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# EMJ

European Edition

## + VACCINES FOR COVID-19: PERSPECTIVES, PROSPECTS AND CHALLENGES

+ FEATURE

The People versus COVID-19

+ EDITOR'S PICK

Chronic Spontaneous Urticaria: A Review of Pathological Mechanisms, Diagnosis, Clinical Management, and Treatment

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# Contents

*"Along with other exciting healthcare innovations seen so far in 2020, national electronic health records are an aspect of medicine that is becoming hugely important."*

Spencer Gore, CEO

# Editorial Board



VIEW IN FULL ←

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# EMJ 5.1.

# Welcome

It is with great delight that I welcome readers to *EMJ 5.1*, containing all the latest advancements in medicine that matter most. Promising a selection of peer-reviewed papers and research articles spanning therapeutic areas from diabetes to dermatology, cardiology to respiratory, the articles enclosed ensure every speciality is accounted for.

As the COVID-19 epidemic rolls out throughout the world, we are publishing a feature article by Prof Linda Saif on the prospects of vaccine development against the new virus. In her article Prof Saif discusses the current strategies for vaccine development against COVID-19, using evidence from SARS and MERS vaccine research.

Our Editor's Pick for this edition is the review presented by Dr Victor Desmond Mandel et al., who examined the current pathological mechanisms, diagnosis, and treatment options for chronic spontaneous urticaria. This lesser understood condition receives a thorough analysis, as the authors collate the evidence from the latest studies to conclude with what we know so far about the disease.

Along with other exciting healthcare innovations seen so far in 2020, national electronic health records are an aspect of medicine that is becoming hugely important. Dr Quin Yong See collected research on the attitudes and perceptions of general practitioners to electronic health records, highlighting the barriers which need to be overcome to ultimately increase adoption of its usage. This can only be good news for patients, who could experience improved safety and co-ordination of care as a result.

For the cardiologists amongst you, we have a review by Dr Ankur Luthra et al. on managing cardiovascular diseases in pregnancy. If dermatology is your speciality, Dr Padmavathi Nagarajan and Dr Devinder Mohan Thappa report on the attitudes of patients with psoriasis towards their illness, and how increasing their knowledge of their condition may increase their compliance with treatments. To peruse the news in diabetes, read the review offered by Dr James Albers and colleagues, who call for better recognition of diabetic amyotrophy to decrease diagnosis and referral time for patients.

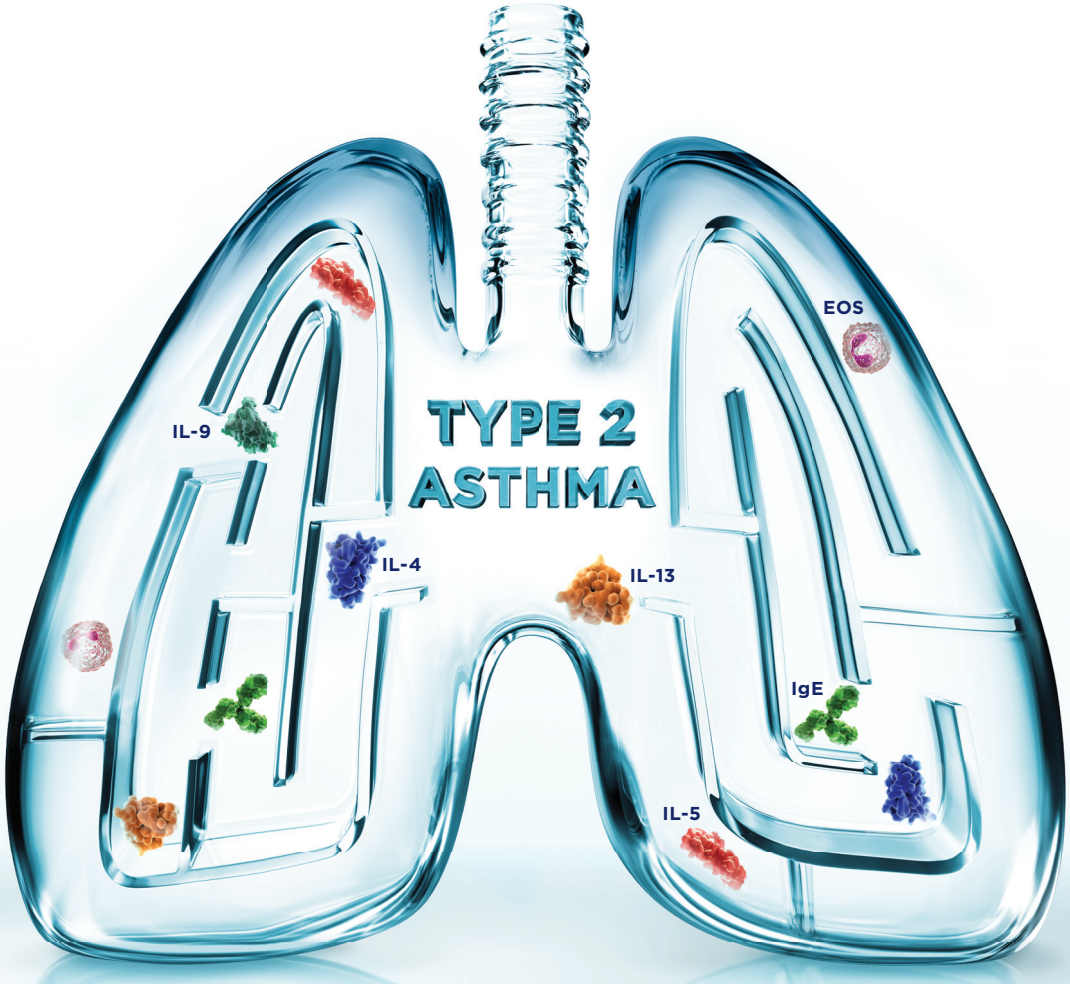
So far, the EMJ team has been working hard in this new decade, which has seen the launch of brand-new journals such as *EMJ Hematology US Edition*, with new therapeutic journals in radiology and microbiology and infectious diseases to follow. We hope you will join us this year in celebrating our wider coverage of therapeutic areas, but for now, happy *EMJ 5.1* reading.

Spencer Gore

Chief Executive Officer, EMG-Health

IN YOUR PATIENTS WITH SEVERE UNCONTROLLED ASTHMA

# LOOK BEYOND EOSINOPHIL AND IgE LEVELS IN TYPE 2 INFLAMMATION




## Cytokines IL-4, IL-5 and IL-13 are key drivers of Type 2 inflammation in asthma[1-3]

**1.** Fulkerson P, *et al. Nat Rev Drug Discov.* 2013;12(2):1–23. **2.** Caruso M, *et al. Curr Opin Allergy Clin Immunol.* 2013;13(6):677–85. **3.** Hammad H, *et al. Nat Rev Immunol.* 2008;8:193–204.

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SANOFI GENZYME

# Foreword

Dear friends and colleagues,

It is with great pleasure that I welcome you to the first flagship journal of 2020: *EMJ 5.1*. promises to bring many advances in medicine, the evidence of which is documented within these pages. I would like to introduce you to the articles presented in this issue and hope that you will be left feeling encouraged by the progress we are all making for patients.

Scientific advances in the understanding of chronic spontaneous urticaria are well overdue and are reviewed expertly by Mandel et al., the Editor's Pick for this publication. Current opinions on the pathological mechanisms are examined, alongside the wider implications on patient quality of life. Treatment options for nonresponders to first-line treatment is considered and advantages offered of the alternative treatments, which include phototherapy, intravenous Ig, and new generation biological drugs.

Chronic obstructive pulmonary disease (COPD) management and control ensures patients do not have to limit their daily activities. Jammes et al. herein present their original research that demonstrates the potential of in-shoe foot orthosis intervention for pulmonary rehabilitation in patients with COPD and foot misalignment. An interesting read and results which will stimulate further discussion, this article is especially relevant for respiratory specialists.

Epigenetic changes to gene expression equate to a number of human disease causations, and Al-Hasani et al. consider its control on nephrogenesis in diabetic nephropathy. The authors highlight the promising results seen with histone deacetylase inhibitors, whilst also calling for further research on the epigenome so that we may be able to decipher the workings of this disease. Additional reviews are provided by Albers et al. on recognising diabetic amyotrophy and Alkhatib et al. on using exercise and nutrition as a diabetes lifestyle prevention.

Without further ado, the *EMJ 5.1* Editorial Board wishes you pleasant reading and looks forward to bringing you further updates from across the healthcare sphere soon. .

**Prof László Véscei**

University of Szeged, Szeged, Hungary

# In patients with mHSPC, ADT alone is not enough...

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**ERLEADA 60 mg film-coated tablets. ACTIVE INGREDIENT:** 60 mg apalutamide**.** Please refer to Summary of Product Characteristics (SmPC) before prescribing. **INDICATION:** ERLEADA is indicated: In adult men for the treatment of non-metastatic castration-resistant prostate cancer (nmCRPC) who are at high risk of developing metastatic disease. in adult men for the treatment of metastatic hormone-sensitive prostate cancer (mHSPC) in combination with androgen deprivation therapy (ADT). **DOSAGE & ADMINISTRATION:** Treatment with apalutamide should be initiated and supervised by specialist physicians experienced in the medical treatment of prostate cancer. ERLEADA is for oral use. The tablets should be swallowed whole and can be taken with or without food. **Adults:** The recommended dose is 240 mg (four 60 mg tablets) as an oral single daily dose. 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Treatment should be discontinued permanently, if a seizure develops during treatment. The risk of seizure may increase with concomitant medication that lowers the seizure threshold. ***Falls & Fractures***: Before initiating treatment with ERLEADA, patients should be evaluated for fracture and fall risk and should be monitored and managed according to established treatment guidelines and use of bone-targeted agents should be considered. ***Ischaemic heart disease***: Ischaemic heart disease, including events leading to death, occurred in patients treated with apalutamide. Patients should be monitored for signs and symptoms of ischaemic heart disease and management of cardiovascular risk factors, such as hypertension, diabetes, or dyslipidaemia should be optimised as per standard of care. ***Concomitant use with other medicinal products:*** A review of concomitant medicinal products should be conducted when apalutamide treatment is initiated. Concomitant use of apalutamide with medicinal products that are sensitive substrates of many metabolising enzymes or transporters should generally be avoided if their therapeutic effect is of large importance to the patient, and if dose adjustments cannot easily be performed based on monitoring of efficacy or plasma concentrations. Co-administration of apalutamide with warfarin and coumarin-like anticoagulants should be avoided, if not avoided International Normalised Ratio (INR) monitoring should be conducted. ***Recent cardiovascular disease*:** If ERLEADA is prescribed, patients with clinically significant cardiovascular disease should be monitored for risk factors such as hypercholesterolaemia, hypertriglyceridaemia, or other cardio-metabolic disorders. Patients should be treated, if appropriate after initiating ERLEADA for these conditions according to established treatment guidelines. ***Androgen deprivation therapy may prolong the QT interval*:** In patients with a history of or risk factors for QT prolongation and in patients receiving concomitant medicinal products that might prolong the QT interval, physicians should assess the benefit-risk ratio including the potential for Torsade de pointes prior to initiating ERLEADA. ***Effects on ability to drive and use machines*:** ERLEADA has no or negligible influence on the ability to drive and use machines. 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ADT=androgen deprivation therapy; mHSPC=metastatic hormone-sensitive prostate cancer; rPFS=radiographic progression-free survival

*Median OS not reached in either treatment arm; HR=0.67; 95% CI, 0.51–0.89; *P*=0.005.[2] **Radiographic progression-free survival: Time from randomisation to first imaging-based documentation of progressive disease or death, whichever occurred first. Median rPFS not reached for ERLEADA® + ADT vs 22.1 months with ADT alone; HR=0.48; 95% CI, 0.39–0.60; *P*<0.001.[2]

