 Over the years that you have been practising as an allergist, how have you seen the field change in terms of advancements to the technology used?

First of all, there are new diagnostic techniques that I mentioned, which is very important. And then the biological treatments that developed were not around when I started. They just started to develop the antigen databases and mobile health technologies, so this is the system. These are the major developments that we have: large databases and mobile health. ■

Maria Jose Torres

Head of the Allergy Department of Malaga Regional University Hospital, Spain; Member of the Governance Committee, European Academy of Allergy and Clinical Immunology (EAACI); and Member of the Advisory Board of the Research and Outreach Committee, EAACI

Q1 Out of all your various responsibilities such as being a member of the European Academy of Allergy and Clinical Immunology (EAACI), principal investigator of 15 national projects, and Deputy Editor of the *Allergy* journal, what do you enjoy doing the most and why?

All working facets of a researcher and scholar have interesting aspects. Designing and writing a research study is a very challenging activity, but it is also very rewarding to get your application approved and funded. Conducting the study is a hard and demanding process but generating new data that helps broaden the knowledge of a specific field is among the most satisfying experiences a scholar can have. Once the results are generated, it is very important to disseminate them in top-level scientific conferences and good quality research papers. Therefore, contributing to the growth and progress of EAACI is just a natural activity for a researcher in the allergy field. Similarly, it is crucial to count on top-quality journals in our field to both disseminate our research in an efficient manner and to increase the interest in allergy and clinical immunology among talented, international scholars.

Q2 You are a member of the local organising committee for this year's hybrid EAACI congress. What have been the biggest challenges of bringing this hybrid congress model into fruition?

First of all, I believe the hybrid model has been a great success. This fact illustrates how crises, like the current COVID-19 pandemic, are also opportunities for improvement and creativity. The capacity of the EAACI to adapt to such a challenging situation is a very good example of the commitment of our members and the excellent 'health' of the organisation. From a practical point of view, it has been difficult to integrate remote and on-site Speakers and Chairs in the same session. Personally, I was amazed by the excellent coordination among the Chairs, Speakers, and technical team. We, as local organising committee members, tried help as much as possible, but the biggest credit should be given to the scientific programme committee coordinators, who did an outstanding job.

Q3 What does your role as coordinator of the Spanish National Network of Asthma and Allergy entail?

The network is called ARADyAL (Asthma, Adverse Drug Reactions, and Allergy Network). This structure functions as a translational network, combining innovative approaches in the field of immunology, genetics, nanomedicine, pharmacology, and chemistry, with a special focus on the search for new biomarkers and designing and evaluating intervention strategies for patients with severe allergic phenotypes. The ARADyAL consortium is composed of 28 groups from different Spanish regions, including clinical and basic researchers, and organised into three different scientific programmes. I am the coordinator of the whole network, and I am also directly in charge of conducting one of the scientific programmes. As the coordinator, I am responsible for the timely completion of the objectives and the smooth functioning of the network, especially guaranteeing a productive collaboration among research groups.

Q4 As a Deputy Editor of the *Allergy* journal and with over 180 publications to your name, what is the most interesting topic in allergy that you have come across and the current gaps in literature you have noticed?

"Our main goal was to provide clinicians and scientists with good quality data, helping them to treat their patients and design proper research studies."

When talking about current hot topics in allergy and clinical immunology, one is obliged to refer to the COVID-19 pandemic. For the last year and a half, the *Allergy* journal performed a significant effort to adapt the journal to the urgent need for good quality research about the COVID-19 pandemic. Our main goal was to provide clinicians and scientists with good quality data, helping them to treat their patients and design proper research studies. We worked very hard to position the *Allergy* journal among the most appealing international journals to publish COVID-19 related studies. I am happy to say that our efforts were successful, as illustrated by the excellent performance of the *Allergy* journal in all bibliographic quality indicators. Nevertheless, there is always room for improvement. Despite having progressed significantly in the understanding of the immunological mechanisms of COVID-19 and in the identification of risk factors for severe disease, we still need evidence-based recommendations for the daily management of patients (e.g., effective preventive measures, suitability of biologicals, and other treatments during the pandemic, optimal time gap between COVID-19 vaccine and allergen immunotherapy, etc.).

Q5 In a recent paper you published in June, you discussed the prevention of severe allergic reactions to COVID-19 vaccines. What are some of the urgent clinical needs in diagnosing and treating patients who have suffered severe allergic reactions after vaccination?

Currently, we encounter many difficulties when approaching patients who experienced reactions after a COVID-19 vaccination. On the one hand, the sensitivity and specificity of any diagnostic test has not been studied. This fact implies that we are unaware of the real value (e.g., positive and negative predictive values) of skin testing or basophil activation test to diagnose allergic reactions to COVID-19 vaccines. On the other hand, the safety of drug challenges with COVID-19 vaccines remains unstudied. There is a really urgent need to address all these aspects in properly designed studies.

Q6 Has the COVID-19 pandemic influenced your current research and opened a new area of unmet research in drug/vaccine allergy?

The COVID-19 pandemic has heavily impacted our research activities, especially those involving lung function test or nasal challenges. For many months, these projects had to stop and only recently we have been able to partially resume, although at a significantly slower speed compared to pre-COVID-19 times. On the other hand, the pandemic has opened the opportunity for very interesting research lines. Our group has been focussed on drug hypersensitivity for decades, and we started investigating adverse reactions to COVID-19 vaccines as soon as the administration programme started in early 2021. Of note, we are correlating the tolerability of the vaccines with their immunological effect on the patient. We really hope these studies will contribute to the prediction of adverse reactions and a better selection of candidate patients for each type of COVID-19 vaccine.

Q7 What are some points of emphasis you incorporate into practice to be the best allergist you can be?

As a physician, it is very important to execute an evidence-based practice. To this end, it is crucial to be up-to-date and to never stop studying and learning. As a matter of fact, regularly conducting research studies is the best guarantee for being aware of new discoveries and trends in clinical science. An evidence-based practice ensures that your patients are managed in the best possible way according to the current state of knowledge in a specific area.

Q8 Finally, what are your ambitions for the future and what future studies are you looking to explore in the next year?

From a research perspective, I am really interested in understanding the underlying mechanisms of allergic reactions to drugs and in searching for diagnostic biomarkers. Moreover, I find the research about prevention of allergic diseases and treatments able to modify their natural history fascinating. In the coming years, I would also like to continue contributing to the growth of the EAACI and *Allergy* journal and to position allergy and clinical immunology among the most appealing scientific disciplines for physicians and researchers. ■

High-Sensitivity Assays for C-Reactive Protein as a Systemic Inflammatory Marker in Assessing Asthma

EDITOR'S PICK

I have selected this review by Ong as my Editor's Pick for this issue because there is a real interest in establishing an early diagnosis for asthma, but also in determining the risk level of severity regarding the underlying inflammatory process. This role could be played by using C-reactive protein, a very simple and relatively low-cost marker. This review aims to explore the potential of this marker for determining the severity of disease, despite the fact that more studies should be undertaken to define the close relationship of those two concerns: inflammatory process and severity of the disease.

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Abstract

Asthma is a chronic inflammatory condition, and the main features include airway hyper-responsiveness and inflammation of the airway with the accumulation of inflammatory cells. Increased level of plasma fibrinogen and serum amyloid A suggests the involvement of systemic inflammation in asthma. C-reactive protein (CRP) is an acute-phase protein that produced mainly by hepatocytes and is an inflammatory marker. CRP levels monitoring is useful in the evaluation of early inflammation and efficacy of treatment in acute-phase illnesses. Several studies show that asthma alone can cause an increase in high-sensitivity CRP (hs-CRP) when compared with the healthy controls. Besides that, steroid-naïve patients have a higher mean value of hs-CRP levels compared with those on inhaled corticosteroid. Furthermore, studies have shown that an elevated hs-CRP level has a positive correlation with non-atopic asthma but not atopic asthma. Additionally, an increase in serum hs-CRP levels correlates with the severity of asthma. Therefore, serum hs-CRP is a useful surrogate marker to predict the severity of inflammation of the bronchus in asthma and assess the asthma status.

INTRODUCTION

Asthma is a chronic inflammatory condition where the main physiological features include reversible airflow narrowing and systemic inflammation.[1] Airway hyper-responsiveness and inflammation of the airway with the accumulation of inflammatory cells are observed in mild and moderate asthma to severe asthma.[2] Involvement of inflammatory cells such as neutrophils, eosinophils, mast cells, neutrophils, cytokines, and T-lymphocytes in asthma induce systemic inflammation.[3] Increased level of plasma fibrinogen and serum amyloid A suggests the involvement of systemic inflammation in asthma.[4] Severe inflammation and exacerbation occur in asthma occur due to narrowing of the airway, airway oedema, bronchial hyper-responsiveness, and hypersecretion, which result from the remodeling process.[2] Several studies have shown that the inflammatory process in asthma correlates with severity of disease and deterioration of pulmonary function.[2, 5-8]

C-reactive protein (CRP) is an acute-phase protein that is produced mainly by hepatocytes and is an inflammatory marker.[9] IL-1, IL-6, and TNF-α are important cytokines that stimulate the activation of CRP.[7] IL-6 is involved in phosphorylation of the transcription factor through gene activation and thus promotes the synthesis of CRP.[10, 11] Besides that, IL-1β stimulates the transcriptional events in the presence of IL-6 which then activates CRP.[12] IL-6 has growth-regulator factors, which are involved in activation, growth, and differentiation of T-cells.[7] Moreover, activation of CRP can stimulate the vascular smooth muscle cells to secrete more TNF-α.[13] CRP is involved in complement activation and activation of phagocytic cells to eliminate bacteria and damaged cells.[7] Increased CRP levels may indicate several conditions such as infection, cancer, and autoimmune conditions such as systemic lupus erythematosus and rheumatoid arthritis, and myocardial infarction.[9] CRP levels monitoring is useful in the evaluation of early inflammation and efficacy of treatment in acute-phase illnesses.[14] High-sensitivity assays for CRP (hs-CRP) which measures the very low amount of CRP in the blood (below 0.2 mg/L) has been used to evaluate the systemic inflammation and prognostic marker for diabetes mellitus and cardiovascular diseases.[2] A population-based study by Kony et al.[15] suggested that increased CRP levels are associated with higher frequency of bronchial hyper-responsiveness and respiratory impairment due to systemic inflammation. Therefore, hs-CRP could be used to detect systemic inflammation and the severity of asthma. The purpose of this paper is to evaluate the association between hs-CRP levels and patients diagnosed with asthma.

Table 1: High-sensitivity C-reactive protein levels in patients with asthma and controls.

Study	Study design	Asthma group		Healthy control	
		Patients (n)	Hs-CRP level (mg/L)	Patients (n)	Hs-CRP level (mg/L)
Galez et al., 2006[16]	Observational study	47	0.55	42	0.23
Kasayama et al., 2008[5]	Observational study	329	0.44	1,684	0.40
Pellizzaro et al., 2010[17]	Observational study	22	4.20±1.32	27	1.74±4.67
Kilic et al., 2012[18]	Observational study	30	$1.97x10^{-6}$	30	$4.50x10^{-6}$
Razi et al., 2012[19]	Observational study	108	5.47±7.33	93	1.46±1.89
Shimoda et al., 2015[20]	Observational study	45	2.21x0.39	40	2.49x0.41

Hs-CRP: high-sensitivity C-reactive protein.

METHODS

A PubMed search was conducted using the MeSH terms "C-reactive protein" OR "systemic inflammation" AND "asthma" retrieving papers from January 2000–December 2020. Additional studies of interest were retrieved from the reference list of selected articles. The search yielded 214 original articles. The titles and abstracts of all studies were screened for their eligibility to be included in this review. A full-text manuscript was examined when the decision could not be made on the basis of the title and abstract solely. A total of 19 articles were deemed relevant and were included in this review.