# PUSH BACK EARLY. EXTEND LIFE.

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**INTERVENE EARLY. TREAT WITH ERLEADA® + ADT IN mHSPC[1]**

women. Animal reproductive studies have not been conducted with ERLEADA. It is unknown whether apalutamide/metabolites are excreted in human milk. ERLEADA should not be used during breast-feeding. Based on animal studies, ERLEADA may decrease fertility in males of reproductive potential. It is not known whether apalutamide or its metabolites are present in semen. For patients having sex with female partners of reproductive potential, a condom should be used along with another highly effective contraceptive method during treatment and for 3 months after the last dose of ERLEADA. **INTERACTIONS:** The elimination of apalutamide and formation of its active metabolite, N desmethyl apalutamide, is mediated by both CYP2C8 and CYP3A4. **Potential for other medicinal products to affect apalutamide exposures:** ***Medicinal products that inhibit CYP2C8:*** No initial dose adjustment is necessary when ERLEADA is co-administered with a strong inhibitor of CYP2C8 (e.g., gemfibrozil, clopidogrel) however, a reduction of the ERLEADA dose based on tolerability should be considered. Mild or moderate inhibitors of CYP2C8 are not expected to affect the exposure of apalutamide. ***Medicinal products that inhibit CYP3A4:*** No initial dose adjustment is necessary when ERLEADA is co-administered with a strong inhibitor of CYP3A4 (e.g., itraconazole, ketoconazole, ritonavir, clarithromycin) however, a reduction of the ERLEADA dose based on tolerability should be considered. Mild or moderate inhibitors of CYP3A4 are not expected to affect the exposure of apalutamide. ***Medicinal products that induce CYP3A4 or CYP2C8:*** No dose adjustment is necessary when ERLEADA is co-administered with inducers of CYP3A4 or CYP2C8. **Potential for apalutamide to affect exposures to other medicinal products:** Apalutamide is a potent enzyme inducer and increases the synthesis of many enzymes and transporters; therefore, interaction with many common medicinal products that are substrates of enzymes or transporters is expected. The reduction in plasma concentrations can be substantial, and lead to lost or reduced clinical effect. There is also a risk of increased formation of active metabolites. ***Drug metabolising enzymes:*** *In vitro* studies showed that apalutamide and N desmethyl apalutamide are moderate to strong CYP3A4 and CYP2B6 inducers, are moderate inhibitors of CYP2B6 and CYP2C8, and weak inhibitors of CYP2C9, CYP2C19, and CYP3A4. When substrates of CYP2B6 (e.g., efavirenz) are administered with ERLEADA, monitoring for an adverse reaction and evaluation for loss of efficacy of the substrate should be performed and dose adjustment of the substrate may be required to maintain optimal plasma concentrations. In humans, ERLEADA is a strong inducer of CYP3A4 and CYP2C19, and a weak inducer of CYP2C9. Concomitant use of ERLEADA with medicinal products that are primarily metabolised by CYP3A4 (e.g., darunavir, felodipine, midazolam, simvastatin), CYP2C19 (e.g., diazepam, omeprazole), or CYP2C9 (e.g., warfarin, phenytoin) can result in lower exposure to these medicinal products. Substitution for these medicinal products is recommended when possible or evaluation for loss of efficacy should be performed if the medicinal product is continued. If given with warfarin, INR should be monitored during ERLEADA treatment. When substrates of UDP glucuronosyl transferase (e.g., levothyroxine, valproic acid) are co-administered with ERLEADA, evaluation for loss of efficacy of the substrate should be performed and dose adjustment of the substrate may be required to maintain optimal plasma concentrations. ***Drug transporters:*** Apalutamide was shown to be a weak inducer of P glycoprotein (P gp), breast cancer resistance protein (BCRP), and organic anion transporting polypeptide 1B1 (OATP1B1) clinically. When substrates of P-gp (e.g., fexofenadine, colchicine, dabigatran etexilate, digoxin), BCRP/OATP1B1 (e.g., lapatinib, methotrexate, rosuvastatin, repaglinide) are co-administered with ERLEADA, evaluation for loss of efficacy of the substrate should be performed and dose adjustment of the substrate may be required to maintain optimal plasma concentrations. Based on *in vitro* data, inhibition of organic cation transporter 2 (OCT2), organic anion transporter 3 (OAT3) and multidrug and toxin extrusions (MATEs) by apalutamide and its N-desmethyl metabolite cannot be excluded. No *in vitro* inhibition of organic anion transporter 1 (OAT1) was observed. ***GnRH Analog:*** In mHSPC subjects receiving leuprolide acetate (a GnRH analog), co-administration with apalutamide had no apparent effect on the steady-state exposure of leuprolide acetate. Medicinal products which prolong the QT interval: Since androgen deprivation treatment may prolong the QT interval, the concomitant use of ERLEADA with medicinal products known to prolong the QT interval or medicinal products able to induce Torsade de pointes such as class IA (e.g., quinidine, disopyramide) or class III (e.g., amiodarone, sotalol, dofetilide, ibutilide) antiarrhythmic medicinal products, methadone, moxifloxacin, antipsychotics (e.g. haloperidol), etc. should be carefully evaluated. Paediatric population: Interaction studies have only been performed in adults. **LEGAL CLASSIFICATION:** Medicinal product subject to medical prescription. **MARKETING AUTHORISATION NUMBER(S):** EU/1/18/1342/001, EU/1/18/1342/002, EU/1/18/1342/003. **MARKETING AUTHORISATION HOLDER:** Janssen-Cilag International NV. **PACKS & PRICE:** *Country specific* Products mentioned in this document may not be registered in all countries. Prescribing Information may vary per country. Health Care Providers must refer to their country prescribing information. Prescribing information generation date or last revised: February 2020. Based on 27 January 2020 EU Summary of Product Characteristics.


**1.** ERLEADA® (apalutamide) summary of product characteristics. Janssen-Cilag International NV, Beerse, Belgium, February 2020.
**2.** Chi KN, *et al. N Engl J Med.* 2019;81(1):13–24.
**3.** Chi KN, *et al. N Engl J Med.* 2019;81(1):13–24. Supplementary information.



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# Vaccines for COVID-19: Perspectives, Prospects, and Challenges Based on Candidate SARS, MERS, and Animal Coronavirus Vaccines

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## INTRODUCTION

Several coronaviruses (CoV) are widespread in humans and cause only mild upper respiratory infections and colds; however, pandemic outbreaks of more severe coronavirus infections in humans have become more prevalent. The severe acute respiratory syndrome (SARS) coronavirus (betaCoV Lineage B) caused the first pandemic of the 21st century in 2002–2003, with its epicentre in China. The Middle East respiratory syndrome (MERS) coronavirus (betaCoV Lineage C) emerged almost a decade later and infections continue in the Middle East. Now, only 7 years after MERS, the COVID-19 SARS-CoV-2 (betaCoV Lineage B) has emerged, again in China, as an even more devastating pandemic. Its occurrence was not unexpected, because like SARS, for which the host origin was bats, scientists had previously identified SARS-like CoV in these animals in China.[1] Based on sequence analysis of the SARS-CoV-2 genome, it is more closely related to SARS (80%) and to one bat RaTG13 SARS-like CoV (96%) than to MERS CoV (54%).[2]

To date we have a limited arsenal to combat these deadly infections, with no approved treatments or vaccines for any of these severe CoV diseases, including COVID-19. Research on these diseases was largely curtailed by lack of interest in vaccines among pharmaceutical companies and lack of sustained government funding, as SARS disappeared and MERS waned. COVID-19 and SARS-CoV bind the same host cell receptor (angiotensin converting enzyme 2 [ACE2]) and may share similar disease pathogeneses and limited cross-neutralising antibodies.[2] This knowledge, as well as our improved understanding of CoV replication strategies and technological advancements in vaccines since SARS,[3] suggests that a first-generation COVID-19 vaccine could be

forthcoming more rapidly than before but unfortunately may not be available to stem the current outbreak.

SARS-CoV-2 has a large single stranded positive RNA genome (29.88Kb) with approximately 12 open reading frames. Like all CoV, it encodes genes for four major structural proteins, specifically the nucleocapsid (N) associated with the RNA genome and three membrane proteins: the large spike (S) glycoprotein (uncleaved in SARS, cleaved in SARS-CoV-2 and infectious bronchitis virus [IBV] into amino-terminal S1 and carboxy-terminal S2); the integral membrane (M) glycoprotein; and the envelope (E) protein. The S protein contains the receptor binding domain (RBD) and functions in viral attachment (via S1) and membrane fusion (via S2) to host cells, as well as induction of neutralising (VN) antibodies that can block binding to the host receptor.[2] It is a major focus in vaccine development. For SARS-CoV, the M protein can also induce VN antibodies and the N protein contains T-cell epitopes, meaning they may also be vaccine targets.[3,4,5]

## COVID-19, SARS, AND MERS CANDIDATE VACCINES

No coronavirus vaccines to prevent respiratory infections in humans have been licensed. In animals, only IBV vaccines are licensed to prevent upper respiratory CoV infections in chickens.[6,7] Like for SARS and MERS, several types of COVID-19 vaccines are proposed or in various stages of development.[8] Major criteria for all vaccines include safety and efficacy plus duration of immunity, but vaccines for pandemics also necessitate rapid development and high production capacity.[3] Although classical inactive and attenuated vaccines are being evaluated, rapid production of these virus vaccines in large quantities in cell culture under biosafety level-3 conditions is challenging, and this limits their rapid deployment in the face of emerging pandemics. However, live attenuated CoV vaccines generated by reverse genetics from infectious virus clones by deletion of multiple key virulence determinants to prevent reversion (ExoN, nsp16, accessory proteins, etc.) remain the most immunologically robust, inducing mucosal, systemic, humoral, and cell mediated immunity and broader cross-protection.[4,5,6,9] As such, they are highly effective for priming immune responses in naïve hosts.[6,10] An ideal approach for an attenuated COVID-19 vaccine would be to first generate a temperature-sensitive mutant virus with restricted replication to the upper respiratory tract, and then apply reverse genetics to construct additional targeted attenuating mutations. In combination with a parenteral heterologous S and N protein booster vaccine, such an attenuated vaccine could also potentially elicit cross-protection against heterologous strains that spill over within a betaCoV lineage, such as bat SARS-like strains.[1]

Newer vaccine technologies include viral proteins (subunits or virus-like particles), recombinant viral vectors, or nucleic acid vaccines. The latter two have the advantage of providing universal vaccine platforms amenable to introduction of new antigenic targets from emerging viruses.[3] They mimic attenuated vaccines by infecting host cells or inducing endogenously produced antigenic proteins to generate both antibody and T-cell immune responses.[3,4,5,9] COVID-19 candidate vaccines under development include S protein or RBD subunit vaccines and replicating or non-replicating vector vaccines expressing mainly S protein or the RBD.[8] Vaccines should be based on a consensus S gene to account for variability among strains. Other vaccines are based on nucleic acid constructs such as DNA plasmids or mRNA vaccines that encode S or RBD proteins expressed in host cells.[4,5,9] Although DNA vector vaccines may be safe, stable, and rapidly produced, their immunogenicity and efficacy in humans is not yet proven. SARS and MERS CoV DNA vaccines often have greater efficacy in DNA prime/heterologous boost (S/S1 proteins, inactivated virus, or recombinant viral vectors) regimens.[4,5,9] DNA vaccine administration by electroporation and its possible genomic integration and persistence are remaining issues.[3] mRNA vaccines are used as templates for endogenous protein production in the vaccine recipient. Delivery of the mRNA vaccine is enhanced by use of lipid nanoparticles for intramuscular (IM) or intradermal administration.[3] An mRNA vaccine for COVID-19 is the first to advance to initial Phase I safety trials in humans in the USA. A potential advantage of mRNA vaccines is the

anticipated development of a portable mRNA 'printing' facility to produce large quantities of mRNA.

Recombinant vector vaccines in various stages of development for SARS or MERS include recombinant adenovirus (Ad) vectors with CHAd63 from chimpanzees used to overcome the widespread pre-existing immunity to human adenoviruses (Ad 5 etc.).[4,5,9] Recombinant Ad vectors expressing SARS-CoV S or N proteins or MERS-CoV S proteins elicited variable levels of protection in mouse, ferret, or nonhuman primate (NHP) challenge models.[4,5,9] Other candidate vectored vaccines for SARS or MERS include poxvirus vectors (such as modified vaccinia ankara [MVA]), parainfluenza, measles virus, Newcastle disease virus (NCD), and vesicular stomatitis virus which express the SARS- or MERS-CoV S protein or S and N proteins.[4,5,9] They induced variable levels of protection, as mostly accessed in mouse models, including transgenic mice expressing human ACE2 or dipeptidyl peptidase 4, the MERS-CoV host receptor. Notably some of the candidate vaccines (MVA and NCD MERS-CoV S) were also tested in the MERS intermediate animal host, dromedary camels, and shown to induce VN antibodies and, for MVA, protection against nasal shedding.[9] It is important to design vaccines for livestock that serve as intermediate hosts to curtail spill over into humans. A similar vaccine strategy would entail use of poultry or swine influenza vaccines to limit transmission of potentially high-risk zoonotic influenza viruses to humans.

Safety is of major concern for vaccines and as such it is important to investigate adverse events or vaccine-induced immunopathology evident during candidate vaccine studies in animal models. Eosinophil-related lung pathology was observed in mice vaccinated with formalin and ultraviolet-inactivated SARS vaccine[4] or γ-irradiated inactivated MERS-CoV vaccine post-murine challenge; however, adding toll-like receptor agonists to an ultraviolet-inactivated SARS-CoV vaccine reduced the Th2-associated lung pathology.[9] In one ferret study, the MVA-S vaccine was associated with liver pathology, but this was not evident in other studies.[4] In tests of a SARS-S protein candidate vaccine, antibody-dependent enhancement (ADE) of infection was reported post-challenge in hamsters,[4] but not in mice using an S protein nanoparticle vaccine for MERS.[9] ADE has remained a long-term obstacle to the development of safe vaccines for feline infectious peritonitis, a systemic CoV infection of cats.[6,10] In feline infectious peritonitis-infected cats, ADE was triggered by antibody-mediated virus entry into macrophages via Ig Fc receptors. The inconsistencies in these events among animal models necessitates an improved understanding of the biological basis for their occurrence and a better knowledge of human immunology to avoid similar reactions in humans.

## CORONAVIRUS VACCINE STRATEGIES BASED ON CORONAVIRUS PATHOGENESIS IN THE HOST

COVID-19 vaccination strategies would be aided by a clearer understanding of SARS-CoV-2 pathogenesis in humans, the correlates of protection, and the duration of natural immunity. An understanding of the pathogenesis of SARS-CoV-2, including the target organs infected and the route of virus dissemination to these organs, will assist in development of vaccines to block viral dissemination and prevent infection of the target organs. An important consideration is if SARS-CoV-2 targets the lungs to cause pneumonia via viraemia or after an upper respiratory infection. If the latter, then IN vaccines using live replicating vectors or attenuated viruses that effectively induce local mucosal immunity could protect the upper and, consequently, the lower respiratory tracts and reduce nasal shedding. An example is the current use of a live attenuated influenza virus vaccine that induces mainly local IgA antibodies and fewer systemic antibodies, yet elicits protection.[11]

Alternatively, if the lungs (or other organs) are the major sites of infection via viraemia, then parenteral (IM) vaccines that elicit sufficient VN antibodies in serum to block viraemia and are also transudated to the lungs (and other target organs) may effectively block infection. This would be equivalent to the IM application of inactivated influenza vaccine to prevent respiratory infections in humans. Additionally, in people who have recovered from COVID-19 and are primed to the virus (again, like seasonal influenza), a parental vaccine alone, such as

a subunit S or RBD protein, may be effective as an annual booster vaccine. This would enhance memory B- and T-cell responses and immunity, and prevent virus reinfections. Diarrhoea and foecal shedding were reported in some COVID-19 patients,[12] so for this scenario, oronasal vaccines may be more effective. Thus, COVID-19 vaccines will likely be used in three populations: naïve susceptible individuals with no immunity; recovered, including subclinically infected, individuals, with various levels of immunity; and in people who have pre-existing immunity to SARS and MERS. Therefore the immunogenicity, protective potential, or adverse effects of candidate vaccines may vary among such populations. Assessing pre-existing levels of immunity will be important to validate vaccine effectiveness and safety in each population and in the various age groups within each population, but especially in the elderly with the highest death rates.

## ANIMAL MODELS FOR SARS, MERS, AND COVID-19 CORONAVIRUSES

A variety of small animal models have been used to test vaccines for SARS- and MERS-CoV.[4,5,9] They include NHP (macques, African Green monkeys), mice, hamsters, and ferrets. These as well as additional animals, including susceptible livestock species, should be studied to define which models best mimic human COVID-19 infections and the potential correlates of protection. As with most animal models, none fully recapitulate the disease pathogenesis in humans or replicate human physiology and immune responses: all have advantages and limitations. The robustness of inbred mice models that do not reproduce SARS or MERS disease has been improved by the use of aged mice, mouse-adapted SARS or MERS strains, and hACE2 or DPP4 transgenic mice, respectively.[4,5,9] Ferrets, also used as an influenza model, reflect SARS pathogenesis in humans including fever, nasal shedding, and lung pathology;[4] however, their less well-characterised immune systems and lack of reagents compared to mouse models are a limitation. Pigs are susceptible to infection with MERS-CoV[13] and SARS[14] and and if susceptible to SARS-CoV-2, they are potentially a relevant model because they are outbred and their physiology, metabolism, respiratory anatomy, and immune responses resemble those of humans.[15] Although NHP better reflect humans, they too do not manifest all of the clinical signs, disease, and immune parameters in humans and are limited in availability.

With all of these models, the major concern is how well they will predict vaccine immune responses, including adverse events, and protection in humans. Because clinical trials are now underway for three MERS vaccines,[8] once they are tested in humans in the ongoing MERS outbreak settings, the results should reveal how well data from these models predict human responses to CoV and protection. Much could be learned about CoV pathogenesis, factors that influence shedding and transmission, and safety and immunity induced by candidate vaccines in the susceptible intermediate host species. Although currently unknown for COVID-19, only limited studies have been done in camels infected or vaccinated with MERS-CoV.[9] Use of an effective MERS vaccine in camels is also an important strategy to block ongoing transmission to humans.

## LESSONS FROM ANIMAL CORONAVIRUS VACCINES

To prevent CoV infections in livestock and poultry, most of the current licensed CoV vaccines are either inactive, attenuated, or live vector (porcine epidemic diarrhoea virus) vaccines (comparisons with SARS and testing of new-generation vaccines are reviewed[6,10]). None are completely efficacious in animals. The gastrointestinal tract is the major site of CoV infection in many animals, and severity is greatest in neonates. As such, oral attenuated vaccines were developed for use in pregnant animals, both to prevent disease in the mother and also to induce high levels of passive IgA antibodies in milk that are transferred to neonates via suckling to prevent intestinal infection. In studies of swine, milk IgA, but not serum IgG antibodies were correlates of passive immunity to enteric CoV infections of neonates.[6,10]

The only licensed animal CoV vaccines targeted to prevent respiratory CoV infections are IBV vaccines for chickens.[6,7,10] However, unlike

SARS-CoV-2 which causes atypical pneumonia, IBV causes an upper respiratory infection with infection of bronchi, severe disease in young chicks, and infection of the kidney and reproductive tract by some strains. Both live attenuated and inactivated IBV vaccines are licensed, with the latter also used in an attenuated prime/inactivated boost vaccine regimen. The correlates of protection against IBV clinical disease are uncertain, but high levels of serum VN antibodies are suggested to prevent viral dissemination from the respiratory tract, thus blocking infection of the reproductive tract and kidneys. Generally, live attenuated or certain replicating vectored vaccines were more effective in fewer doses than inactivated IBV or subunit vaccines. Problems encountered in vaccine protection include the existence of multiple serotypes/subtypes of IBV which fail to cross-protect, variation in virulence among IBV field strains, and reduced but not eliminated nasal shedding.

Bovine CoV (BCoV) is pneumoenteric and causes diarrhoea and respiratory disease in cattle.[6,10] Upper respiratory reinfections are common with repeated nasal shedding episodes. It is endemic and most cattle are seropositive, but antibody titers wane unless boosted by reinfections or vaccines. In spite of its economic impact, no respiratory vaccines have been developed to prevent BCoV-associated pneumonia in calves or in feedlot cattle. In cattle naturally infected with BCoV, high serum antibody titers have been correlated with protection of feedlot cattle against BCoV-induced pneumonia and shedding associated with the bovine respiratory disease complex. Also application of a live attenuated BCoV vaccine (licensed for oral use to prevent BCoV diarrhoea) in cattle on entry to a feedlot reduced the risk of treatment for bovine respiratory disease complex. Because MERS-CoV is endemic in camels (which, like cattle, are ruminants) in Saudi Arabia and camels are mostly seropositive to MERS-CoV, a similar approach, but using an IM or IN S protein subunit or inactivated MERS-CoV as a safe booster vaccine, could be successful to reduce virus shedding and transmission to humans.

The porcine respiratory coronavirus (PRCV) resembles SARS-CoV-2 infections in many important clinicopathological aspects (aerogenic spread via droplets, tropism for the lung, and interstitial pneumonia affecting 5–60% of the lung).[6,10] Despite the lung lesions, many PRCV infections are clinically mild. Respiratory coinfections, dose, route of infection, and immunosuppression (corticosteroids) are cofactors that exacerbate the severity of PRCV and also BCoV infections.[6,10] These cofactors may play a role in the severity of COVID-19 or enhanced virus transmission by superspreaders. Although no vaccines have been developed for PRCV, an Ad5 vector vaccine expressing the PRCV S protein inoculated oronasally into pigs reduced but did not prevent PRCV nasal shedding and elicited a rapid anamnestic VN antibody response post-challenge.

## CONCLUSIONS AND CHALLENGES FOR COVID-19 VACCINEDEVELOPMENT

In the face of a pandemic, rapid development, production, and deployment of first-generation vaccines are critical. Synthetic nucleic acid (DNA, mRNA) priming vaccines in combination with S (and possibly N) protein booster vaccines are leading candidates based on the above criteria. An approach used to expedite veterinary vaccines during epidemics is to issue conditional licensures; for COVID-19 these could be based on human clinical data confirming safety and adequate levels of protection to reduce fatalities in the highest risk groups (elderly and patients with comorbidities, healthcare workers). Second-generation, more potent, or efficacious vaccines to prevent disease, deaths, and reduce shedding, as discussed, should be developed in parallel for future deployment.

Most candidate vaccines are predicated on the induction of serum VN antibodies and systemic cell-mediated immune responses in the animal models as indicators of protection, but the correlates of immunity to COVID-19 in humans are unknown. Mucosal immune responses may be important, particularly to reduce nasal shedding. A possible scenario is that vaccines will prevent severe disease and deaths, but may not eliminate nasal shedding, allowing continued transmission. Achieving sterilising immunity at mucosal surfaces is a major challenge to prevent virus shedding and mucosal immunity is often short-lived, requiring multiple booster vaccine doses.

The elderly and those with chronic conditions or comorbidities are at greater risk of severe disease or mortality, yet many existing vaccines (such as for influenza) have reduced efficacy in these groups. Alternative vaccination approaches such as better adjuvants and multiple or higher doses, like for high-dose inactivated influenza vaccines, may be needed to confer protection in these vulnerable groups. Animal models also need to mimic these parameters.

The pathogenesis of COVID-19 in humans is unclear, and as such vaccine strategies may need to be altered if the virus infects both the respiratory and intestinal tracts (pneumoenteric, like BCoV) and is also shed in faeces. Oronasal vaccine prime and parenteral S vaccine booster may be optimal to prevent both enteric and respiratory infections and faecal and nasal shedding as used for some animal CoV vaccines.