C-REACTIVE PROTEIN IN PATIENTS WITH ASTHMA AND HEALTHY INDIVIDUALS

Several studies have shown that serum levels of hs-CRP are increased compared with healthy controls (Table 1).[5,16-20] Both systemic inflammation and airway inflammation may occur in asthma due to an increase in the concentration of acute-phase protein.[21] A study by Jousilahti et al. showed that acute-phase proteins of systemic inflammation such as serum amyloid A and plasma fibrinogen were positively associated with asthma prevalence.[22]

Additionally, the study by Büyüköztürk et al. also showed that the level of acute-phase reactant serum amyloid A was significantly higher in patients diagnosed with asthma compared to healthy controls.[21] Hs-CRP measurement may be used as an inflammatory marker to assess the severity of systemic inflammation asthma.[5,16,17,19] CRPs can activate macrophages by binding with Fc receptors for antibodies, and act on monocytes and neutrophils by interacting with CRP receptors on their surface.[19] Therefore, CRP can be a useful surrogate marker to assess subclinical, airway inflammation, and systemic inflammation in asthma.[5,16,17,19]

The study by Sävykoski et al.[23] suggested that serum hs-CRP levels were higher in patients with mild and moderate asthma compared to the healthy controls.[23] Besides that, the study by Wu et al.[24] demonstrated that hs-CRP level gradually increased in asthma of various severity from mild, moderate to severe in children.[24] A population-based study by Shaaban et al.[25] demonstrated that increase serum CRP levels over time were associated with a decrease in forced expiratory volume in 1 second (FEV_1) and pulmonary function.[25] Furthermore, Kony et al.[15] showed that increased serum hs-CRP was associated with lower FEV_1.[15] However, the study by Ramirez[26] showed no significant correlation between hs-CRP and asthma with mild severity.[26]

However, several other factors may contribute to the elevation of serum hs-CRP levels other than asthma.[17] For example, obesity, cardiovascular diseases, atherosclerosis, diabetes mellitus, and ageing may elevate the hs-CRP levels whereas smoking cessation may reduce the hs-CRP levels.[17,19] Therefore, an increase in CRP level in patients with asthma may be caused by an underlying chronic inflammatory state of the patients or infections.[17] Moreover, hs-CRP levels can be affected by the interval between the beginning of the asthma exacerbation and the sample collection for measurement.[27] Hence, CRP is a sensitive but non-specific marker of inflammation.[28] Nevertheless, an increase in hs-CRP level in children is primarily attributed to respiratory allergic diseases because the prevalence of these risk factors is very low in children.[7] Besides that, serum level C3 is elevated in children with asthma, and the serum C3 level has a positive correlation with the severity of asthma.[29]

Despite these confounding factors, results from several studies suggest that asthma alone can cause elevated CRP levels.[16,17,19] CRP levels are also increased in patients with undiagnosed asthma even with the absence of pulmonary events.[17]

C-REACTIVE PROTEIN IN STEROID-NAÏVE ASTHMA AND INHALED-CORTICOSTEROID ASTHMA

Several studies demonstrated that steroid-naive patients have a higher mean value of hs-CRP compared to those on inhaled corticosteroid (ICS; Table 2).[4,6,7,9,30-33] In a study by Takemura et al.,[4] serum levels of hs-CRP in steroid-naïve patients correlated positively with numbers of sputum eosinophils and negatively with indices of pulmonary function. However, no significant correlation was found between serum hs-CRP levels, pulmonary function, or sputum indices in steroid-inhaling patients.[4]

Table 2: High-sensitivity C-reactive protein levels in patients with steroid-naïve asthma and treated with inhaled corticosteroids.

Study	Study design	Steroid-naive asthma		Inhaled corticosteroid asthma	
		Patients (n)	Hs-CRP level (mg/L)	Patients (n)	Hs-CRP level (mg/L)
Takemura et al., 2006[4]	Observational study	22	1.30±1.5	23	0.90±1.0
Allam et al., 2009[9]	Observational study	24	2.63±2.1	26	2.35±1.66
Zietkowski et al., 2009[7]	Observational study	20	1.36±0.54	19	1.07±0.28
Deraz et al., 2011[6]	Observational study	30	3.15	30	1.55
Girdhar et al., 2011[30]	Observational study	30	48.0±60.0	30	24.00±54.00
Halvani et al., 2012[31]	Observational study	30	3.32	31	2.60
Hoshino et al., 2014[32]	Observational study	48	0.90	51	0.50
Karthikeyan et al., 2014[33]	Observational study	18	0.93±1.18	22	0.17±0.18

Hs-CRP: High-sensitivity C-reactive protein.

In a study by Zietkowski et al.,[7] serum hs-CRP levels in patients diagnosed with mild-to-moderate asthma treated with ICS was lower compared to steroid-naive mild asthma but there was no correlation with serum eosinophil cationic protein.[7] However, the study by Allam et al.[9] showed that serum hs-CRP level had a significant positive correlation with sputum eosinophil percentage.[9] The contradictory results from different studies can occur due to non-compliance of medication, suboptimal doses of ICS, or development of asthma that resistant to steroids.[9]

Decreased CRP levels in patients treated with steroids can be due to the interaction between corticosteroids with IL-1β and IL-6 which reduce the production of ILs.[17] Corticosteroids inhibit the synthesis of IL-6 is induced by cytokines such as IL-1β and TNF-α.[17,34] The study by Hashino and Nakamura[35] demonstrated that inhaled beclomethasone dipropionate improved the symptoms of asthma and hyper-responsiveness by inhibiting the infiltration of inflammatory cells in the airway tissue.[35] In addition, Sin et al.[36] showed that discontinuation of inhaled fluticasone increased the serum CRP level in mild-to-moderate chronic obstructive pulmonary diseases.[36] Therefore, based on the result of these studies, ICS should reduce the systemic inflammatory marker in asthma.[31]

Furthermore, a study by Kasayama et al.[5] also suggested that prolonged ICSs treatment for 3 months reduces the plasma CRP levels significantly due to the clinical effect of corticosteroids on the inflammatory process of airway tissue.[5] Deraz et al.[6] showed that serum hs-CRP had a sensitivity of 72% and a specificity of 93% in assessment of different grades of asthma severity and control.[6]

The study by Kadakal et al.[3] demonstrated that the hs-CRP decreased in post-treatment of corticosteroid in asthma compared to pre-treatment where the hs-CRP levels pre-treatment and post-treatment were 4.7 mg/L and 2.1 mg/L respectively.[3] This study also showed that the hs-CRP levels had a negative correlation with FEV_1 following corticosteroid treatment due to improved pulmonary function parameters and decreased hs-CRP levels.[3] In addition, the

study by Girdhar[30] also showed that hs-CRP levels decreased after treatment in patients with asthma where the hs-CRP levels before and after treatment were 4.8±6.0 mg/dL and 2.4±5.4 mg/dL, respectively.[30] Some studies did not show a significant decrease in hs-CRP level because of short duration and therapy, and inclusion of patients diagnosed with mild or moderate asthma where the inflammatory markers would be detected even at lower levels of inflammation.[3] Qian et al.[27] showed that hs-CRP is a sensitive marker only in cases of severe asthma.[27] Patients with a BMI >25 kg/m^2, especially females, have a higher risk of developing moderate to severe degrees of asthma.[30] The study by Boulet and Franssen[37] suggested that obese patients were less likely to achieve asthma control with ICS or ICS with a long-acting β-agonist compared to patients who were non-obese.[37] All those factors may cause only slight reduction in hs-CRP levels after treatment.

C-REACTIVE PROTEIN IN ATOPIC AND NON-ATOPIC ASTHMA

The study by O'Lafsdottir et al.[38] suggested that higher hs-CRP levels are associated with non-allergic asthma but not in allergic asthma.[38] Besides that, hs-CRP levels were also affected by age, BMI, and smoking.[38] The study by Sahoo et al.[39] also showed that increase in hs-CRP levels was observed in non-atopic asthmatics but no association of increased hs-CRP in atopic asthmatics.[39] The mean hs-CRP levels in atopic asthma and non-atopic asthma were 2.9±2.1 mg/L and 8.3±2.5 mg/L respectively, in the study.[39] Furthermore, the study by Butland et al., also demonstrated that hs-CRP levels had a positive correlation with non-atopic asthma but not atopic asthma.[4]

Lack of correlation between hs-CRP and atopic asthma suggests that the mechanism underlying the bronchial hyperresponsiveness is different.[38] The study by Lúdvíksdóttir et al. showed that atopic asthma was more hyper-responsive to adenosine 5′-monophosphate (AMP) compared to non-atopic asthma.[41] Another study by Lúdvíksdóttir et al., showed that atopic asthma had higher levels of exhaled nitrogen oxide compared to non-atopic asthma.[42] Moreover, the study by Amin et al. showed that the number of eosinophils, T-lymphocytes (CD3-, CD4-, CD8-, CD–25-positive cells), IL-4, and IL-5 increased more significantly in atopic asthma compared to non-atopic asthma.[43] Furthermore, atopic asthma has a higher degree of epithelial damage compared to non-atopic asthma.[43] Negative correlation between hs-CRP levels and atopic asthma suggest that the systemic inflammatory process in atopic asthma is low.[38]

The study by Wood et al. demonstrated that asthmatic patients with neutrophilic airway inflammation had increased systemic inflammation.[44] Therefore, CRP can be a systemic marker for patients with neutrophilic asthma.[44] Moreover, the study by Ko et al., also reported that hs-CRP had a significant association with patients with asthma with high neutrophil and low eosinophils.[36] The study also showed that hs-CRP levels were correlated with small airway obstruction in patients diagnosed with neutrophilic asthma.[45] Inflammatory mediators results from inflammation or repair mechanisms may 'spill-over' into circulation which may lead to systemic inflammation.[46] Fu et al. showed that systemic inflammation in neutrophilic asthma had altered genes involved in IL-1, TNF-α/nuclear factor-κB, and Kit receptor pathways which were associated with immune response, inflammatory responses, and defense.[47] Furthermore, Wood et al. showed that patients with neutrophilic asthma had increased receptors α for IL-8 (IL-8-RA) which are highly selective for neutrophil chemotaxis.[44] Meanwhile, there were several studies suggested that there was no correlation between hs-CRP level with history of atopy, eosinophilia inflammation, and allergic sensitisation.[4,31,44]

C-REACTIVE PROTEIN IN PREDICTING ASTHMA CONTROL

Four studies showed that serum hs-CRP increases with the severity of asthma and the highest among patients with poorly controlled and exacerbating asthma (Table 3).[18,26,48,49] However, studies by Ramirez et al. and Sigari et al. demonstrated no significant correlation between hs-CRP levels and clinical indices of asthma control.[26,50] The studies by Fujita et al. and Al Obaidi et al. classified patients to stable asthma and exacerbating asthma, the study by Ramirez et al. classified the patients according to the National Asthma Education and Prevention Program (NAEPP) control scores.[51]

Table 3: Correlation between high-sensitivity C-reactive protein levels and asthma control.

Study	Study design	Well-controlled asthma		Partly controlled asthma		Poorly controlled or exacerbating asthma	
		Patients (n)	Hs-CRP level (mg/L)	Patients (n)	Hs-CRP level (mg/L)	Patients (n)	Hs-CRP level (mg/L)
Fujita et al., 2007[48]	Observational study	NA	0.473	NA	NA	NA	0.908
Al Obaidi et al., 2013[49]	Observational study	126	16.970	NA	NA	52	225.230
Ramirez et al., 2010[26]	Observational study	27	2.040	NA	NA	27	1.990
Kilic et al., 2012[18]	Observational study	7	$0.700x10^{-6}$	NA	NA	23	$2.2x10^{-6}$
Sigari et al., 2013[50]	Observational study	59	10.600	30	11.41	11	11.200
Monadi et al., 2016[2]	Observational study	80	1.980±1.700	NA	NA	40	3.300±3.100

Hs-CRP: High-sensitivity C-reactive protein; NA: not available.

The study by Sigari et al. classified the patients according to Global Initiative for Asthma (GINA) control[52] and the studies by Kilic et al. and Monadi et al. classified the patients according to Asthma Control Test (ACT).[53]

The management goals of patients diagnosed with include suppression of airway inflammation and the achievement of well-controlled asthma.[54] ICS treatment was associated with decreased hs-CRP levels and therefore serum hs-CRP levels may be used to differentiate between poorly-controlled asthma and well-controlled asthma.[2] However, the study by Ramirez et al. suggested that there was no correlation between hs-CRP level with wheeze, the National Asthma Education and Prevention Program (NAEPP) control score, fractional exhaled nitric oxide, and FEV_1.[26] The study by Khalili et al. also showed that the fractional exhaled nitric oxide level was not associated with asthma control based on Asthma Control Questionnaire (ACQ).[55] Nevertheless, a study by Hancox et al. demonstrated that CRP level had a negative correlation with FEV_1 and forced vital capacity.[56] Moreover, two observational studies have shown that hs-CRP levels have a positive correlation with the severity of asthma.[23,27] A study by Tonelli et al. also demonstrated that hs-CRP levels are significantly higher in patients with severe asthma compared to patients without any respiratory symptoms.[57]

Several studies showed that hs-CRP can be used as a surrogate marker for evaluation and monitoring of asthma, estimation of disease severity, and response to corticosteroid therapy.[2,3,7,15] The synthesis rate of CRP reaches highest at around 48 hours and the CRP level decreases rapidly after the pathology ceases.[50] Elevation levels of hs-CRP in well-controlled asthma compared to healthy controls suggest that the continuous eosinophilic inflammation.[49] Several studies have shown that patients diagnosed with asthma are likely to have higher CRP concentrations compared to individuals who were diagnosed formerly with asthma or never had asthma.[56,58] Moreover, two studies have shown that increase levels of hs-CRP are associated with respiratory symptoms of asthma such as wheeze, dyspnoea after effort, and nocturnal cough.[15,38] Therefore, patients with asthma with exacerbation or that is poorly controlled may have elevated CRP concentration compared to patients with stable asthma or healthy control.[50]

The study by Kony et al. showed that higher frequency of bronchial hyper-responsiveness was associated with higher CRP levels while FEV_1 had a negative correlation with CRP.[15] The study by Fujita et al., demonstrated the serum hs-CRP levels have a positive correlation with the degree of airway obstruction as measured by FEV_1/FVC.[48] Furthermore, the study by Al Obaidi et al. showed that patients diagnosed with asthma who had frequent bronchial hyper-responsiveness or reduced FEV_1 tend to have both local inflammation in the bronchus and systemic inflammation.[49] Therefore, increased CRP levels are associated with impairment of FEV_1, decline of lung function, and high frequency of bronchial hyper-responsiveness.[49]

Several studies have shown that hs-CRP levels are correlated with asthma control.[18,26,48,49] Therefore, hs-CRP levels can be used as an adjunct in assessment of asthma control in routine clinic follow-up since measurement is relatively simple and non-invasive.[18] Moreover, hs-CRP can be a marker for systemic inflammation that reflects on the control of the patient's symptoms and severity.[18] Furthermore, hs-CRP level can also be used as a clinical indicator for dosage of ICSs and disease management.[18] However, hs-CRP levels in some patients during asthma exacerbation remains unchanged or decreased compared with the levels during asymptomatic period.[48] Several infectious and inflammatory conditions may alter the hs-CRP levels, which may impair its accuracy as an indicator for asthma control.[27] Therefore, hs-CRP level may be used as an additional clinical marker for asthma control in routine follow-up but not during asthma exacerbation in acute settings because it is not specific. Hs-CRP can be used as a tool for monitoring the asthma status but is not recommended for the diagnosis of asthma. Further longitudinal research is required to compare the efficacy and accuracy of hs-CRP levels with other investigations such as arterial blood gas, peak flow, and pulse oximetry in assessing asthma exacerbation. Further follow-up cohort studies are warranted by assessing asthma control periodically and measuring hs-CRP levels prospectively to confirm the correlation.

CONCLUSION

In conclusion, an increase in hs-CRP level in asthmatic patients suggest that asthma involves systemic inflammation rather than the location inflammation of the airway. ICS can reduce the local inflammation of the airway and systemic inflammation where the level of hs-CRP shows reduction after treatment. Additionally, a high hs-CRP level is associated with a decline in pulmonary function and increased sputum eosinophils. Moreover, increased hs-CRP level has a significant association with non-atopic asthma and severity of respiratory symptoms but not with atopic asthma. Nevertheless, serum hs-CRP is an effective surrogate marker to predict the severity of inflammation of the bronchus in asthma. Furthermore, hs-CRP can be used as a marker to assess asthma status because increased serum hs-CRP has a positive correlation with the severity of asthma. Hence, serum hs-CRP can serve as an effective marker to assess the control and severity of asthma and response to treatment.

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Combatting SARS-CoV-2: Potential Therapeutic Candidates Against COVID-19

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Abstract

Background: COVID-19, a global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in December 2019 in China and has spread to 210 countries and territories. Since then, it has infected >187.3 million people worldwide, causing >4.0 million deaths, and numbers are continuing to rise. Fever, dry cough, shortness of breath, and pneumonia are the main symptoms of this disease, which does not have any specific antiviral treatment or vaccines to date, and clinical management is mainly symptomatic treatment.

Summary: The global spread of SARS-CoV-2 has necessitated the development of novel therapeutic agents against the virus to stop the pandemic. Drugs targeting viral as well as host factors may have a potential antiviral effect. The development of novel drugs may take years; hence, the best alternative available is to repurpose existing antiviral drugs with a known safety profile in humans. Further, compounds with known *in vitro* and *in vivo* efficacy against SARS-CoV and Middle East respiratory syndrome coronavirus have been included in recent clinical trials and exhibited encouraging results against SARS-CoV-2. Here, the authors provide a summary of therapeutic compounds that have shown antiviral effects against SARS-CoV-2 infections in cell lines, animal models, and patients.

Key Messages: With every passing day, knowledge about SARS-CoV-2 is increasing due to continued efforts of scientists working in this area globally. Approximately 15% of patients with COVID-19 are affected by severe illness and treatments are desperately needed. In this time of global pandemic, collective and co-ordinated efforts are needed to develop therapeutic agents against this disease.

INTRODUCTION

Severe cases of pneumonia of unknown aetiology from Wuhan, in the Hubei province of China, were reported to the World Health Organization's (WHO) China Country Office on 31st December 2019. Later, public health officials reported that the causative agent of these pneumonia cases was a novel coronavirus, which was later renamed as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV) on 11th February 2020.[1] The WHO then named the disease caused by SARS-CoV-2 as the coronavirus disease 2019 (COVID-19). As of 14th July 2021, a total of 187,296,646 cases have been reported from 210 countries and territories, causing 4,046,470 deaths. Further, 3,327,841,570 vaccine dosages have been administered worldwide (at the time of writing).[2]

Most of the patients exhibited fever, dry cough, breathing difficulties (dyspnoea), headache, and pneumonia, bearing some resemblance with infections caused by SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). In severe cases of COVID-19, progressive respiratory failure due to alveolar damage, caused by acute respiratory distress syndrome (ARDS), led to the death of patients.[3,4]

Coronaviruses (CoVs), a group of enveloped, positive-sense, single-stranded RNA viruses, cause respiratory, hepatic, and enteric infections in several animal species including humans.[5,6] Novel coronavirus SARS-CoV-2, of probable bat origin,[7] belongs to the *Coronaviridae* family, which includes two subfamilies: *Letovirinae* and *Orthocoronavirinae*. The *Orthocoronavirinae* sub-family is further divided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*.[8] The past two decades have witnessed the emergence of three novel zoonotic *Betacoronavirus*: SARS-CoV, MERS-CoV,[9] and the most recent SARS-CoV-2. Although most human CoVs do not cause severe disease, >10,000 cumulative cases were reported in the epidemics caused by SARS-CoV and MERS-CoV in the past two decades, with a mortality of 10% in the former and 37% in the latter.[3]

After the emergence of novel coronavirus SARS-CoV-2, several treatment and vaccination strategies have been envisaged and scientists across the globe are racing against time to develop successful antiviral drugs and vaccines. To date, seven vaccines have been launched globally; however, the surge of mutated strains of SARS-CoV-2 has deteriorated the situation further in many parts of the world, even 18 months into this pandemic. Vaccinating the whole population may take several years, hence the focus on the development of novel therapeutic interventions should be a global priority. Any new therapeutic intervention may require years to develop, so is of no use in the current scenario. Here, the authors have summarised the information available related to various treatment options, either approved or already in the developmental phase, for the infections caused by SARS-CoV or MERS-CoV. This review also focuses on the potential drugs that can be repurposed for the management of COVID-19.

THERAPEUTICS TARGETING VIRAL FACTORS AND PATHWAYS

The life cycle of SARS-CoV-2 consists of three main stages: entry into host cells through interaction with angiotensin-converting enzyme 2; replication of genome; and assembly of virions by exploiting the host cellular machinery. Potential therapeutic agents targeting these critical viral pathways exhibit antiviral effects. In this section, the authors have discussed the antiviral drugs or compounds that target viral factors and pathways required for their survival and spread inside the host (Figure 1).

Nucleoside analogues

Nucleotides/nucleosides are the building blocks of nucleic acids including viral genomes. Nucleoside analogues are synthetic analogues of purine and pyrimidine bases, with altered sugar moiety or heterocyclic ring. These analogues are administered as prodrugs, which are converted into the active triphosphate inside the host cell by the action of host or viral kinases, and inhibit the viral replication by mis-incorporation of nucleotides in viral genomes during replication, causing chain termination that leads to the disruption of genome replication or subsequent transcription.

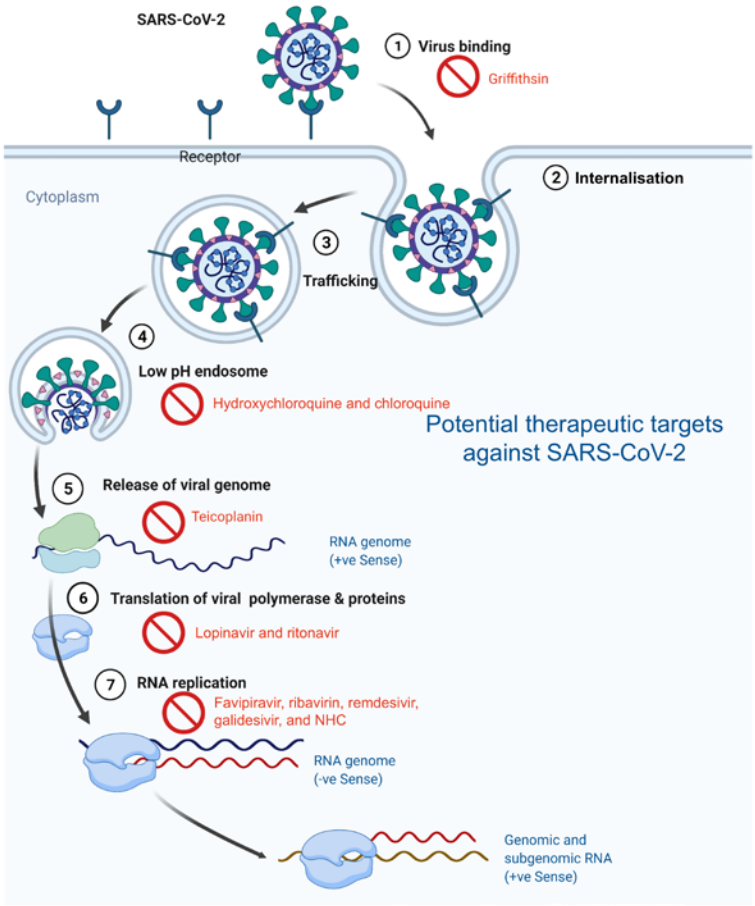

Figure 1: Directly acting therapeutic options against SARS-CoV-2.

NHC: an orally bioavailable cytosine analogue; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.
Image created using BioRender.

Further nucleoside analogues may introduce mutations in the genome by substitution of natural nucleotides during chain elongation, leading to impairment of RNA synthesis, structure, and functions. Loss of viral fitness and viability by accumulation of mutations is called lethal mutagenesis.[10,11] Drugs with the potential to target nucleosides interfere with viral genome synthesis and exhibit a broad range of activity against viruses including CoVs.