Future spill over of SARS or SARS-CoV-2-like CoVs from animal reservoirs is likely. New approaches are needed to generate vaccines that can induce broader heterologous and cross-protective immunity against CoV within each betaCoV lineage. This will require focus on additional proteins (S2, N, etc.) and conserved epitopes that induce broad cross-reactive and cross-protective immunity.

The lack of vaccines to induce active immunity warrants the rapid development and application of passive immunisation approaches to treat patients both prophylactically and therapeutically. The most timely would be convalescent plasma therapy using plasma containing antibodies from recovered patients as an empirical treatment during the COVID-19 outbreak followed by confirmatory double blind trials. A shorter hospital stay and reduced mortality was reported in plasma-treated versus untreated SARS patients.[16] If effective, plasma banks with blood donated by the substantial numbers of recovered COVID-19 individuals could be established promptly.

Monoclonal antibodies (MAB) against the target viral proteins can be produced rapidly and are important to map the epitopes that confer protective and cross-reactive immune responses as confirmed by administration of the MAB to SARS-CoV-2 challenged animals. The protective MAB can guide vaccine design and most importantly can provide passive immunoprophylactics for COVID-19 in patients.[17] A recent relevant strategy used mRNA encoding respiratory syncytial virus MAB (palinozumab) delivered to the lung via intratracheal aerosols.[18] Nanobodies (camelid variable heavy-chain antibodies) developed for MERS provided passive immunotherapy in a mouse model, and represent another promising approach.[19]

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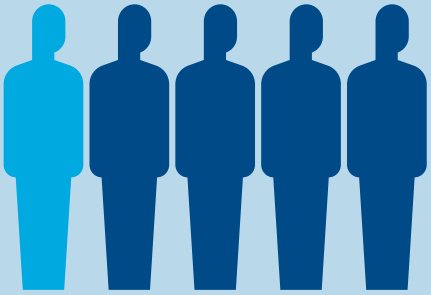

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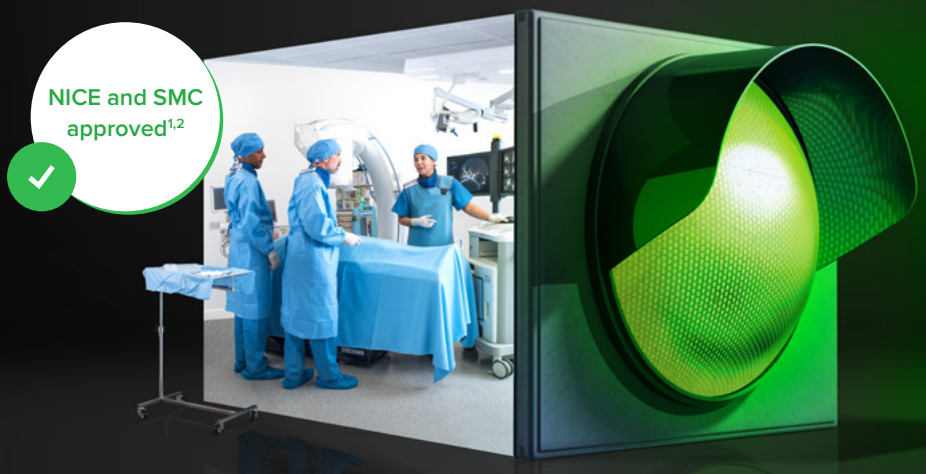

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# The People Versus COVID-19

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DOMINATING headlines since January has been coronavirus and its global spread. Geographical knowledge has been sharpened by daily maps bleeding red further across borders, and the average person has become an expert on mortality counts, public health measures, and handwashing. But something is missing from the headlines: the incredible and unsung scientific efforts of laboratory scientists sequencing viral DNA, health economists and epidemiologists mapping disease spread and recommending public health measures, and clinicians on the frontlines caring for those affected, to name just a few. There is a global effort of public and private sectors stepping up to the challenge of this pandemic.

## THE EMERGENCE OF COVID-19

Coronaviruses predominantly affect animals; however, they are capable of cross-infection in humans. First identified in 1966, in recent years coronaviruses have been responsible for two outbreaks: SARS-CoV (*Betacoronavirus*, subgenus *Sarbecovirus*) in 2002, and MERS-CoV (*Betacoronavirus*, subgenus *Merbecovirus*) in 2012.[1]

The current coronavirus outbreak is due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that causes the illness referred to as novel coronavirus disease 2019 (COVID-19). SARS-CoV-2 was first identified in December 2019 by analysis of bronchoalveolar lavage fluid samples from a cluster of patients affected by an acute respiratory illness in Wuhan, China. Genetic analysis of SARS-CoV-2 revealed it to be similar to SARS-CoV, within the genus *Betacoronavirus*; SARS-CoV-2 joins two bat-derived SARS-like strains in lineage B of the subgenus *Sarbecovirus*.[1]

In analysing the original HIV outbreak of the 1980s, it took more than 2 years for the culprit virus to be identified.[2] Improvements to DNA sequencing technologies and progress in international scientific collaboration have led to the much more rapid recognition of SARS-CoV-2. Rather than being reliant on data from local cases, the open publication of data from the original cases in China has meant that a diagnostic test was developed and released within days.

## TRANSMISSION

Two-thirds of the original reported 41 cases in Wuhan, China had direct exposure to the Huanan Seafood Wholesale Market where live animals are sold; this is believed to be the original point of animal-to-human transmission.[3] SARS-CoV-2 is capable of human-to-human transmission via exposure to infected respiratory droplets, which is the driving force behind its pandemic spread.[4] High-risk exposure occurs only in those having direct physical contact with infected cases, or contact within 2 metres of infected cases for greater than 15 minutes.[4] The incubation period from exposure to the virus to development of symptoms has been recorded as 0–24 days, with a mean of five days[5] although other studies report incubation is more likely 1–14 days.[3]

Transmissibility of a virus can be described through multiple measures, including $R_0$. $R_0$ describes how many unvaccinated people will catch the disease from one infected person. If the $R_0$ is less than 1, the disease will decline and eventually disappear. If the $R_0$ is greater than 1, the disease will grow exponentially and potentially cause a pandemic. The $R_0$ for SARS-CoV-2 is estimated to be between 3 and 5. SARS-CoV associated with the 2002 epidemic had an $R_0$ of 2–5, while measles has an $R_0$ of 12–18.[3]

SARS-CoV-2 has been found to be easily inactivated by cleaning of contaminated surfaces for one minute with a solution of ethanol (62.0–71.0% alcohol), hydrogen peroxide (hydrogen peroxide 0.5%), or hypochlorite sodium (0.1% bleach).[5] Frequent handwashing is an effective measure against the spread of the virus.

## EPIDEMIOLOGY

Following recognition of the first cluster of cases in Wuhan, China in December 2019, new cases of COVID-19 have been identified in every continent. At the time of writing, international cases of COVID-19 exceed 110,000 with more than 4,000 deaths reported.[6] Over 80% of those affected have mild disease, with fewer than 3% of cases affecting children.[7]

The speed of the global scientific effort tracking COVID-19 is unprecedented. China first announced affected cases of COVID-19 on 31st December 2019, identified the culprit virus by 9th January 2020, and released the genome sequencing for SARS-CoV-2 on 11th January. Details for a diagnostic test were published as a free, open-access resource for international use on 13th January.[8] Whole genome sequencing was conducted on samples from cases in France on 24th January and revealed similarity to sequencing from China that confirmed both international spread and low mutation rate of the virus.[9]

Multiple factors have contributed to COVID-19 spreading at greater rates and in greater numbers than the SARS-CoV epidemic of 2002.[10] Wuhan is a megacity and transport hub for much of central China, and the initial outbreak coincided with significant rates of travel for New Year celebrations. Initial measures tracked cases of pneumonia, with a delay in recognising the high rates of mild infections and delay in isolating these cases. A high proportion of mild cases also meant a greater number of cases were present and managed in the community, which posed greater difficulty for containment strategies. Additionally, the SARS-CoV-2 virus is transmissible during asymptomatic or mild disease, unlike the previous SARS-CoV where the infectious period of each case coincided with more severe symptoms.[10]

## PUBLIC HEALTH BURDEN

Travel has been the determining factor for the international spread of COVID-19. Public health measures first focussed on addressing potential exposures from international movement. Computer modelling studies have suggested that travel restrictions successfully delayed international transmission by 2 weeks, with this impact strengthened by local public health interventions and behavioural modifications of individuals.[11]

Secondary infections, affecting close contacts of infected cases and healthcare workers, are an additional target of public health strategies. Rapid identification, isolation, and optimal care for infected cases helps reduce secondary infections. It is prevention of these cases that is addressed through use of screening diagnostic tests, community isolation measures, and personal protective equipment such as masks.

Public health measures, including contact tracing, population education, and dissemination of resources, are dependent on pre-existing infrastructure. Provision of personal protective equipment, diagnostic tests, and critical care technologies is a challenge in rural settings and low-income economies.[12] Declaration of a Public Health Emergency of International Concern by the World Health Organization (WHO) on 30th January will allow for international support of vulnerable nations' health systems and infrastructure.[13]

## FUTURE TREATMENT AND VACCINE

No specialised treatment for COVID-19 has yet been identified and internationally validated. Current treatment for severe cases employs the use of existing antiviral therapies, and supportive respiratory and critical care treatments. Multiple

*"The COVID-19 challenge has allowed us a glimpse into the future of global scientific research, and highlighted the power of the shared efforts of the global scientific community"*

open-access articles have been published discussing treatment strategies used by clinicians to address existing cases, with global peer review and analysis ongoing as more cases develop.

The process by which SARS-CoV-2 infects the respiratory system has been found to be similar to other viruses: receptor-mediated endocytosis via the angiotensin-converting enzyme II (ACE2) as an entry receptor.[5] Recognition of this entry process allows for production of targeted drug therapies, with multiple therapies already under development. Whole genome sequencing of the virus also contributes to the ability of research institutions and private companies to create targeted treatments or potential vaccines. However, any new therapy or vaccine developed must undergo rigorous testing in clinical trials to have confidence in its safety and efficacy, so is likely over a year away from global availability.

## GLOBAL SCIENTIFIC COMMUNITY

Transparency of research, shared data and insight, and supportive spirit have been hallmarks of the global scientific response to COVID-19. Greater numbers of scientific journals now provide for early, free dissemination and real-time global peer review of research and insights. This has been utilised in tackling the COVID-19 pandemic to support rapid growth of knowledge and foster collaborative work across institutions internationally.

"It feels like things are transitioning to a completely new culture of doing research," said virologist Isabella Eckerle of the Geneva Centre for Emerging Viral Diseases, Geneva, Switzerland, "It's exciting."[14] A PubMed search for COVID-19 or SARS-CoV-2 reveals 360 articles; the majority are open-access, free publications from scientists and clinicians across the world supporting the global effort to combat this pandemic. During the SARS-CoV outbreak in 2002, it took a year for one-third of that number of publications to be released.[2]

Historical efforts have built an invaluable foundation of knowledge, technology, and scientific techniques that have been vital to the success of the current response. Rapid whole-genome sequencing of the virus was possible

due to original recognition of DNA structure in the 1950s, development of sequencing machines in the 1980s, and exponential leaps in processing speeds and sequencing technology over the last two decades.[15] Artificial intelligence has been used to predict molecular targets for potential treatment and vaccine pathways.[6] More than 80 clinical trials are currently underway to assess the effect of using pre-existing antiviral treatments against SARS-CoV-2.[16]

Viral genome sequencing tends to take days to weeks, however laboratories around the globe ran analyses during the night and through weekends to provide data for global efforts as quickly as possible.[3] Editorial assessment and peer review of scientific research can take months, but many international journals have pushed to release articles within days of submission to contribute to the global body of knowledge.[14] A WHO forum in early February saw 400 scientists generate a research roadmap to direct collaborative and parallel research efforts for the international scientific community.[17]

Contributors to every aspect of the scientific process have acted with unprecedented speed and transparent collaboration over the past three months. The COVID-19 challenge has allowed us a glimpse into the future of global scientific research, and highlighted the power of the shared efforts of the global scientific community.