Favipiravir

Favipiravir (T-705), a guanosine analogue with approval for influenza treatment, inhibits the nucleic acid synthesis in RNA viruses such as influenza, Ebola, yellow fever, chikungunya, norovirus, and enterovirus.[12] Its triphosphate form is incorporated in the viral RNA strand by the viral RNA-dependent RNA polymerase, resulting in chain termination and lethal mutagenesis.[13,14] A recent study by Wang et al. reported the *in vitro* activity of favipiravir against SARS-CoV-2 in Vero E6 cells.[15] Randomised clinical trials have been initiated in patients with COVID-19 to evaluate the safety and efficacy of a favipiravir and interferon-α combination (ChiCTR2000029600[16]), and the efficacy and safety of a combination of favipiravir with two other drugs: baloxavir and barboxil (ChiCTR2000029544[17]). Data obtained from these clinical trials will give a clear picture of the future usage of these drugs against SARS-CoV-2 infections. Meanwhile, on 19th June 2020, the Drugs Controller General of India (DCGI) granted approval to Glenmark Pharmaceuticals, Mumbai, India, to manufacture and market favipiravir for restricted emergency use for the treatment of patients with mild to moderate COVID-19, considering the unmet medical demands in India due to the ongoing pandemic.[18]

Ribavirin

Ribavirin, a guanosine analogue, exhibits antiviral activity by inhibiting the viral RNA synthesis and mRNA capping in a broad range of viruses, including CoVs. The monophosphate form of ribavirin interacts with nucleotide biosynthesis enzyme inosine monophosphate dehydrogenase, resulting in inhibition of guanosine production, which impairs the viral RNA synthesis.[19] Moreover, its triphosphate form gets incorporated in the viral RNA by the viral polymerase, causing lethal mutagenesis.[20] It has been approved for the treatment of hepatitis C virus and respiratory syncytial virus.[21,22] However, when used in patients with SARS and MERS, it exhibited no obvious survival benefits. In a recent study, ribavirin exhibited antiviral effects against SARS-CoV-2 in Vero E6 cells.[15] Pharmacokinetics and bioavailability data of this drug are available, and findings from ongoing clinical studies in patients with COVID-19 may highlight its antiviral efficacy against SARS-CoV-2 infections. In one completed clinical trial assessing the efficacy of triple combination of ribavirin, interferon β-1b, and lopinavir–ritonavir, Hung et al.[23] reported that the ribavirin triple antiviral combination was superior and safer compared to lopinavir–ritonavir alone in treatment of patients with COVID-19.

Remdesivir

Remdesivir (GS-5734), one of the most promising drugs against SARS-CoV-2, is an adenosine analogue and a phosphoramidate prodrug with broad-spectrum activity against filoviruses, pneumoviruses, paramyxoviruses, and CoVs such as SARS-CoV and MERS-CoV.[24] The triphosphate active form of remdesivir mimics adenosine triphosphate and acts as a substrate for viral polymerase. It is incorporated into the growing strand of viral RNA and causes the premature chain termination of viral RNA.[25,26] Remdesivir is currently in clinical trial stage to evaluate its efficacy against Ebola.[27] Furthermore, it reduced the viral load in the lungs of a murine model of SARS and MERS upon prophylactic and early therapeutic administration.[28] Holshue et al.[29] recently reported that a patient with COVID-19 recovered in the USA after intravenous treatment of remdesivir in January 2020. In a recent randomised, Phase III trial of remdesivir (Veklury®, Gilead Sciences, Foster City, California, USA), wherein 53 severely ill patients with COVID-19 received remdesivir on compassionate use basis, clinical improvement in 36 patients was observed; however, this study is limited by the lack of viral load data.[30] In May 2020, Veklury received emergency use authorisation (EUA) from the U.S. Food and Drug Administration (FDA) for the treatment of hospitalised patients with severe COVID-19, after successful Phase III clinical studies.[30] Additionally, many Phase III clinical trials have been initiated to evaluate intravenous remdesivir in patients with COVID-19 (NCT04252664,[31] NCT04292730,[32] NCT04292899,[33] NCT04280705,[34] and NCT04257656[35]), and the results are awaited.

Galidesivir

Galidesivir (BCX4430), an adenosine analogue, inhibits RNA polymerase activity in various RNA viruses including SARS-CoV, MERS-CoV, filoviruses, Ebola, and Marburg viruses.[36] It is currently undergoing clinical studies for the evaluation of its safety and efficacy against yellow fever.[37] In preclinical studies, it exerted antiviral activities in SARS and MERS, hence its efficacy against SARS-CoV-2 should be investigated. Recently, the National Institute of Allergy and Infectious Diseases (NIAID) initiated a randomised, double-blind clinical trial to investigate the antiviral effects of galidesivir in yellow fever and/or patients with COVID-19 and results are awaited (NCT03891420[38]).

β-D-N4-hydroxycytidine

NHC (β-D-N4-hydroxycytidine/EIDD-1931), an orally bioavailable cytosine analogue, has shown broad-spectrum antiviral effects against many RNA viruses, including Ebola, influenza, CoV, and Venezuelan equine encephalitis virus.[39] Its antiviral effects are mainly attributed to the mutagenesis of viral RNA.[40,41] In a recent study, Sheahan et al.[39] reported that NHC/EIDD-1931 exhibited antiviral activity against MERS-CoV, SARS-CoV, SARS-CoV-2, and other Group 2b or 2c bat-CoVs, as well as the remdesivir-resistant CoV. Furthermore, prophylactic and therapeutic administration of EIDD-2801, an orally bioavailable NHC prodrug (β-D-N4-hydroxycytidine-5'-isopropyl ester), reduced the viral load and improved lung pathology in mice infected with SARS-CoV or MERS-CoV. This study could not demonstrate *in vivo* efficacy of NHC/EIDD-2801 against SARS-CoV-2 due to the lack of a suitable animal model.

Collectively, broad-spectrum antiviral effects and oral bioavailability of NHC/EIDD-2801 highlight its therapeutic potential as an antiviral drug against SARS-CoV-2 infections.[39] Currently, the efficacy of EIDD-2801 (molnupiravir) in patients with COVID-19 is being investigated in two clinical trials (NCT04405739,[42] NCT04405570[43]).

Protease Inhibitors

The proteases expressed by viruses are involved in proteolytic cleavage of polyprotein precursors, a crucial step in the formation of fully functional viral proteins and processing of proteins essential for the assembly of viral particles. Thus, targeting the viral proteases by protease inhibitors interferes with the binding of substrates to binding sites of proteases and results in immature virus particles.[44]

Lopinavir and ritonavir

Lopinavir (LPV) is an approved HIV protease inhibitor, which is administered in combination with ritonavir (RTV), a cytochrome P450 3A4 (CYP3A4) inhibitor that enhances the efficacy of LPV. This combination has exhibited antiviral activity against SARS and MERS in addition to HIV-1.[45] There are two proteases in SARS-CoV: a 3C-like proteinase and a papain-like cysteine proteinase.[46] The antiviral effects of LPV/RTV against SARS-CoV-2 are believed to be mediated partially through the inhibition of the 3C-like proteinase.[22,47]

Recently, a controlled, randomised, open-label clinical trial was carried out to evaluate the efficacy of LPV/RTV in patients with the SARS-CoV-2 infection (n=199), which yielded no significant clinical benefit in those patients.[48] However, another controlled clinical trial was initiated in China to evaluate the efficacy of a combination of LPV/RTV and IFNα-2b in patients with COVID-19 (ChiCTR2000029308[49]) and outcomes are awaited. Despite the poor treatment outcomes in patients with COVID-19, the combination of LPV/RTV is still being prescribed to hospitalised patients in many countries and their efficacy is under investigation in several clinical trials across the world.

Griffithsin

Griffithsin is a red-alga-derived antiviral protein that attaches to the oligosaccharide moieties of viral glycoproteins such as glycoprotein 120 of HIV and SARS-CoV spike glycoprotein 'S', a major immunogenic protein of human CoVs, which plays an essential role in virus and host cell receptor interaction, and hence, offers a potential drug target.[22] It inhibits a broad spectrum of viruses including SARS-CoV, human CoV NL63, and human CoV 229E *in vitro* as well as in mice infected with SARS-CoV.[50,51] Further, griffithsin has been evaluated in a Phase I clinical trial as a gel or an enema for the prevention of HIV; nonetheless, safety and potency against COVID-19 should be evaluated.

Chloroquine and hydroxychloroquine

Chloroquine (CQ) and hydroxychloroquine (HCQ), age-old antimalarial drugs from the group of amino-quinolones, have shown activity against SARS-CoV-2 in virus-infected Vero E6 cells.[15,52]

CQ causes alkalinisation of the phagolysosomes by increasing endosomal pH, which blocks virus–cell fusion.[53] Other mechanisms may include the inhibition of the glycosylation of viral proteins and cellular receptors of SARS-CoV. Few studies have reported the reduction of lung damage in patients with COVID-19 by chloroquine treatment, which may be attributed to modulation of cytokine release, an added benefit besides its antiviral properties.[52]

HCQ possesses a hydroxyl group in the side chain, which differentiates it from CQ.[54] Like CQ, HCQ also increases endosomal pH and exhibits antiviral effects and modulates immune cells; however, HCQ is less toxic than CQ.[55] The mechanism of action of HCQ is similar to that of CQ and it demonstrated a higher *in vitro* efficacy compared to CQ. The comparison of the half-maximal effective concentration values for HCQ (6.25 µM at 24 hours; 5.85 µM at 48 hours) and CQ (100 µM at 24 hours; 18.01 µM at 48 hours) revealed that HCQ exhibited a higher *in vitro* antiviral effect compared to CQ.[53]

CQ and HCQ are the most hyped drugs among all the potential therapeutics against COVID-19. The whole world seems divided on the role of these drugs in treatment of COVID-19. On the one hand, these drugs have been praised as

the magic drugs and biggest game-changers in history of medicine by the then-president of the USA, Donald Trump. On the other hand, these drugs have been criticised as useless and dangerous in COVID-19 management.[56]

Two studies, each from France and China, were among the earliest to claim the therapeutic benefits of HCQ in COVID-19.[57,58] However, the French study by Gautret et al.[57] that used a combination HCQ and antibiotic azithromycin[57] received criticism from the scientific community for drawing bold conclusions despite the lack of placebo arm, small sample size, and other limitations. Later, the International Society of Antimicrobial Chemotherapy (ISAC), which published this study in its journal, issued a statement that findings of the French study did not meet the society's expected standards.[59] On 28th March 2020, the U.S. FDA issued an EUA for the use of CQ and HCQ in the treatment of COVID-19.[56]

More than 100 clinical trials have been initiated in different parts of world to investigate the efficacy of CQ and HCQ in the treatment of COVID-19. The SOLIDARITY trial, co-ordinated by the WHO; DISCOVERY trial (SOLIDARITY in France); PRINCIPLE trial, conducted by the University of Oxford, UK; RECOVERY trial, also by the University of Oxford; and ORCHID trial, by the National Heart, Lung, and Blood Institute (NHLBI) in the USA, are the major clinical trials investigating the clinical benefits of HCQ in patients with COVID-19.[60] Major controversy erupted when analysis of a multicentre registry of 96,032 patients by Mehra et al.[61] reported enhanced ventricular arrhythmias and reduced survival of hospitalised patients with COVID-19 receiving CQ and HCQ, with or without a macrolide. Consequently, the DISCOVERY and SOLIDARITY trials were suspended immediately. However, this article was retracted within two weeks of publication and the WHO resumed the SOLIDARITY trial. Further, the RECOVERY investigators concluded that HCQ failed to exhibit any beneficial effect in patients with COVID-19 and discontinued the HCQ arm of the trial immediately. In mid-June 2020, the U.S. FDA determined that HCQ and CQ were ineffective in treatment of COVID-19 and revoked the EUA, leading to the discontinuation of the HCQ arm from the SOLIDARITY and ORCHID trials.[60]

Teicoplanin

Teicoplanin, a glycopeptide antibiotic currently used to treat staphylococcal infections, has shown broad-range antiviral activity against influenza virus, flavivirus, hepatitis C virus, Ebola virus, and human CoVs such as SARS-CoV and MERS-CoV.[62] Teicoplanin inhibits the cathepsin-L-mediated cleavage of the viral spike protein in the late endosomes, thus blocking the release of viral RNA and subsequent virus replication cycle of CoVs.[62] A recent study by the same group reported that teicoplanin exerts *in vitro* antiviral effect against SARS-CoV-2.[63] Further investigation of this drug should be carried out to evaluate its efficacy in animal models.

HOST-DIRECTED THERAPEUTICS AGAINST SARS-CoV-2

SARS-CoV, MERS-CoV, and SARS-CoV-2 induce unusual and extreme host immune responses, causing severe lung pathology. Similar to SARS and MERS, some patients with COVID-19 develop ARDS and in many of the moribund patients cytokine storm is observed, which is characterised by the release of IL-2, IL-7, IL-6, granulocyte-macrophage colony-stimulating factor, IFN-γ, IL-12, IFN-γ-induced protein 10, monocyte chemoattractant protein-1, macrophage inflammatory protein-1α, and TNF-α.[3,64,65] This aberrant immune response causes fibrosis and long-term lung damage, leading to reduced pulmonary function and a low quality of life in the patients who survive the intensive care.[66,67]

Development of therapeutics specific to SARS-CoV-2 may take years; in the meantime, existing host-directed therapeutics with proven safety profiles may be repurposed for use in treatment as adjuncts to combinational therapies with cyclosporine, remdesivir, lopinavir–ritonavir, IFN-β-1b, antiviral peptides, and monoclonal antibodies targeting SARS-CoV-2. These agents can help in boosting the immune system, reducing lung immunopathology, and preventing the COVID-19-associated ARDS.

Tocilizumab

Tocilizumab, a monoclonal antibody against the IL-6 receptor (licensed for both rheumatoid arthritis and cytokine release syndrome) with a proven safety profile, can be used as an adjunct

to antiviral treatments. IL-6, a proinflammatory cytokine secreted by macrophages, fibroblasts, and T- and B-cells, plays an important role in several immunological processes such as Ig secretion, T-cell activation, and initiation of hepatic acute-phase protein synthesis. Tocilizumab binds to both soluble and membrane-bound IL-6 receptors, and thus interferes with IL-6-mediated signalling. A recent retrospective study of 21 patients with COVID-19 using tocilizumab reported improved lung condition on CT scans, oxygen concentration, normalised lymphocyte counts, and C-reactive protein levels in most of the patients.[68] Furthermore, a randomised clinical trial of tocilizumab has been initiated in China in patients with COVID-19 pneumonia and elevated IL-6 levels (ChiCTR2000029765[69]). A retrospective, observational cohort study reported reduced mortality in patients with COVID-19 admitted to the intensive care unit after treatment with tocilizumab.[15] Another retrospective cohort study from India reported survival benefits of tocilizumab in severely ill patients with COVID-19 with persistent hypoxia.[70] Recently, the Italian Medicines Agency (AIFA) has also approved a Phase II clinical trial of tocilizumab in an estimated 330 patients with COVID-19 pneumonia.[68]

Anakinra

Anakinra is a IL-1 receptor antagonist that blocks the activity of cytokine IL-1, which is critical to the cytokine storm; recent studies have reported that SARS-CoV-2 infection leads to pyroptosis with the increase in the levels of IL-1β.[71,72] A Phase III randomised clinical trial of anakinra in patients with severe sepsis and macrophage activation syndrome demonstrated significantly improved incidences of patient survival.[73] Currently, data related to the potential use of anakinra in SARS-CoV-2 infections are limited; nonetheless, its evaluation in patients with COVID-19 and ARDS and cytokine storm may be worthwhile as it may provide an alternative therapeutic option in the management of patients with COVID-19. In a recent cohort study, anakinra significantly reduced the mortality and ventilation in patients with COVID-19 with advanced complications.[74]

Adalimumab

Adalimumab is a monoclonal antibody against human TNF-α, marketed for the treatment of rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, and ulcerative colitis. The spike protein of SARS-CoV induces shedding of the angiotensin-converting enzyme 2 ectodomain in a TNF-α-converting enzyme-dependent manner that is crucial for viral entry into the cell.[75] TNF-α is placed at the centre of this process; hence, it has been hypothesised that the use of TNF blockers may have an effective role in the reduction of SARS-CoV-2 infection and subsequent organ damage.[76] Lately, a study to evaluate the efficacy of adalimumab in patients with COVID-19 has been initiated in China (ChiCTR2000030089[77]).[68]

Convalescent plasma

Convalescent plasma is plasma obtained from patients who have recovered from infection, and it contains neutralising antibodies against that particular microbe. It has been used as a last resort in patients with SARS to improve survival benefit. Furthermore, several studies have reported lower mortality and shorter hospital stays in admitted patients receiving convalescent plasma treatment compared to control groups.[78,79] In 2014, the WHO recommended use of convalescent plasma as empirical treatment in patients infected with the Ebola virus.[80] Antibodies present in the convalescent plasma might reduce the viraemia in patients, which may be the reason for the efficacy of convalescent plasma therapy.

In most viral diseases, viraemia peaks within the first week of infection, followed by generation of primary immune response by Day 10–14 in most patients, leading to viral clearance. Therefore, convalescent plasma therapy should be more effective if it is started at the early stage of the disease;[78] however, other treatments including steroids and antiviral drugs might affect the antibody level in the convalescent plasma.[81]

In a preliminary, uncontrolled case study, Shen et al.[82] administered convalescent plasma to five patients who were severely ill with COVID-19 with ARDS, and reported an improvement in their clinical status. Briefly, body temperature was normalised in 4/5 patients within 3 days of plasma transfusion. Viral loads decreased significantly and ARDS was resolved within 12 days post-transfusion. Due to the limited sample size of the study, definitive statements about the

efficacy of convalescent plasma therapy cannot be made and these findings should be evaluated in clinical trials.[82] This evidence suggests that convalescent plasma therapy may have a potential role in the management of patients critically ill with COVID-19. Therefore, it seems plausible to evaluate the safety and efficacy in future clinical trials.

Corticosteroids

Corticosteroids, a class of anti-inflammatory drugs, have been proven to be useful in severe cases of COVID-19 associated with aberrant immune activation leading to cytokine storm and subsequent ARDS. A recent trial (NCT04327401[83]) using dexamethasone in patients with COVID-19 with severe ARDS reported significant survival benefits compared to the placebo group.[84] The RECOVERY trial data suggested that dexamethasone lowered the 28-day mortality in patients with COVID-19 receiving oxygen support or mechanical ventilation; however, it is not recommended for use in patients with COVID-19 with mild or moderate symptoms without the need for oxygen support.[85] Due to the dynamic nature of the immune activation, corticosteroids should be given at the right time and right stage of disease progression to achieve maximum benefits.

CONCLUSION

As SARS-CoV-2 continues to spread and the death toll rises exponentially across the globe, rapid development of therapeutic interventions against SARS-CoV-2 infections to minimise COVID-19-related deaths poses a major challenge. In the present scenario, repurposing existing antiviral drugs with known safety profiles and, in few cases, their efficacy against other human CoVs, seems to be the most appropriate short-term strategy to tackle COVID-19.

Currently, several potent antivirals such as lopinavir–ritonavir, remdesivir, CQ/HCQ, etc., with or without other supporting medications, are being administered to patients with COVID-19 in various clinical trials; however, sufficient data are still lacking. It is pertinent to mention that, besides identifying suitable therapeutic options, intense and concerted efforts are also being directed towards development of appropriate vaccines. In this direction for controlling this ongoing pandemic, several vaccine candidates, including spike protein, attenuated live viral particles, and *Mycobacterium bovis* bacillus Calmette-Guérin-based interventions are either launched in the market or being tested in clinical trials. However, since the present article focuses only on the therapeutic options against COVID-19, this aspect has not been discussed.

The whole world is united in its efforts to prevent the spread of SARS-CoV-2 with a hope that this pandemic meets the fate of the previous two pandemics of this century; SARS and MERS have subsided on their own. However, only the future can tell the endpoint of this pandemic. Moreover, with the rapid rise of mutant variants across the globe, one thing is clear: there is a pressing need to develop wide-spectrum antiviral therapeutics against SARS-CoV-2 to reduce the mortality it is causing. Therefore, intense research and development efforts and development of multi-pronged therapeutic approaches for combating this pandemic will constitute a top priority research area in virology.

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Lymphatic Filariasis: An Immunologic Perspective

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Abstract

Introduction: This paper provides an overview of the current immunologic research findings of lymphatic filarial worms, which affect millions of people worldwide.

Objectives: This paper aims to discuss the immunologic features of lymphatic filarial worms. It also aims to highlight their potential anti-inflammatory actions and the use of anti-filarial drugs against COVID-19.

Methods: A literature review was performed to obtain insights on the immunologic features of lymphatic filarial worms.

Results: The CD4+ (Th2) response profile is the main defence against filarial worms. Modulation of human immune responses are primarily mediated by parasite-secreted peptides. Lymphatic filarial worms have anti-inflammatory potentials. Drug repurposing of diethylcarbamazepine, doxycycline, and ivermectin can be looked upon against COVID-19.

Conclusion: Lymphatic filarial worms have several immunologic effects on host immune systems, which promote chronic infection and curtail anti-inflammatory responses. Insights in this paper can serve as a guide for the understanding of immunologic aspects of lymphatic filarial worms.

BASIC EPIDEMIOLOGY

According to the World Health Organization (WHO), approximately 120 million people situated in the tropical and subtropical regions were affected by lymphatic filariasis.[1] Walker et al.[2] noted that the majority of lymphatic filariasis cases worldwide are caused by *Wuchereria bancrofti*, while *Brugia malayi* and *Brugia timori* cause many of the cases in Asia. The Philippines has 45 provinces that are endemic for lymphatic filarial worms *W. bancrofti* and *B. malayi*.[3]

Walker et al.[2] noted that the migration of people from rural to urban areas contributed to an increase in distribution of *W. bancrofti* and *B. malayi*.[2] This leads to an increase in vector breeding sites. Turkington et al.[4] noted that males are usually affected due to frequent exposure to areas with mosquitos, such as agricultural areas. Specifically, older males (>35 years of age),

farmers, and those living near rivers have a higher risk of infection.[5,6]

BIOLOGY OF THE FILARIAL WORMS

Filarial nematodes belong to the phylum Nematoda, class Secernentea, order Rhabditida, superfamily Filarioidea, and family *Onchocercidae*.[7] They are usually differentiated by the location, habitat of the worms, geographical distribution, periodicity, or time of diagnosis of the microfilaria (offspring) of the adult worms, morphology (presence of sheath, appearance of tail and head regions), plus the type of vectors and periodic biting preferences. Filarial nematodes, in terms of adult worm locations, are usually found in the lymphatics, subcutaneous, and bodily cavities. Table 1 summarises the filarial worms, their microfilarial morphology with periodicity, along with vector details.[7-10]

Filarial nematodes are dioecious and exhibit sexual dimorphism.[11,12] Adult males have corked tails with two spicules and are smaller than females.[7] In the Philippines, *Aedes poicilus* is the main vector for *W. bancrofti* in most provinces.[3]

Table 1: Different classifications of the filarial nematodes.

Filarial worm	Microfilarial morphology[7]	Microfilarial periodicity[8]	Vectors[9]	Vector biting periodicity[7,10]
Lymphatic filarial worms			**Mosquitos**	
Wuchereria bancrofti	Sheath is present; no nuclei to tail tip	Nocturnal (10 p.m.–2 a.m.)	*Aedes*, *Anopheles*, and *Culex*	Mostly nocturnal
Brugia malayi	Sheath is present; two nuclei in tail	Nocturnal (10 p.m.–2 a.m.)	*Aedes*, *Anopheles*, and *Mansonia*	Mostly nocturnal; *Mansonia* can be sub-periodic
		Sub-periodic	*Coquillettidia*	Mostly nocturnal
Brugia timori	Sheath is present (does not stain with Giemsa); two nuclei at tip; cephalic space 3:1	Nocturnal (10 p.m.–2 a.m.)	*Anopheles*	Mostly nocturnal
Subcutaneous filarial worms			**Flies and midges**	
Loa loa	Sheath is present; continuous nuclei to tail tip	Diurnal	*Chrysops*, fruit fly, mango fly	9 a.m.–11 a.m. 2 p.m.–4 p.m.
Onchocerca volvulus	Unsheathed; no nuclei at tail tip	None	*Simulium* or black fly, buffalo gnat	10 a.m.–11 a.m. 4 p.m.–6 p.m.
Mansonella streptocerca	Unsheathed; nuclei to tip of tail and is bent ('shepherd's crook' appearance)	None	*Culicoides* (midge)	Dawn and dusk
Mesenteric/Deep tissues/Skin			**Flies and midges**	
Mansonella perstans	Unsheathed; nuclei to tip of tail	None	*Culicoides* (midge)	Dawn and dusk
Mansonella ozzardi	Unsheathed; no nuclei; tail is shorter/slender/ tapered	None	*Culicoides* (midge), *Simulium*	Dawn and dusk

Other vectors include *Anopheles minimus* var. *flavirostris* and *Culex* spp. *Mansonia bonnae* and *Mansonia uniformis* are the main mosquito vectors for *B. malayi*, although *Aedes* spp. and *Anopheles* spp. can be utilised.[13] Partono et al.[14] mentioned that *Anopheles barbirostris* is the vector for *B. timori*. In humans, the third stage filariform enter the skin from mosquito bites and migrate into lymphatic vessels, where they molt into their adult forms for >1 month in the case of *W. bancrofti* and 2–3 weeks for *B. malayi*. The adults are viviparous in nature (parasite species that lay larva instead of eggs) and release first-stage larva called microfilaria. A gravid (pregnant) female is able to discharge about 50,000 microfilaria per day.[7] The incubation period is about 8–16 months, with females capable of laying eggs for 5 years, and adults can generally live for 9 years.[15,16]

IMMUNOLOGIC FEATURES OF THE LYMPHATIC FILARIAL WORMS

Normal Host Response Against Lymphatic Filarial Worms

The immune response of the human host to filarial infections is characterised by CD4+ (Th2) response, humoral response (antibodies: IgG1, IgG4, IgM, and IgE), and cytokine response (IL-4, IL-5, IL-9, IL-10, IL-13). Cellular responses involve the action of mast cells, basophils, eosinophils, and macrophages.[17] Chronic infections involve the action of T regulatory cells and macrophages. Th2 is deemed generally protective for filarial infections.