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# Preparing Patients for Intervention: Interviews with Key Opinion Leaders in Hepatology and Interventional Radiology

**Interviewees:** Edoardo G. Giannini,[1] Sachin Modi[2]

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## Interview Summary

For this article, EMJ conducted interviews with two key opinion leaders, Prof Edoardo G. Giannini and Dr Sachin Modi, world experts in hepatology and interventional radiology, respectively, to gain insight and perspective on the issues surrounding how to adequately prepare patients for intervention. Prof Giannini is the Academic Director of the Gastroenterology Unit and Director of the Emerging Gastrointestinal and Liver Diseases Programme at the University of Genoa in Italy, and Dr Modi is a consultant interventional radiologist and oncologist at University Hospital Southampton in the UK.

The article begins by discussing the challenges faced by specialists in preparing patients with platelet transfusions for intervention, then exploring the unmet needs as described by the two experts. Finally, new developments and how they may positively alter current procedures and enhance co-operation between the specialities are also examined.

## INTRODUCTION

### Crucial Issues in Preparing Patients for Intervention

#### Professor Edoardo G. Giannini and Doctor Sachin Modi

Among the various options available to increase platelet count in patients with chronic liver disease (CLD) who require invasive procedure or surgery, only platelet transfusion is commonly employed in clinical practice. EMJ asked both experts about the importance of raising platelet levels in patients, and their views on the limitations of the options currently available.

Prof Giannini shared that when the clinician performing the procedure considers that the presence of thrombocytopenia may present a risk of bleeding, only platelet transfusion is used to increase platelet count in patients with cirrhosis before an invasive procedure is performed. However, platelet transfusions are not entirely free

of possible shortcomings. In thrombocytopenic patients the aim of platelet transfusion is to prevent bleeding, although any increase in platelet count may be transient or insufficient. It is known that there are challenges in evaluating the effectiveness of platelet transfusion. Additionally, for those in need of repeated transfusions, the risk of alloimmunisation may also be relevant.

Dr Modi added that the interventional radiologist (IR) will generally follow the consensus guidelines on preprocedural management of coagulation status and haemostatic risk in obtaining image-guided interventions. The guidelines are written in co-operation between the Society of Interventional Radiologists (SIR) and the Cardiovascular and Interventional Radiology Society of Europe (CIRSE). Dr Modi added: "We know that platelets are important in terms of risk of bleeding and that thrombocytopenia or low platelets result in an increased risk of bleeding with image-guided interventions. Based on the complexity of the procedures, we like to have a certain platelet level to ensure that the procedures can be performed safely with minimum risks of bleeding."

Liver biopsy comes under 'Category 2/High Risk' in the guidelines for coagulation status and haemostasis risk,[1,2] procedures with a moderate risk of bleeding, and in the management of these cases, Dr Modi explained that he would like the platelet counts to be above 50,000 units. "If the level is below 50,000 units, we recommend a transfusion to make sure that the platelet count is sufficiently high enough to perform the biopsy safely," he clarified. Prof Giannini added: "There are few alternatives to platelet transfusions; procedures such as partial splenic embolisation carry their own risks, the effect on raising platelet levels is not absolutely reliable, and these procedures are rarely performed. However, this invasive procedure is not free from potential complications such as the formation of splenic abscesses, and the majority of patients who undergo the procedure experience abdominal pain and fever."

From the perspective of the IR, the interviewees were asked which risks come with low platelet levels for the patient, and for the success of the intervention. Dr Modi explained how a liver biopsy is a technically relatively simple procedure and that there are commonly two types of liver biopsies performed: a general liver biopsy, and targeted biopsies of liver lesions to identify the lesions. The main risk of performing a liver biopsy is bleeding, with the significance being that patients can have life-threatening bleeding following this procedure, in the worst-case scenario, leading to death. He said: "Although this is quite rare, it is a significant risk and can result in patients requiring emergency procedures such as surgery to stop the bleeding. However, it is more likely to be embolisation, finding the bleeding point within the blood vessels and stopping it with minimal invasion."

Most IR will mention that there is a rare but possible risk of mortality associated with liver biopsies, but their primary concern is the risk of bleeding. To ensure these risks are minimised it is important that all bleeding parameters are in line before biopsies are carried out. Risks are slightly higher for patients with CLD if they have an increased risk of bleeding, or if tumours in the liver are hypervascular, i.e., have a rich blood supply. The location is also relevant: there is a slight reduction in risk of bleeding if tumours are based within the centre of the liver versus the periphery, near the liver capsule. Often, peripheral liver tumours are more likely to cause significant bleeding.

## Current Options for Obtaining Platelet Threshold

### Professor Edoardo G. Giannini and Doctor Sachin Modi

An important question is whether a patient can be properly prepared with platelet transfusions to reach the targeted platelet level. Many factors may influence the increase in platelet count following platelet transfusions, such as the level of thrombocytopenia and the number of platelets transfused, in addition to other factors unique to patients with advanced CLD such as the presence of splenomegaly. Taking all these factors into account, a clinically meaningful increase in platelet count is often not obtained and highlights that the usual dose of platelets administered is barely able to increase platelet counts before an invasive procedure. Prof Giannini stated that one of the unresolved questions in patients with advanced CLD is what may be the 'safe' platelet count threshold

needed before a procedure that carries the potential for bleeding. He recently evaluated patients within his institution to approximate a threshold where bleeding events are more frequent, and this work has recently been published.[3] "We don't have the ultimate threshold, but we do have data that reveal platelet levels associated with a lower risk of bleeding."

Also, whether platelet transfusions or other pharmacological or non-pharmacological means used to increase platelet counts may be associated with a lower likelihood of procedural and postprocedural bleeding is a challenge faced by hepatologists when they need to prepare a patient for an intervention with platelet transfusions. Prof Giannini added: "Patients often need to be hospitalised which may increase both direct and indirect costs associated with the procedures. Additionally, platelets may not be readily available, and this may delay performing the procedures."

On the same question of obtaining patient platelet thresholds, Dr Modi continued by saying: "Ideally, we are talking about two different patient groups here: inpatients at the hospital, and outpatients who come in for the liver biopsy as an elective procedure. For both groups we want blood parameters including the international normalised ratio and a platelet count of at least 50,000 units on the day of the procedure, to enable us to perform the procedure. If a patient has liver dysfunction or CLD, preferably we want the blood tests within 24 hours so we can be confident that the risk of bleeding will be minimal. It is easier for a hepatologist to manage this in an inpatient; the problem lies when we have outpatients for elective liver biopsies. In the latter group, patients have their blood tests on the morning of the procedure, and if these are deranged then it can be quite difficult to correct them in the timeframe required to perform the biopsy."

## IMPORTANCE OF DEVELOPING ALTERNATIVE OPTIONS

### Issues Experienced in Co-Operation Between Each Expert Group

**Professor Edoardo G. Giannini and Doctor Sachin Modi**

Prof Giannini and Dr Modi were asked whether the co-operation between the hepatologist and IR is optimal for preparing a patient for an intervention, or if there are options for improvement. Prof Giannini explained that co-operation is essential to schedule the intervention adequately and to prepare the patient appropriately, stating: "Close contact between specialists and a shared view of the (bleeding) risks inherent to the procedure, including a desirable platelet count threshold that should be obtained to safely carry out the procedure, are fundamental." Dr Modi agreed that there are indeed options for improvement: "In our hospital we use an IR checklist which we send up to the ward on the day before the procedure. One of the specialist nurses looking after the patient signs that they have received it and completes all the information requested; this includes confirming simple things such as whether the patient is 'in a gown' and 'appropriately clean,' to more important requests such as the date and results of blood tests, and whether the patient is on anticoagulation. If there are any issues, these can be identified at an early stage, i.e., the day before the procedure, and can be corrected in time for the procedure the next day," he affirmed.

Within Dr Modi's hospital, patients coming in for elective liver biopsies are dealt with by a radiology booking team. Usually these patients have a telephone preoperative assessment with a nurse, again looking to identify any factors that may indicate an increased bleeding risk. Problems are highlighted to the IR who then has an opportunity to find a solution before the patient comes in. Unfortunately, the blood tests are usually done the morning of the procedure so deranged platelet count on the day of the procedure is difficult to manage. He continued by saying: "The co-operation between the IR and hepatologist is really important and not just a simple case of a hepatologist submitting a request for a liver biopsy. They need to be aware that these procedures have significant risks associated with them, and anything they can do to try and help this process through will be best for the patient."

Addressing what happens if the hepatologist cannot raise the platelet level with platelet transfusion, Prof Giannini confirmed that most of the time the procedure needs to be rescheduled,

and this may have an impact on both the IR and clinical ward workloads. It is understood that some patients are unable to have the intervention due to an inability to raise platelets to a sufficient level for the procedure. Prof Giannini responded by saying: "It is essential for patients with very low platelet counts in need of particular interventions to achieve a safe platelet count threshold, due to the high risk of bleeding associated with the procedure." Dr Modi confirmed this, saying that some patients are unable to have the procedure because IR are becoming increasingly busier in hospitals. "We have a set number of patients allocated for the morning and for the afternoon while making provision within our day to deal with emergencies, but generally, if the platelet count is not correct, these procedures are often cancelled because they cannot simply be slotted in later during the day. So, yes, it is an issue that these patients then have their procedures cancelled because platelet counts or other blood parameters are not at the right level," he added. Additionally, when platelet levels cannot be raised sufficiently, the IR may then have a discussion with the hepatologist about alternatives to a liver biopsy, e.g., performing a transjugular liver biopsy. An IR is generally happier to perform this type of procedure when a patient's platelet counts are lower because the risk of bleeding from this is significantly reduced, although targeting of lesions cannot be done with this technique.

For an intervention that needs to be repeated shortly after the first, the experts were asked for their opinion on whether the preparation of the patient needs to be completely repeated with platelet transfusions to raise the platelet levels. "The whole preparation usually needs to be repeated because in patients with CLD, the increase in platelet count tends to be short-lived," Prof Giannini said. "The use of thrombopoietic drugs may obviate this need as they are able to maintain an adequate platelet count for a longer period of time compared with platelet transfusions. This removes the need for repeated preparations and transfusions." Dr Modi responded that it is dependent upon the type of procedure. "For percutaneous biopsies we are quite strict about the platelet count being 50,000 units or above, whereas for transjugular liver biopsies we generally allow some more leeway. However, we would not want to perform a transjugular biopsy, for example, on someone with a platelet count of less than 30,000 units, so we would check they had blood tests within 24 hours in case they needed a transfusion. There is an unmet need here, as currently all we have are platelets. We give patients platelets and that takes time. There are often delays because levels need to be rechecked; there are delays with transfusions and some nurses are trained in transfusions while others are not so there is a definite need for an alternative, or a new agent that can help raise platelet levels more efficiently."

## New Developments

### Professor Edoardo G. Giannini

It is interesting to consider what might change for the experts, the co-operation between the two specialities, and the patient preparation with new developments such as the use of thrombopoietin receptor agonists (TPO-RA). This class of drug has been approved, both in the USA and the European Union (EU), for the treatment of severe thrombocytopenia in patients with CLD undergoing invasive procedures. "In several trials, their use was associated with a significant decrease in the need for platelet transfusion; moreover, due to their modality of action, TPO-RA are able to increase platelet count for a longer period of time compared with platelet transfusion, thus allowing for a safer postprocedure window where an adequate platelet count is maintained," Prof Giannini said. "In this regard, the available studies did not describe a statistically significant association between the use of TPO-RA and the occurrence of relevant side effects, such as thrombotic events, although I feel that these drugs should be carefully administered in patients with a higher risk for thromboembolic events, and with appropriate monitoring."