Macrophage presentation with CD4+ cells during filarial infections activates the latter to induce secretion of cytokines (IL-3, IL-4, IL-9) to activate mast cells, as well as IL-5 for eosinophils and IL-4 to induce plasma cell secretion of antibodies IgM (acute), IgG (chronic), and IgE.

IgG antibodies (IgG1, IgG4, and IgM), with their Fab portions, can bind to the surface antigens of filarial worms. The effector cells such as macrophages and eosinophils utilise an antibody-dependent cytotoxicity for destroying parasitic membranes, either by production of nitric oxide, secretion of perforins, or other lytic enzymes.[18] The immunological function of IgE entails mast cell degranulation to release eosinophil and neutrophil chemotactic factors for continued parasitic clearance. High levels of IgG4 are indicative of *W. bancrofti* infection while IgG1 can be protective against *B. malayi*.[19,20] Platelet activating factors are also released by eosinophils and neutrophils that in turn activate formation of a clot that may block filarial worm migration.[21]

Complement proteins are involved in parasitic membrane lysis, production of opsonins for enhancement of phagocytosis, and anaphylatoxins, which enable mast cells to be activated. Senbagavalli et al.[22] noted that classical, mannose-binding lectin (MBL), and alternative pathways are activated in filarial infections, with classical and MBL pathways being elevated during evident or active infection.[22]

Immunomodulatory Effects of the Lymphatic Filarial Worms

Figure 1 summarises the immunobiological features of lymphatic filarial worms. Filarial worms have characteristic secreted products that can affect the immune functioning of the host. Proteins found in filarial worms such as phosphorylcholine (ES-62) can prevent proliferation of CD4+ T cells and B cells.[23] In this way, the release of activating cytokines such as IL-4 may be hampered, and normal activation of the anti-parasitic mechanisms might not take place. Antibody-dependent cytotoxicity will also be affected. It is also noted that filarial infections, in terms of chronic states, affect the antigen presentation of the human host. Semnani et al.[24] noted that filarial worms can down-regulate *MHC I* and *II* genes.

Analysis of the filarial genome revealed that filarial worms secrete homologues that impair cytokines such as IL-5, IL-16 as well as IFN regulatory factors, and suppress cytokine signalling.[23,25] Antigen processing, presentation, and immune activation are also affected, with inhibition of proteins and signalling compounds such as serpins, cystatins, indoleamine 2,3-dioxygenase genes, and Wnt signalling regulators. Liu et al.[21] noted that eicosanoids released from filarial worms can inhibit platelet aggregation.

Immunology of Asymptomatic Infections

The prelude to a chronic, debilitating, disfiguring lymphatic filariasis is the asymptomatic state.

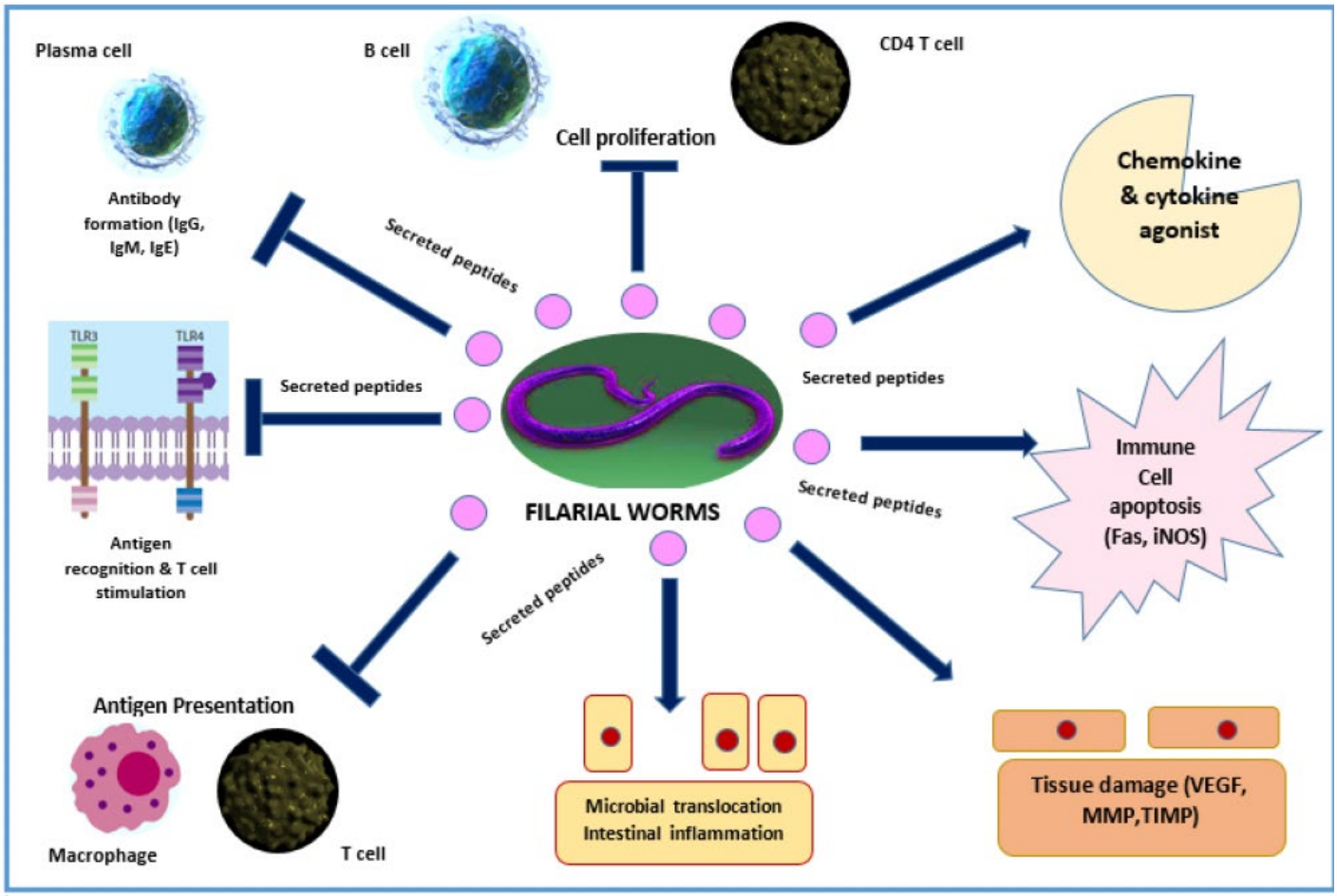

Figure 1: Summary of the immunobiological aspects of the lymphatic filarial worms.

Fas: transmembrane receptor protein; iNOS: inducible nitric oxide synthase; MMP; matrix metalloproteinases; TIMP: tissue inhibitor metalloproteinase; VEGF: vascular endothelial growth factor.

Products secreted by lymphatic filarial worms affect the expression of the Toll-like receptor proteins (TLR 3 and 4) in cells such as B cells, T cells, and monocytes, which in turn leads to poor antigen presentation and immune response.[26] Filarial worms can also stimulate the expression of negative co-stimulatory molecules (receptors) such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) or programmed cell death-1 (PD-1) for T-cell downregulation.[27]

Cellular Effects of the Lymphatic Filarial Worms

Lymphatic filariasis and T cells

Transcriptional activators T-bet (master Th1 cell activation factor) and GATA-3 (principal Th2 activation factor) are needed for CD4+ T-cell differentiation. Babu et al.[28] noted that chronic filarial state affects the activities of these factors, leading to diminished T-cell differentiation and increased expression of factors such as FOXP3, TGF-β, CTLA-4, PD-1, ICOS (inducible T-cell co-stimulator), and indoleamine 2,3-dioxygenase with the addition of anergy-inducing factors to human hosts such as cbl-b, c-cbl, Itch, and Nedd4.[28] An increase also in the frequency of CD8+ T cells was observed by Kroidl et al.,[29] which can be critical, especially during active infection. One potential mechanism is the filarial worm-induced modulation of the expression of chemokine receptor CCR9, which later promotes CD8+ formation.[30]

Lymphatic filariasis and T regulatory cells

T regulatory cells, especially with the phenotype (CD25+FOXP3+), are activated by filarial worm infection.[23] These cells can

down-regulate other CD4+ subsets by the release of immunoregulatory cytokines such as IL-10 and TGF-β. This can help the immune evasion of filarial worms by down-regulating effector T cell responses. Another mechanism is by induction of negative co-stimulatory molecules such as CTLA-4 and PD-1 to turn off T-cell activation.[27]

Lymphatic filariasis, macrophages, and dendritic cells

Macrophage presentation with CD4+ T cells is also down-regulated, especially during the chronic phase. Babu et al.[23] noted that filarial infections stimulate macrophages to release arginase-1 to inhibit the release of proliferation cytokines in T cells and induce expression of negative co-stimulatory molecules. The *MHC I* and *II* genes are down-regulated, leading to diminished T-cell activation to mount normal anti-filarial immune response.

During an early phase of infection or during tissue damage, filarial worms can trigger an inflammatory response. A 70 kDa microfilarial protein from *W. bancrofti* sheath was isolated by Mukherjee et al.[31] that can bind to macrophage-TLR4 and activates nuclear factor (NF-κB), which then upregulates the release of pro-inflammatory cytokines. Similarly, Mukherjee et al.[32] noted that microfilarial protein can also activate dendritic cells and drive Th1 responses.

Lymphatic Filariasis and Microbiota

Increased levels of inflammatory cytokines can stimulate damage to the mucous layer of the intestinal barrier, promote microbial dysbiosis and cause microbial translocation across different sites distal to the intestinal tract, especially in the lymphatics. The presence of microbial products like lipopolysaccharide can trigger activation of host responses such as the complement pathway (alternative pathway) in which systemic effects such as fever and inflammation can add burden to the current state of the disease. Inflammatory compounds seen in chronic lymphatic filariasis include C-reactive protein, TNF-α, IL-2, IL-6, IL-8, endothelin-1, MIP-1α, MIP-1β, MCP-1, TARC, and IP-10.[23]

Lymphatic Filarial Worms and Tissue Damage

Physical damage due to the migration of adult worms and larvae can induce damage to the connective tissue of the host. The filarial worms along with their secreted products cause imbalance between the matrix metalloproteinases and their inhibitors. This can cause progression into a fibrotic state and matrix remodelling.[23] Babu et al.[23] noted that the lymphangiogenic potential (or formation of new lymph vessels within a current lymph tissue) is also brought about by the host secretion of vascular endothelial growth factors A and C.[23] These growth factors can stimulate an increased vascular diameter, secretion of pro-inflammatory cytokines, and fluids (lymph and plasma), which altogether can result in oedema, lymphatic dysfunction, and could be one of the underlying mechanisms of hydrocele formation in lymphatic filarial worm infection. Filarial worms can also stimulate apoptosis by up-regulation of inducible nitric oxide synthase and Fas for apoptosis of dendritic cells, natural killer cells, and CD4+ T cells in mice models.[33] This will be another mechanism of immune evasion as these cells play a big role in human host defence against filarial infections.

Anti-inflammatory Potential of the Lymphatic Filarial Worms

It is known that helminths such as filarial worms can skew Th1 responses into the Th2 phenotype. The former entails activation of cytotoxic T cells and inflammatory cytokines while the latter invokes IL-4 and antibody production.[31] Hence, Th1 can be essential for the elimination of filarial worms during acute infections or to activate the immune system. Th2 is required to shift from an inflammatory into an anti-inflammatory state and entails the production of protective antibodies.