Undoubtedly, TPO-RA will modify procedures and schedules and may help avoid unnecessary costs related to prolonged patient hospitalisation and delayed or cancelled procedures. Prof Giannini recommends that clinicians and IR should familiarise themselves with the modality of action of drugs, patient monitoring, and timeliness of the procedure. He added: "The advancement brought along by these drugs may radically modify how procedures are planned, and this will initially require close co-operation

among the various specialists involved in the process. The ultimate goal is improving the quality of assistance provided to patients."

## CONCLUSION

It is important that platelet levels are raised not only for the intervention, but also for up to 3 weeks after the procedure. Patients need raised platelet levels during the healing process too: some bleedings can occur 5–10 days after the intervention, an intervention may need to be repeated, or an additional intervention may need to be performed. Dr Modi commented on possible developments that would improve the situation for the patient saying: "When the hepatologist requests the liver biopsy in a patient who they know may be at a slight risk of having thrombocytopenia, it would be good if they were able to prescribe them with a medication which can be taken in the days leading up to the biopsy. On the morning they come in, they have their blood tests, and then, hopefully, their platelet count would be at the appropriate level." Continuing, he said: "I think this would be positive for all parties involved: the hepatologist, the IR, and the patient. It would mean that less biopsies would be cancelled, and it would be safer for patients and for IR. Overall, it would be a very positive thing."

## Biographies

### Prof Edoardo G. Giannini

**Professor of Medicine, Department of Internal Medicine, University of Genoa, Genoa, Italy**

Prof Giannini is an expert in liver pathophysiology with application to the prognostic study of the liver functional reserve. His research interests include the noninvasive staging of chronic liver disease, haemostasis in liver patients, staging, and therapeutic management of hepatocellular carcinoma. He is author of >250 publications in international journals and has written several book chapters.

### Dr Sachin Modi

**Consultant Interventional Radiologist/Oncologist, University Hospital Southampton, Southampton, UK**

Dr Modi joined the University Hospital Southampton in 2016 and plays a key role in the chemoembolisation (transarterial cheomembolisation: delivering beads with chemotherapy into liver arteries) and radioembolisation services (selective internal radiation therapy: delivery particles with radiation into liver arteries). His primary passion is interventional oncology delivering liver-directed treatment for primary or metastatic liver cancer, as well as prostate and fibroid embolisation. He is currently co-investigator in several research trials being carried out at University Hospital Southampton and is author of numerous publications and presentations at national and international IR conferences.


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# Chronic Spontaneous Urticaria: A Review of Pathological Mechanisms, Diagnosis, Clinical Management, and Treatment

EDITOR'S PICK

Our Editor's Pick for this EMJ flagship is the review paper by Mandel et al. Chronic spontaneous urticaria has received a lot of attention recently as researchers have aimed to become more knowledgeable on this little-known condition. Despite this, the authors provide a positive summary of the promising treatment options currently being developed, as well as a detailed breakdown of the existing diagnosis methods and clinical management. This is a timely review article, as several new-generation biological drugs to treat this disease are now entering clinical trials.

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## Abstract

Urticaria is a poorly understood and underestimated clinical condition characterised by the sudden onset of itchy wheals and/or angioedema, which usually resolve within 24 and 72 hours, respectively. It is generally classified as being acute (lasting <6 weeks) or chronic (continuous or intermittent for

≥6 weeks). Chronic urticaria can be further classified as chronic spontaneous urticaria (CSU) and chronic inducible urticaria, appearing in response to specific eliciting factors, such as heat, cold, or sun exposure, or following the application of pressure. Scientific advances have been made in the understanding of pathological mechanisms and treatment, especially associated with CSU. The exact pathological mechanism of how urticaria develops is still not yet fully understood, but the clinical implications on the patients' quality of life are severe and have been associated with mental disorders and metabolic diseases. The diagnosis of urticaria is based on medical history and clinical manifestations. The treatment pathway begins with the administration of second-generation, nonsedating, nonimpairing histamine 1 receptor antihistamines and, in case of nonresponse, with new-generation biological drugs. The current review presents an update of the pathological mechanisms, diagnosis, clinical management, and treatment of CSU. It also focusses on the future implications of new-generation drugs and their effects on the clinical practice.

## INTRODUCTION

Urticaria is a common mast cell-driven disease characterised by wheals and/or angioedema (Figure 1), defined as chronic when symptoms occur continuously or intermittently for ≥6 weeks and as spontaneous when specific eliciting stimuli, such as thermal agents, vibration, cholinergic factors, aquagenic, and delayed pressure, have been excluded as possible triggers.[1] When symptoms last <6 weeks, a diagnosis of acute urticaria can be made, and when the above mentioned triggers are identified, a diagnosis of chronic inducible urticaria is assigned.[1] Urticaria has a strong impact on patients' quality of life, and has been associated with anxiety, depression, somatoform disorders, metabolic syndrome, obesity, and sleep difficulties.[2-4] Patients can also present associated clinical symptoms, such as joint pain, headache and fatigue, flushing, breathlessness, gastrointestinal symptoms, and palpitations.[5] The prevalence of acute urticaria is assessed to be 2-fold higher than chronic urticaria.[1] Approximately 50% of cases of acute urticaria are idiopathic (i.e., a specific trigger is not identified) and this condition, which is referred to as acute spontaneous urticaria, can progress to chronic spontaneous urticaria (CSU) in up to 36% of patients.[6,7] Chronic urticaria is considered more common in adults, with a peak age of onset between 20 and 40 years, and women are affected twice as often as men.[6,8] Two recent studies, however, suggested that the prevalence of chronic urticaria and CSU in the paediatric population is similar to that of the adult population.[9,10] Urticaria cases in children and in adolescents might be treated by parents using over-the-counter medications and can possibly explain this underestimation in different studies.[9]

The average duration of chronic urticaria is 3–5 years[11] and its prevalence has been estimated to be 0.5–5.0%,[12] while CSU affects approximately 0.5–1.0% of the global population.[13]

Scientific advances have been made in the understanding of pathological mechanisms and treatment, especially associated with CSU. The current review presents an update of the pathological mechanisms, diagnosis, clinical management, and treatment of CSU. It also focusses on the future implications of new-generation drugs and their effects on the clinical practice.

## PATHOLOGIC MECHANISMS

The pathogenesis of CSU is complex and many different factors have been proposed as possible triggers including infections, food and drugs allergies, and genetic factors such as human leukocyte antigen Class II alleles associated with autoimmunity and the coagulation cascade. However, these proposed triggers have not been proven to be the causal immunologic mechanism, which today still remains unknown.[1] Nevertheless, a strong association is found between CSU and major autoimmune diseases, including autoimmune thyroid diseases, rheumatoid arthritis, Sjögren syndrome, coeliac disease, Type I diabetes mellitus, and systemic lupus erythematosus,[14] and there is evidence pointing towards a potential autoimmune aetiology in ≤50% of patients with CSU.[15]

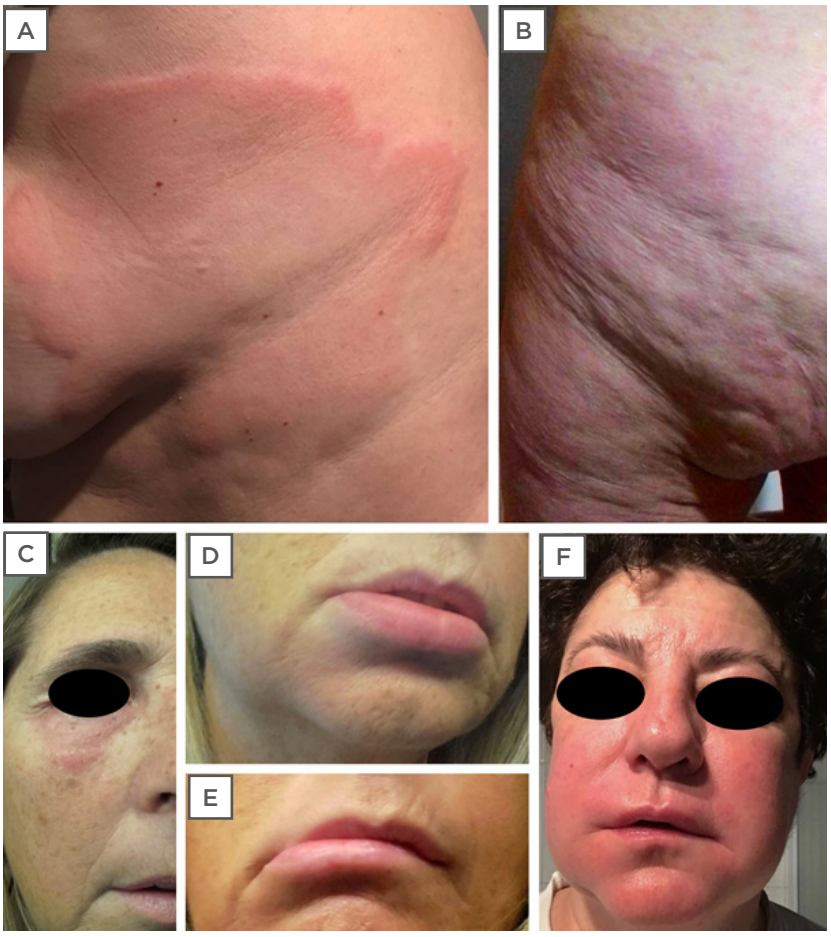


**Figure 1: (A-B)** Urticaria is characterised by an outbreak of swollen, pale red bumps or plaques on the skin (wheals). **(C-F)** Urticaria can also manifest as deep swelling around the eyes, lips, and face (angioedema) that appears suddenly.

At histological evaluation, wheals and angioedema present features common to inflammation including the vasodilatation of postcapillary venules, oedema, and a cellular infiltrate characterised by mast cell degranulation and migration of CD4+ T lymphocytes, monocytes, neutrophils, eosinophils, and basophils.[16] The oedema develops in the upper- and mid-dermis in the form of wheals, while angioedema involves the subcutaneous or submucosal tissue.[1] Cutaneous mast cells play a key role in CSU because their degranulation leads to the relapse of histamine; different proinflammatory cytokines, such as IL-1, IL-4, IL-5, IL-6, IL-8, and TNF-α; platelet-activation factor; vascular endothelial growth factor; matrix metalloproteinase-9; neuropeptides; and other vasoactive substances.[5] This is a standard inflammatory mechanism common to many inflammatory diseases.[1]

Specifically associated with CSU, some authors have demonstrated that upregulation of adhesion molecules for eosinophil cells, in particular P-selectin, alters cytokine expression and microvascular changes in nonlesional skin, and additionally alters the detection of blood basophils in lesional skin exhibiting suppressed IgE receptors, responsible for the release of histamine and upregulation of its release by IL-3.[17-19]

The recruitment of basophils into wheals results in blood basopenia.[20] Following successful treatment, CSU remission has been associated with an increase in blood basophil numbers and IgE receptor-triggered histamine response.[20-22] The IgE receptor-triggered histamine response is also observed during anti-IgE treatment.[1]

It has also been suggested that autoimmunity could be relevant for mast cell activation, specifically for some types of immunological mechanisms.[23] Hypersensitivity reactions have been classified into four types (I–IV) according to immunological mechanisms (Gell and Coombs classification of hypersensitivity reactions).[24] Type I hypersensitivity is due to the presence of an allergen that binds IgE, present on mast cell and basophil surfaces. This link induces the degranulation of the mast cells and basophils, and the release of mediators. Type II is mediated by antibodies, typically IgG or IgM, which link either IgE and/or high affinity IgE receptor (FcεRI) on mast cells and basophils or low-affinity IgE receptor (FcεRII) on eosinophils leading to vasoactive mediator release.[23]

The coagulation cascade has also been hypothesised as a possible CSU immunologic mechanism.[25] In fact, it has been observed that some CSU subjects have high levels of D-dimer, secondary to activation of the coagulation cascade by the activated eosinophils hyper-expressing tissue factor and other activated coagulation factors that amplify the release of histamine from mast cells and basophils.[26] Another possible immunological mechanism recently studied is the endocrine abnormalities of fatty tissue in overweight CSU patients. The fatty tissue may lead to the production of several adipokines that directly target human mast cells and also play a role in endothelial inflammation leading to the production of atherosclerotic plaque.[27] Chronic inflammatory skin diseases are known to be a risk factor for metabolic syndromes, and patients with metabolic syndromes and CSU exhibit high levels of prothrombin fragment 1+2, D-dimer, and inflammatory markers such as IL-6, IL-1, TNF-α, and C-reactive protein.[28]

## DIAGNOSIS AND CLINICAL MANAGEMENT

According to the European Academy of Allergology and Clinical Immunology (EAACI), the European Union (EU)-founded network of excellence, the Global Allergy and Asthma European Network (GA²LEN), the European Dermatology Forum (EDF), and the World Allergy Organization (WAO) guideline, diagnostic work-up starts from medical history and physical examination of the patient.[1] It is important to know the time of onset, timing, frequency, symptom duration, the features of the disease (i.e., wheals only, or wheals and angioedema), characteristics of the lesions (shape, size, site, distribution, and pattern of recurrence), other associated symptoms, familial disease history, and response to previous therapies used.[1,12]

The identification of known potential causes and/or possible triggers (e.g., food, medications, physical stimuli, infections, insect stings, and stressful occurrences) of urticaria is essential. Current drug assumption, especially nonsteroidal anti-inflammatory drugs and angiotensin-converting enzyme inhibitors, should also be established, because these drugs have been associated with the aggravation of pre-existing CSU.[1] Food avoidance with elimination diets is not helpful for CSU, while alcohol should be sidestepped because it can significantly exacerbate this condition.[12]

The EAACI/GA²LEN/EDF/WAO guideline recommended laboratory tests such as differential blood count, erythrocyte sedimentation rate, and C-reactive protein.[1] Furthermore, concomitant autoimmune disorders, thyroid dysfunction, and acute or chronic bacterial (e.g., *Helicobacter pylori*), viral (e.g., hepatitis virus), parasitic (e.g., *Anisakis simplex*), or fungal infections need to be investigated.[1] Screening for neoplastic diseases is not recommended but, if there are atypical features, assessment of serum tryptase, complement levels, and serum protein electrophoresis should be considered and a skin biopsy can be performed.[1,12]

Recent studies have highlighted that CSU may in some cases be associated with elevated BMI, obesity, diabetes, hyperlipidaemia (high levels of serum total cholesterol, triglycerides, low density lipoprotein, and decreased high density lipoprotein), arterial hypertension, metabolic syndrome, and gout.[28,29]

Differential diagnoses from other conditions in which wheals and angioedema can occur, such as exercise-induced anaphylaxis, auto-inflammatory syndromes, urticaria pigmentosa, urticarial vasculitis, or hereditary angioedema, are made clinically.

Several biomarkers have been investigated in association with CSU activity. Currently, the autologous serum skin test (ASST) and the basophil activation test (BAT) are the most commonly available auto-antibodies screening tests.[1] ASST is a relatively simple *in vivo* nonspecific screening test in which the autologous serum is injected back into the patients' skin intradermally to evaluate serum auto-reactivity, mostly due to any type of endogenous proinflammatory or wheal-inducing factors.[30] A positive ASST has been associated with prolonged disease, which is poorly responsive to routine therapy, and related to a delayed response to omalizumab.[31] BAT is an *in vitro* test that assesses the histamine upregulation or release of activation markers of donor basophils following stimulation from CSU patients' serum;[32] therefore, it helps to co-assess disease activity in CSU patients and a negative BAT is correlated with a better response to omalizumab.[33] Indirect BAT is a safe and reliable diagnostic tool which is also helpful in monitoring treatment, however it is usually not routinely available in daily clinical practice.[32,34-36] Another test proposed by Asero et al.[37] is the autologous plasma skin test (APST). Although the APST cannot be considered a screening test for histamine-releasing autoantibodies, it has recently been shown to a have a greater positive predictive value than ASST and has been correlated with antinuclear antibody positivity and angioedema.[38]

Moreover, D-dimer is related to disease activity in CSU patients due to the activation of the coagulation cascade and it seems to be the most promising biomarker.[25,26] This observation was confirmed by Kolkhir et al.,[39] who suggested that the evaluation of not only D-dimer but also fibrinogen, C-reactive protein, and erythrocyte sedimentation rate should be considered before starting treatment, because high levels of these markers may predict an unsatisfactory therapeutic response.

However, none of these biomarkers are currently implemented routinely in clinical practice. The still low level of evidence to support the available biomarkers is probably due to the wide variability and heterogeneity in the data collected from the published studies, which often show differences in methodology, design, selection of patient populations, and/or data analysis.

CSU patient management begins with the compilation of patient-reported scoring.[40] The most frequently utilised scoring system is the 7-day Urticaria Activity Score (UAS7).[1,40] The UAS7 is based on the patient self-assessment of key urticaria signs and symptoms (wheals: 0 = none; 1 = mild [<20 wheals/24 h]; 2 = moderate [20–50 wheals/24 h]; 3 = intense [>50 wheals/24 h or large confluent areas of wheals] and pruritus: 0 = none; 1 = mild [present but not troublesome]; 2 = moderate [troublesome but does not interfere with sleep]; 3 = severe [sufficiently troublesome to interfere with normal daily activity or sleep]) once a day for 1 week.

The UAS7 is the sum of the recorded scores over the period of 7 consecutive days, so disease activity and eventually response to treatment can be determined. The sum of score is 0–6 for each day with a maximum of 42 if summarised for a week. Additionally, for patients with recurrent angioedema, the EAACI/GA$^2$LEN/EDF/WAO guideline also suggested the use of the Angioedema Activity Score (AAS).[1] The AAS consists of five items regarding the characteristics of angioedema to have occurred in previous 24 hours.[41] A score between 0 and 3 is assigned to every answer field. The question scores are added up to produce a daily score. Daily AAS can be summed to give 7-day scores, 4-week scores, and 12-week scores.[41] The minimum and maximum possible AAS scores are 0–15 (daily), 0–105, 0–420, and 0–1,260, respectively.

To evaluate the impact of urticaria on patients, the Urticaria Control Test that assesses patient's disease status (the cut-off value for a well-controlled disease is 12 of 16 possible points), and specific disease quality of life questionnaires (the Chronic Urticaria-Quality of Life Questionnaire [CU-Q2oL] and Angioedema-Quality of Life Questionnaire [AE-QoL]) can also be used.[1,40]

## TREATMENT

The aim of pharmacological treatment is to obtain complete symptom relief. EAACI/GA$^2$LEN/EDF/WAO guideline suggests regular administration of second-generation, nonsedating, nonimpairing H1-receptor antihistamines as first-line symptomatic treatment for urticaria because of their good safety profile.[1] Compared

to the first-generation antihistamines, these antihistamines have greater receptor specificity, lower penetration of the blood–brain barrier, and are less likely to cause drowsiness or psychomotor impairment.[10,42] First-generation antihistamines should therefore be avoided due to their sedating, impairing, and anticholinergic side effects, while H2-receptor blockers are not felt to be of benefit in the treatment of urticaria.[43]

In nonresponders (adult or paediatric patients), the second-line treatment is the up-dosing of the second-generation H1-receptor antihistamines by as much as 4-fold. The leukotriene receptor antagonists, in particular montelukast, can be used as an add-on to second-line treatment in H1-antihistamine refractory CSU, but their administration is not recommended by the EAACI/GA²LEN/EDF/WAO guideline.[1]

For patients (aged 12 years and older) with CSU who have not responded to four-times the standard dose of second-generation H1-receptor antihistamines, omalizumab, a humanised monoclonal anti-IgE antibody, as add-on therapy is now considered the third-line treatment. Omalizumab was the first biologic agent approved by the U.S. Food and Drug Administration (FDA) for CSU. This drug has been widely proven to be very effective and well-tolerated in patients with antihistamine-refractory CSU.[1] Omalizumab binds to free IgE at the fragment crystallisable region (Fc region) preventing interaction with FcεRI receptor on mast cells and basophils. However, the exact mechanisms for the therapeutic effects of this drug for CSU remain unclear. Both 150 and 300 mg of omalizumab injected subcutaneously every 4 weeks have been shown to be effective for refractory CSU (the licensed dosage in Europe is 300 mg, while in the USA this is either 150 or 300 mg).[40] Dosage is currently recommended independently of total serum IgE count or patient body weight. Instead, only the dosage of 300 mg every 4 weeks has been proven to be effective in case of angioedema.[44]

Alternative dosages (off-label) of omalizumab have been used successfully in refractory CSU and reported in small case series: low doses of omalizumab (150 mg every 4 weeks) for long-term management of patients following initial therapy, and high dosages (450 or 600 mg every 4 weeks) for partial or nonresponders.[40] Omalizumab nonresponders are considered those with no symptom control after four doses of omalizumab 300 mg every 4 weeks, because the response rate is similar to placebo after this 16-week period.[45] To date, strategies or duration of omalizumab therapy, once disease control is optimised, has not found a universal agreement.

For all patients with wheals and angioedema, corticosteroid administration, in particular prednisone (dosage 0.3–0.5 mg/kg/day), over restricted periods of time (typically ≤10 days) can be prescribed as add-on treatment.

Finally, the fourth-line treatment (if there is no response to omalizumab within 6 months, or if the condition is intolerable) is cyclosporine A (CsA). CsA inhibits the production of IL-2, IL-3, IL-4, and TNF-α in lymphocytes and inhibits the IgE-mediated release of histamine from mast cells. High doses of CsA and long duration treatment are associated with adverse events such as abdominal pain, nausea, vomiting, paresthesia, headache, hirsutism, elevated serum creatinine, and hypertension; however, these effects resolve after reducing dose.[46] Nevertheless, CsA should be avoided in patients with chronic kidney disease or poorly controlled hypertension. CsA at the dose of 3–5 mg/kg/day has been shown in small, double-blind, randomised controlled trials to be effective in patients with CSU who do not adequately respond to antihistamines.[47,48] During CsA treatment, given the significant side effects, the blood pressure, renal function, and serum cyclosporine levels should be monitored regularly. Simplified stepwise algorithm for the treatment of CSU adapted from the EAACI/GA²LEN/EDF/WAO guideline is summarised in Figure 2.

## OTHER THERAPEUTIC OPTIONS (TABLE 1)

Phototherapy reduces the number of cutaneous mast cells in the superficial dermis and it has been used for the treatment of antihistamine-refractory corticosteroid-dependent CSU in combination with antihistamines for periods between 1 and 3 months, but published data are still limited.[49,50] Recently, 50 patients with steroid-dependent CSU were randomised to receive either narrowband ultraviolet B (NB-UVB) or psoralen plus ultraviolet A phototherapy in addition to licensed doses of antihistamines for 90 days.[51] The reduction in symptoms was maintained in both groups during

a 90-day post-treatment observation period, but NB-UVB phototherapy was found to be statistically better than psoralen plus ultraviolet A at different time points. Bishnoi et al.[51] proposed the combination of antihistamines with NB-UVB prior to third-line treatment with omalizumab.

Intravenous Ig (IVIg) has been used successfully for the treatment of antihistamine-refractory CSU due to its immunoregulatory effects. Its mechanisms for the immune modulation and anti-inflammatory actions include Fc receptor blockade (IVIg blocks FcεRI activity on mast cells, which prevents IgE binding and degranulation), inhibition of complement deposition, enhancement of regulatory T cells, inhibition or neutralisation of cytokines and growth factors, accelerated clearance of autoantibodies, modulation of adhesion molecules and cell receptors, and activation of regulatory macrophages through the FcγRIIb receptor.[52] However, due to the high cost, prolonged infusion times, and limited data on efficacy, the clinical use of IVIg is somewhat limited and alternative biologic agents should be considered.

CSU patients have been proven to have an upregulated TNF-α in the lesional and nonlesional skin.[53] TNF-α inhibitors (etanercept, infliximab, and adalimumab) have been reported to be effective in the treatment of CSU and may be a therapeutic option to those who have failed other alternative therapies.[53,54] However, no head-to-head studies have been performed to date and these drugs may be limited by their increased risk for infections, including tuberculosis and fungal infections, along with an increased risk for lymphomas and other malignancies.