Filarial worm infected individuals have lowered Th1 and Th17 responses and this could be attributed to expression of negative co-stimulatory molecules such as CTLA-4 and PD-1 receptor.[34] Metenou et al.[36] noted that filarial worms can decrease inflammatory genes brought by malarial infection and Babu et al.[35] noted that exposure of macrophages to filarial worms reduces the important receptors needed for invasion of *Mycobacterium tuberculosis*. An exciting area worth exploring for immunological

research and development is to utilise the metabolites of filarial worms, notably anti-inflammatory secretory peptides against pathogens such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Lymphatic Filarial Worms and Bacterial Endosymbiosis

Wolbachia spp., an intracellular alphaproteobacteria, are essential for filarial worm fertility, viability, and are commonly found in the female reproductive tract of filarial worms. Phylogenetic analysis noted that *Wolbachia* spp. are transferred after the divergence of nematodes and arthropods.[37] Drug regimens for *Wolbachia* spp. are of long duration and contraindicated for children and pregnant individuals. Microbial and possibly filarial metabolites can potentially be used in alternative treatments for antimicrobial-resistant pathogens. Another important note is that the presence of *Wolbachia* spp. in mosquitos can reduce infections brought by *Plasmodium* spp., dengue, Chikungunya, and West Nile virus.[38] This can be due to reducing the mosquito's life span or triggering the mosquito to mount an immune attack against these pathogens.

CO-INFECTIONS: FILARIASIS, MALARIA, AND TUBERCULOSIS

Tuberculosis is a communicable respiratory disease caused by the aerobic acid-fast bacteria *M. tuberculosis*.[36] Bacillus Calmette–Guérin vaccination offers a Th1 response. Exposure to helminths may dampen the response against the vaccine by TGF-β production and possibly shifting to Th2 responses.[36] Patients with onchocerciasis have lowered responses to purified protein derivative.[39] In a study by Potian et al.[40] using mouse models infected with a helminth model *Nippostrongylus brasiliensis*, macrophages were activated by IL-4R and caused resistance to tuberculosis. Latent tuberculosis is exemplified by release of pro-inflammatory cytokines such as IL-1B, IL-6, IL-8, and IL-12; however, with an onset of early to chronic filarial infection, a mixed to down-regulatory cytokine phenotype is noted by Potian et al.[40]

Babu et al.[35] demonstrated that macrophages and dendritic cells previously infected with filarial worms and exposed to *M. tuberculosis* showed decreased surface expression of receptors used by the bacterium in invasion.

Malaria is a protozoan infection caused by the genus *Plasmodium* spp., and co-infections with lymphatic filarial worms have been noted. These two parasites differ in terms of human immune responses.[36] *Plasmodium* spp. infections are characterised by production of pro-inflammatory cytokines and Th1 responses while filarial infections are characterised by Th2, IL-10, and T-regulatory cell profiles. Yan et al.[41] showed that using irradiated *B. pahangi* with *Plasmodium berghei* infected erythrocytes protected CBA/J mice models against cerebral malaria and caused resistance. This activity could be explained by the IL-10 mechanism and reduced cerebral T cell infiltration. There is also down-regulation of the Th1 response during co-infections, which could possibly be due to IL-12 modulation and a decrease in malarial-induced inflammatory cytokines such as IFN regulatory factor, TNF-α, Th17, IL-12p70, CXCL-10, and IFN-γ.[36]

With regards to lymphatic filarial worm and malarial parasites on the same vector, the filarial infection in mosquitos can physically damage the midgut tissue and, as a consequence, the ookinete cannot develop.[42] Filarial worms can also induce immune attack by mosquitoes against invading *Plasmodium* and leakage of resident bacteria, which produces anti-parasitic metabolites against malaria. Muturi et al.[43] noted that a filarial worm and a malarial parasite can infect the same human host, as seen in *W. bancrofti* and *P. falciparum* co-infection.

FUTURE PERSPECTIVES

Trial vaccines are available for lymphatic filarial worms. Joseph et al.[44] determined that multivalent fusion protein vaccine (rBmHAT) with heat shock proteins, larval transcript-2, and tetraspanin conferred >95% protection against *B. malayi* infective larvae in mouse and gerbil models. It also enhanced Th2 response against filarial worms. In a related study by Dakshinamoorthy et al.,[45] vaccinated monkeys developed significant titres of antigen-specific IgG antibodies against each of the component antigens rBmHSP12.6, rBmALT-2, and rBmTSP-LEL.[45] Altogether, this is promising for vaccination against lymphatic filarial worms. The impact of

lymphatic filarial worms in host microbiota and genetic polymorphisms for vaccine development can also be integrated in the future.

Circulating antigens of *W. bancrofti* are detected by monoclonal antibodies against Og4C3 and AD12 antigens. The determination of the circulating filarial antigens is now considered by the WHO as the 'gold standard'. Both enzyme-linked immunosorbent assay and immunochromatography test formats can detect IgG-4 antibodies against the recombinant BmR1 antigen of *B. malayi*. Polymerase chain reaction-restriction fragment length polymorphism-based assay using *ITS1* rRNA gene as primer can differentiate all the species of human and animal filarial parasites. *COX1* gene can also identify between *B. malayi* and *B. timori*.[7,46,47]

Diethylcarbamazine has been the drug of choice for lymphatic filariasis.[48] It disrupts the parasitic membrane and is effective for adult worms and microfilaria. Diethylcarbamazine can potentially be used against COVID-19, as it displays antiviral (RNA) activity against murine leukaemia virus. The anti-inflammatory mechanism works by inhibiting cyclooxygenase, promoting antibody production and cytokine release, as well as enhancing Th1 release.[49-51]

Ivermectin is noted for the treatment of lymphatic filariasis, loaiasis, and onchocerciasis. Heidary and Gharebaghi[52] noted that this drug causes paralysis of the microfilaria and hyperpolarisation of glutamate-sensitive channels. It could potentially be used against COVID-19 as it can inhibit nuclear import of SARS-CoV-2.[52] In a randomised, double-blind, placebo-controlled trial by Chaccour et al.[53] using a single dose of 400 mcg/kg ivermectin, a reduction of viral load, IgG titres, and anosmia were observed. This highlights the potential positive impact of repurposing anti-filarial drugs against COVID-19.

With the discovery of the bacterial endosymbiont, *Wolbachia* spp., studies have shown that upon treatment with doxycycline, it effectively decreased and caused the death of adult worms and improved the severity of the lymphoedema.[38] Doxycycline is also a promising drug against COVID-19 as it can potentially target host proteases (which are needed for pre-processing of SARS-CoV-2) and inhibit matrix metalloproteinases critical for viral fusion.[54] It also has anti-viral (RNA) and anti-inflammatory effects.[55] Repurposing of anti-filarial drugs against COVID-19 is a potential area worth exploring.

SUMMARY

The normal host immune response against lymphatic filarial worms entails CD4+ (Th2) cells, antibodies, complement, phagocytes, and cytokines. Immunological actions of filarial worms entail down-regulation of B and T cells, inhibiting antigen recognition, and stimulation of apoptosis. Secreted peptides of lymphatic filarial worms have potential anti-inflammatory effects. Drug repurposing will also be vital against COVID-19.

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The Role of Infection and Autoimmunity in Urticaria and Angioedema as a Common Entity

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Abstract

Chronic spontaneous urticaria with angioedema is prevalent, affecting approximately 1% of the general population, and has a significant impact on quality of life, according to epidemiological data. This article aims to broaden the view on the mechanisms of urticaria and the role of infection in the current environment. It is not easy to identify the cause of urticaria but appropriate steps to treat an underlying infection can, in some cases, improve the symptoms of urticaria and angioedema, reduce severity and duration, or lead to remission.

Although chronic spontaneous urticaria with angioedema is a multifactorial condition involving inflammation, autoimmunity, and coagulation, IgE-mediated autoimmunity, or autoallergy, is thought to play a major role. Every year, more is learnt about the role of cells releasing mediators, underlying autoimmune processes that lead to the development of mast cell activation and urticaria.

It has become increasingly clear that mast cell roles in immune system responses are not limited to an allergic role; they are key players in protective immune responses, both innate and adaptive, to various pathogens and in defence of some infections.

Several guidelines, consensus papers, and practice parameters have been developed for the management of chronic urticaria. The Dermatology Section of the European Academy of Allergology and Clinical Immunology (EAACI), the Global Allergy and Asthma European Network (GA^{2}LEN), the European Dermatology Forum (EDF), and the World Allergy Organization (WAO) produce a guideline, which is revised every 4 years by a global panel of experts in the field. Infections may be a cause, aggravating factor, or unassociated bystander in chronic urticaria.

The author looked at evidence, using a keyword search, for the role of viral and bacterial infections in acute, acute recurrent, and chronic urticaria and angioedema, including COVID-19, herpes, viral hepatitis, and *Helicobacter pylori*.

INTRODUCTION

Urticaria is a highly prevalent, mast-cell-driven skin disorder with recurrence of transient wheals that can occur with or without angioedema.[1,2] Urticaria affects between 15% and 25% of the population at some point during their lifetimes.[3] Urticaria is characterised by the development

of wheals, angioedema, or both. Chronic urticaria is defined by recurrently appearing signs and symptoms for >6 weeks. The disease activity of all chronic urticaria subtypes can markedly change over time and differ between individual patients.[4]

The condition tends to be more common in adults than in children and in females than in males, with peak occurrence in the third to fifth decades of life. This condition is marked by the onset of pruritic wheals, which represent well-circumscribed areas of non-pitting oedema with blanched centres and raised borders that involve only the superficial portions of the dermis and are seen in conjunction with surrounding erythema of the skin.[5]

The discussion of the role of infectious diseases in urticaria has been continuing for more than 100 years. It is evident that the eradication of the infection could lead to the resolution of urticaria; however, a causal relationship with underlying or precipitating infection is difficult to establish.[6]

Different studies have reported that multiple infections range from 37% to 58% among patients diagnosed with urticaria.[7] There are several theories for the pathogenesis of the potential autoimmune nature of this condition, associated with approximately 50% of cases.[8] It is understood that the underlying mechanism of urticaria is caused by activation of mast cells and basophils, which release pro-inflammatory mediators that result in increased permeability of blood vessels and irritation of nerve endings, leading to swelling and pruritus.[9]

Current research in chronic spontaneous urticaria (CSU) targets the role of cells and released mediators that lead to the development of urticaria through mast cell activation, with a focus on the underlying autoimmune processes.[10] Although CSU is a multifactorial condition involving autoimmunity, coagulation, and inflammation,[11] IgE-mediated autoimmunity, or autoallergy, is thought to play a major role.[12]

Histamine release likely occurs through cross-linking receptors by IgG-specific and specific auto-immune IgE autoantibodies derived against antigens, on mast cells and basophils.[13]

IgG anti-FcεR1α induces histamine release irrespective of the degree of IgE sensitisation of the basophils. As proof of concept, histamine release was effectively neutralised in a concentration-dependent manner by pre-incubating donor basophils with a soluble fragment of FcεR1α prior to the addition of purified IgG from sera of patients diagnosed with CSU.[12] It was shown that circulating IgG antibodies against IgE and the high-affinity IgE receptor FcεR1 likely played a role.[14] Approximately 40% of patients have circulating antibodies to one of these targets, with a higher frequency of positivity in patients who are autologous serum skin test positive.[15]

Anti-FcεR1 antibodies are thought to be the more common of the two. FcεR1 is found on the surface of both dermal mast cells and basophils, and autoantibodies to this receptor can provoke chronic stimulation and degranulation of these cells in an IgE-independent fashion.[16] In contrast, IgG anti-IgE antibodies may bind to and cross-link receptor-bound IgE on the surface of mast cells and basophils, leading to activation and degranulation of these cells.

Protein microarray analysis showed that patients diagnosed with CSU have higher IgE levels than healthy individuals, and IgE are directed mainly to thyroid antigens and double-stranded DNA.[12] Cholinergic urticaria is a frequent form of inducible urticaria, characterised by small, itchy wheals induced by physical activity or passive warming. The underlying causes are not completely understood. Several recent studies have provided evidence that IgE-mediated mast cell activation is of major importance in the pathogenesis.[13]

In approximately 10% of patients, urticaria is linked to rare factors: allergic urticaria,[17] salt-dependent aquagenic urticaria that has been reported in adults and two cases have now been reported in children.[18] Twenty five reports of chronic urticaria and malignancy raised the possibility that chronic urticaria and malignancies are linked in some patients.[15] Recently, two additional cases of cancer and chronic urticaria have also been reported, with resolution of urticaria once the tumour was removed.[19,20]

Exposure to phthalates (substances used primarily to soften polyvinyl chloride) was shown to increase the risk of acute urticaria in children.[21] Nearly all of the numerous studies reporting

evidence for infectious agents triggering acute or recurrent acute urticaria were retrospective observational studies without appropriate controls or were case reports.[22] There were reports of *Mycoplasma pneumoniae* infection in 32% of 65 children with acute urticaria.[23] There were observations of urticaria symptoms after influenza vaccination.[24] Upper respiratory or digestive symptoms are common with urticaria associated with infections.[25,26]

Other reports focused on an association between acute urticaria and streptococcal infection,[30] hepatitis A[28] and B viruses,[30] *parvovirus B19*, *cytomegalovirus* (CMV),[29] *Coxsackie A9* virus,[31], *enterovirus*, influenza A,[24] and parainfluenza viruses. Viral infection can be a potential trigger and sometimes the main aetiologic agent in causing acute or chronic urticaria.[32]

Several hypotheses exist that hepatitis B or C infection may enhance IgE-induced mediator release from mast cells and basophils.[33]

Protein factor V, which is produced during viral hepatitis, can activate human basophils and skin mast cells to release histamine and other mediators.[34] Factor V protein acts as an endogenous super-antigen by interacting with the VH3 domain of IgE to induce the activation of mast cells.[35]

As only <5% and 2% of patients diagnosed with CSU have hepatitis B and C, respectively, the rates of infection do not appear to be increased in patients diagnosed with CSU, suggesting that viral hepatitis and CSU are not usually linked.[36]

Acute infection with viral pathogens in the *Herpesviridae* family can trigger acute urticaria, and reactivation of *Herpesviridae* is associated with cutaneous urticarial-like syndromes. Reactivation of latent *Herpesviridae* has not been studied systematically in chronic idiopathic urticaria and CSU.[37] CSU is an inflammatory disorder with autoimmune features (termed chronic viral urticaria) based on serology, consistent with the hypothesis that reactivation of a latent human *herpesvirus* (HHV) or viruses may play a role in CSU.[37]

Patients diagnosed with CSU also exhibited serological evidence of increased immune response to HHV-4 (*Epstein–Barr* virus) but not all patients diagnosed with CSU were infected with Epstein–Barr virus. These observations, combined with case reports of CSU response to antiviral therapy, suggest that HHV-6, possibly interacting with HHV-4 in cutaneous tissues, is a candidate for further prospective study as a co-factor in CSU.[37]

In the beginning of the COVID-19 outbreak in Wuhan, urticaria was self-reported among community-acquired cases in 1.4% of patients, with an approximately 1:1 ratio of male (50.7%) and female patients, with an overall median age of 57.0 years.[38] From a series of 88 patients, 20% developed cutaneous manifestations including erythematous rash, widespread urticaria, and chickenpox-like vesicles.[39] In a later publication, cutaneous manifestations of COVID-19 infection included a papulovesicular rash (34.7%; 25/72), and urticaria (9.7%; 7/72).[40]

Many bacterial infections have been associated with urticaria manifestation, such as *Helicobacter pylori*, *Streptococcus*, *Staphylococcus*, *Mycoplasma pneumoniae, Salmonella*, *Brucella*, *Mycobacterium leprae, Borrelia*, *Chlamydia pneumoniae*, and *Yersinia enterocolitica.* In some cases, the skin manifestations, described as urticaria, could be caused by the presence of the microorganism in the skin, the action of their toxins, or complement activation mediated by circulating immune complexes. Although only a weak association with urticaria of unclear pathogenesis exists, clinicians should consider these bacterial agents in the work-up of the patients diagnosed with urticaria. The eradication of the infection could, in fact, lead to the resolution of urticaria.[6]

Seropositivity of anti-*H. pylori* antibodies was higher in the urticaria-diagnosed patients than in control groups. *H. pylori* is a spiral-shaped micro-aerophilic Gram-negative bacterium that colonises the gastric mucosa and induces a strong inflammatory response with release of various bacterial and host-dependent cytotoxic substances.[41] The existence of a correlation between *H. pylori* and urticaria may help clinicians to find more effective methods to treat patients diagnosed with chronic urticaria.[32]

H. pylori is a risk factor for developing chronic urticaria; therefore, stool test for *H. Pylori* antigen is recommended.[42] Meta analysis showed that *H. pylori* might be associated with the occurrence

and persistence of CSU. The effectiveness of *H. pylori* eradication therapy in suppressing CSU symptoms was significant. Interestingly, it was found that resolution of CSU was not associated with successful eradication of *H. pylori* infection. Patients diagnosed with urticaria who had undergone antibiotic therapy for *H. pylori* eradication showed significantly higher CSU remission, with or without *H. pylori* eradication.[43]

Autoimmune mechanisms are contributing to the pathogenesis of chronic urticaria; different pathogenic autoantibodies, causing a release of histamine after reaction with IgE epitopes, or with the α-chain of Fc epsilon RI receptors, are considered.[44] Lesions can be as small as a few millimetres in diameter but can coalesce to form wheals as large as several centimetres wide. They often remit within 24 hours from time of onset. Urticaria may be accompanied by the presence of angioedema, which is a similar process that occurs at submucosal surfaces of the upper respiratory and gastrointestinal tracts and deeper layers of the skin including subcutaneous tissue.[45]

Urticaria is mainly classified based on clinical criteria: acute and chronic urticaria. Chronic urticaria comprises both CSU and chronic inducible urticaria that includes physical and non-physical urticarias.[46] CSU is a common and complex condition lasting for more than six weeks, and occurs without an identifiable causative factor. This skin disease in a sub-group of patients is related to autoreactive IgE, but the nature of this autoreactive IgE is still poorly characterised.

Formerly referred to as chronic idiopathic urticaria, CSU refers to recurrent urticaria lasting more than 6 weeks, that occurs in the absence of an identifiable trigger. Urticaria that are incited by a well-defined eliciting factor (e.g., pressure, temperature, vibration) are referred to as inducible urticaria and will not be further discussed in this review. Prevalence of chronic urticaria is estimated to be anywhere from 0.5–5% in the general population but is not truly known.[47] Recent guidelines now include isolated idiopathic angioedema within the definition of CSU provided that other causes of angioedema, particularly those that are bradykinin mediated, have been excluded.[48] Epidemiological data indicate that CSU in the general population is prevalent in approximately 1%[49] and has a significant impact on quality of life.[50]

CLINICAL APPROACHES

Diagnosis and Treatment Approaches in Chronic Spontaneous Urticaria Differ in Various Parts of the World

There are no currently available biomarkers that can be used for the evaluation and management of patients diagnosed with CSU. Potential biomarkers of CSU severity and/or duration include basophil numbers and susceptibility to activation, inflammatory markers, markers of activation of the extrinsic coagulation pathway, immunoglobulin E, and vitamin D. Although the described markers are promising, further studies on representative and well-characterised patient populations are needed to determine the value of these clinical and biological markers for predicting the severity and course of disease in patients with CSU.[51]

An 'urticaria diary' can be very helpful for assessment. It should be used over several weeks to document information on the frequency and intensity of symptoms (e.g., wheals, itch, swelling, and systemic symptoms), possible relevance with physical factors, food intake and other activities (e.g., physical or emotional stress), and patient's medication.

The Urticaria Activity Score (UAS) is based on the evaluation of numbers of wheals and the intensity of itching using a 0–3-point scale. It is calculated as the daily sum of the wheal and itch score, with a maximum score of six points per day and 42 points per week (for the UAS 7).

There are three possible underlying causes of CSU: infections, food intolerance, and auto-reactivity.[52] Bacterial infections, as well as viral, fungal, or parasitic infections can be a cause of CSU. The current guidelines recommend differential blood count analyses, determination of blood sedimentation rate and C-reactive protein, together with a focused patient history for discovering potentially relevant infections.[2]

Testing for *H. pylori* (stool, breath test, or demonstration of antigen/antibodies) are recommended.

Higher levels of C-reactive protein (CRP) were associated with autologous serum skin test positivity, arterial hypertension, urticaria activity, quality of life impairment, inflammatory and coagulation markers, and poor response to antihistamines. Elevated levels of the sensitive inflammatory biomarker CRP are suggested for diagnosis and disease activity of CSU.[53] Recent findings have demonstrated that IgE anti-thyroid antibodies are present at higher frequency and amounts in patients diagnosed with CSU and have greater potential to induce thyroid autoantibody-mediated skin reactions in these subjects versus healthy controls.[54]

A systematic review that assessed the relationship between vitamin D and CSU showed statistically significant lower serum vitamin D levels in CSU-diagnosed patients compared to controls, and disease improvement after high-dose vitamin D supplementation. Vitamin D deficiency was reported more commonly for patients diagnosed with CSU (34.3–89.7%) than in controls (0.0–68.9%).[55] When history indicates a possible inducible pattern, physical skin tests (e.g., cold, heat, ultraviolet light, and pressure) as well as exercise tests should also be performed in order to verify or rule out inducible urticarias. To perform these tests antihistamines must be discontinued for at least 2–3 days.

If high or low temperature contact urticaria is suspected, skin testing with TempTest® (MOXIE GmbH, Berlin, Germany) can be used as a diagnostic tool as well as to monitor responses to treatment. The provocation testing is performed for 5 min with a temperature 4–45° on the volar forearm. The provocation time and temperature can be adapted individually. If a palpable, clearly visible wheal and flare-type skin reaction occurs, the test reaction is rated positive. Eosinopenia in patients diagnosed with CSU is associated with Type IIb autoimmunity (markers include autoantibodies, basophil tests, and/or autologous serum skin test), high disease activity, and poor response to treatment. Eosinophils should be explored as biomarkers and investigated for their contribution to the pathogenesis of CSU.

Subjects with IgE autoantibody-mediated CSU appear to have a faster onset of improvement in response to omalizumab than those with IgG-mediated disease, due to the unique mechanisms by which this drug sequentially affects IgE levels and FcεR1 status.

On the contrary, subjects who display a slow response to omalizumab are thought to have IgG antibodies against FcεRI since down-regulation of this receptor occurs only after free IgE is first complexed by the drug. The authors validated this hypothesis by demonstrating a high correlation between length of time to the onset of omalizumab efficacy and positive basophil histamine release activity, with the latter predicting slower response times to treatment.[56]

A recent study in 49 Caucasian patients diagnosed with CSU found elevated levels of specific IgE against a mix of *Staphylococcus aureus* enterotoxins in 51% of patients compared to 33% in healthy controls.[57] Total serum IgE levels and CSU disease activity were correlated with *Staphylococcus* enterotoxin B IgE levels. These results suggest a role of *S. aureus* enterotoxin IgE antibodies in the pathogenesis of CSU, in keeping with the current hypothesis of autoallergy being important in some patients.

In cholinergic urticaria that is actively (e.g., due to exercise) or passively (e.g., having a hot bath) induced, increases of the body temperature result in the appearance of itching and formation of wheals. Typically, the wheals are tiny, short-lived, and accompanied by a pronounced flare reaction, which is often localised on the limbs and trunk.[58] This form of urticaria should be differentiated from exercise-induced urticaria/anaphylaxis, in which exercise but not passive warming provokes symptoms (cutaneous and more frequently than in cholinergic urticaria, systemic symptoms). In the differential diagnosis, attention should be given to food or drug-dependent exercise-induced anaphylaxis.

CONCLUSION

The understanding, knowledge, and management of urticaria and angioedema are rapidly increasing. It was noted that in some patients, treatment of the underlying pathology led to clinical improvement. Further research is required to gain understanding of the mechanisms of interaction between infection and urticaria and angioedema. Interdisciplinary co-operation with dentists and ear, nose, and throat specialists, and X-ray and serological analysis for streptococcal (anti-streptolysin) or staphylococcal infection should be performed to identify bacterial infections

of the nasopharynx, e.g., recurrent sinusitis or tonsillitis.[51,59]

Data obtained indicated viral infection as a potential trigger and sometimes as the main aetiologic agent in causing acute or chronic urticaria. In every case, urticarial manifestation cleared up after either healing or controlling of the viral infection. However, prospective studies and well-structured research are needed to better clarify the role of viruses in the pathogenesis of urticaria and their relative prevalence.[60]

The author can hypothesise that the underlying lasting immune response to an infection, rather than infection itself, is the causative factor for persistence of urticaria, alongside autoimmune factors. Antibodies and co-factors acting together reduce the threshold of reactivity leading to symptoms.

Many questions and unmet needs remain to be addressed, such as the development of routine diagnostic tests for autoimmune urticaria and angioedema; the global dissemination and consistent use of tools to assess disease activity, impact, and control; and the development of more effective and well-tolerated long-term treatments for all forms of urticaria and angioedema.

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