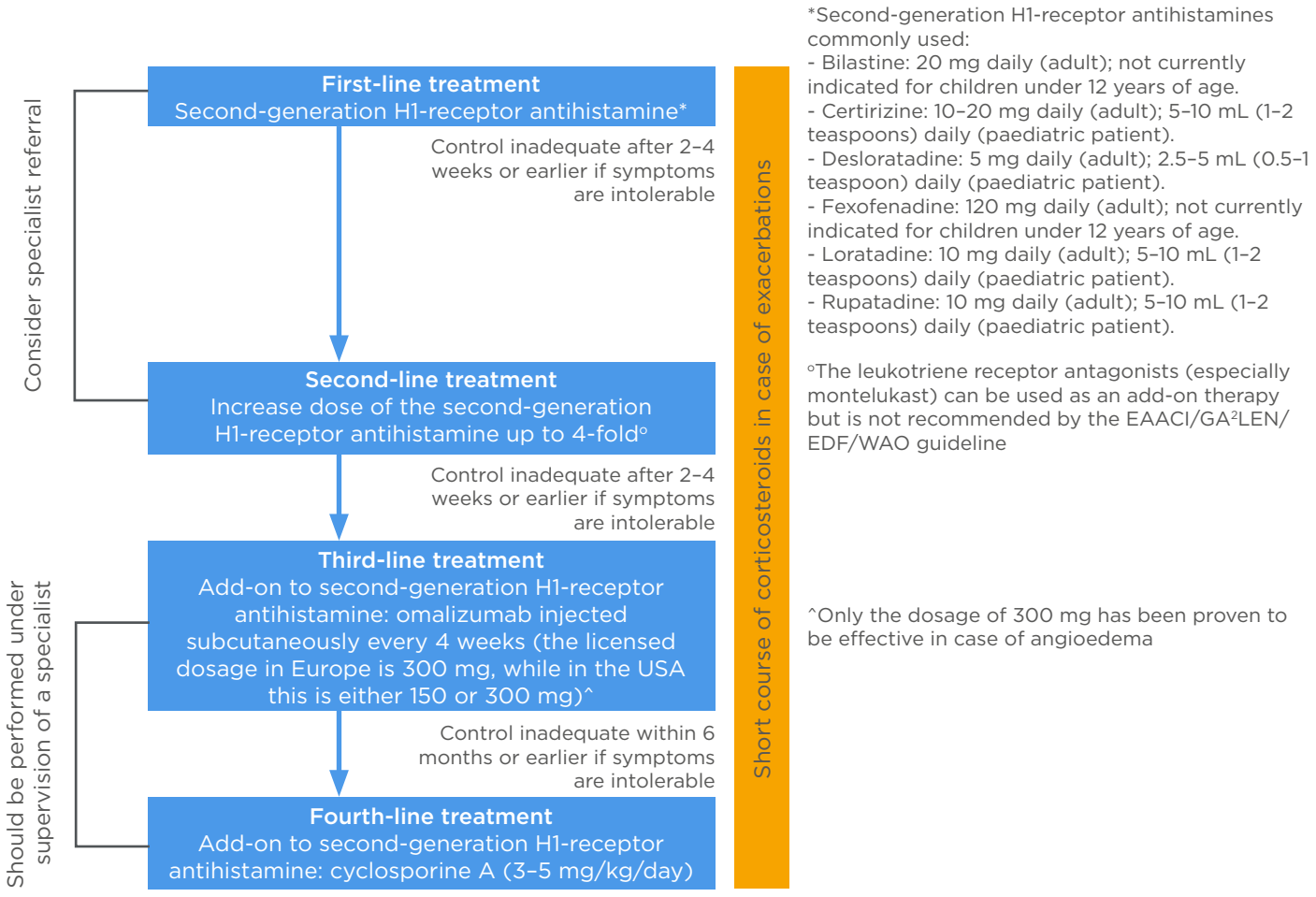


**Figure 2:** Simplified stepwise algorithm for the treatment of urticaria adapted from the EAACI/GA²LEN/EDF/WAO guideline 2018.

**Table 1: Other therapeutic options in chronic spontaneous urticaria.**

| Treatment | Mechanism of action in chronic spontaneous urticaria | Dosing/frequency in chronic spontaneous urticaria |
|---|---|---|
| Phototherapy | Reduces the number of cutaneous mast cells in the superficial dermis | Narrowband ultraviolet B three times weekly in addition to licensed doses of antihistamines for 3 months |
| Intravenous Ig | Blocks FcεRI activity on mast cells, which prevents IgE binding and degranulation and may decrease B-cell autoantibody production | 0.4 mg/kg/day intravenous infusion for 5 days every 4–6 weeks but lower doses (0.15 mg/kg/day) have also been explored with good results |
| TNF-α inhibitors | Block upregulation of TNF-α production in the lesional and nonlesional skin | Etanercept 50 mg injection subcutaneous once weekly, infliximab 5 mg/kg intravenous infusion every 8 weeks, and adalimumab 40 mg injection subcutaneous every 2 weeks |
| Rituximab | B-cell depletion via complement and antibody-dependent cytotoxicity results in decreased circulating autoantibody levels | Rituximab 375 mg/m2 intravenous infusion once weekly for 4 weeks |
| Ligelizumab | Similar in function to omalizumab, but produces a greater and longer suppression of free IgE and IgE on the surface of basophils | Ligelizumab 72 mg or 240 mg injection subcutaneous every 4 weeks |
| Bruton tyrosine kinase inhibitor | Unknown | GDC-0853 oral administration twice daily for a total of 56 days |
| Spleen tyrosine kinase inhibitor | Blocks upregulation of transcription factors responsible for the synthesis and degranulation of proinflammatory mediators | GSK2646264 topical application for 28 days |
| IL-1 inhibitors | Inhibition of IL-1β may modify the clinical course of urticarial lesions | Canakinumab 150 mg injection subcutaneous every 8 weeks, Anakinra 100 mg injection subcutaneous once a day |
| Prostaglandin D2 receptor antagonist | Inhibition of chemoattractant receptor homologous molecule expressed on Th2 cells could reduce the frequency and severity of urticarial lesions because of its anti-inflammatory properties | AZD1981 40 mg oral administration three times daily for a total of 7 days |

Rituximab, a mouse-human chimeric anti-CD20 monoclonal antibody, induces B-cell depletion by targeting the CD20 antigen on the B lymphocytes. This mechanism results in inhibition of autoantibody production and some promising results have been demonstrated in patients with CSU;[55] however, given the lack of randomised, double-blind, placebo-controlled trials, rituximab is not licensed for the treatment of antihistamine-refractory CSU.

Future therapeutic options currently under investigation include new-generation biological drugs. Ligelizumab, a humanised IgG1κ monoclonal antibody targeting the third heavy chain constant region domain of IgE, is similar in function to omalizumab, but has been proven to bind free IgE with greater affinity.[56] A Phase IIB dose-finding trial[57] evaluated the efficacy and safety of this drug compared with placebo and omalizumab, showing complete control of symptoms in a higher percentage of patients with ligelizumab therapy of 72 mg or 240 mg.[58] Currently, an extension study[59] is investigating the long-term safety of this drug in CSU patients who completed the trial, remained in the follow-up period for at least 32 weeks, and had an active disease (UAS7≥12).

Another humanised IgG1 monoclonal antibody is quilizumab, which targets the M1 prime segment of membrane-expressed IgE resulting in diminished IgE-switched B cells and plasmablasts. The effects appeared to last up to 6 months after completion of therapy,[60] however, further development of this drug has been discontinued.

Other agents are currently under experimentation in clinical trials. Bruton tyrosine kinase is a nonreceptor tyrosine kinase that transmits signals crucial for B-cell development and its genetic deletion causes B-cell immunodeficiency.[61] Although the role of B cells in urticaria is not well understood, it is believed that bruton tyrosine kinase inhibitors could potentially play a role in refractory CSU management. GDC-0853, a bruton tyrosine kinase inhibitor, is currently under investigation in a Phase IIA multicentre, randomised, double-blind, placebo-controlled pilot study[62] that is evaluating the efficacy, safety, and pharmacodynamics of this drug compared with placebo in individuals with anthistamine-refractory CSU.

Spleen tyrosine kinase upregulates transcription factors that are responsible for the synthesis and degranulation of proinflammatory mediators and its expression increases in certain subsets of patients affected by CSU.[63-65] GSK2646264, a topical spleen tyrosine kinase inhibitor, is currently being tested in a randomised, double-blind, single and repeat ascending trial in order to determine its efficacy in patients with CSU and cold urticaria.[66]

Inhibition of IL-1β may modify the clinical course of urticarial lesions in patients affected by CSU and various studies are underway in order to elucidate the role of IL-1 inhibitors in CSU.[67] Canakinumab, a fully human anti-IL-1β antibody, is currently under investigation in a Phase II randomised, double-blind, placebo-controlled, single-centre study evaluating the use of this drug compared with placebo in CSU patients.[68] Finally, inhibition of chemoattractant receptor-homologous molecule expressed on $T_H2$ cells could reduce the frequency and severity of urticarial lesions because of its anti-inflammatory properties. CSU patient eosinophils overexpress this prostaglandin D2 receptor.[69] A Phase IIA, randomised, placebo-controlled, double-blind study is evaluating the efficacy of AZD1981, a prostaglandin D2 receptor antagonist, as a potential therapeutic option in CSU.[70]

Further investigations are needed for identifying strategies for the prevention and symptomatic treatment of CSU, identification of the best therapy, and development of new drugs. Moreover, the exploration of novel therapeutic targets can help to better understand the aetiopathogenesis of the disease.

## CONCLUSIONS

There is currently a low level of evidence on the exact pathological mechanism in CSU, and as a result clinical and diagnostic indications have not recently changed. New-generation treatment options should be available in the near future and seem promising. Future studies should investigate personalised treatment, with recommended dosages considering disease severity and treatment responsiveness.

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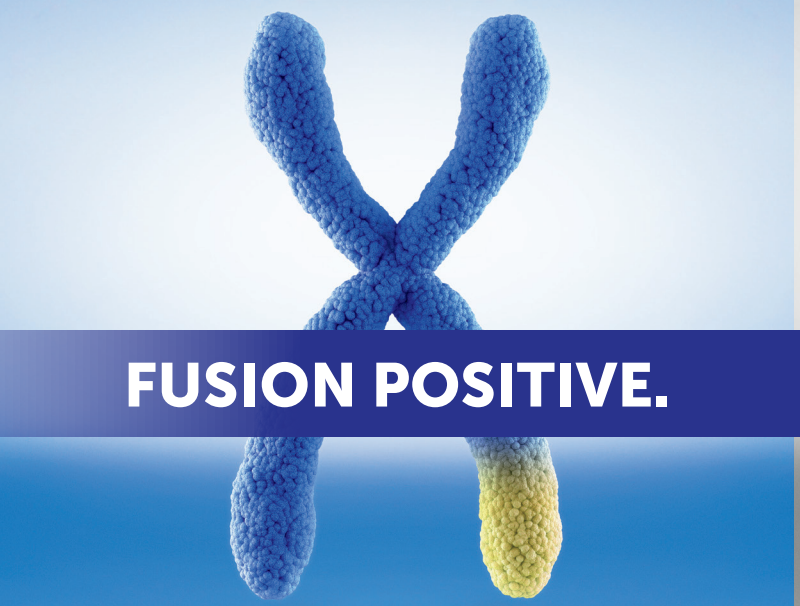
FOR ADULT AND PAEDIATRIC PATIENTS WITH *NTRK* GENE FUSION-POSITIVE SOLID TUMOURS[1]



# FUSION POSITIVE. POSITIVE RESULTS.[1]

VITRAKVI received a conditional marketing authorisation valid throughout the EU on 19 September 2019. This means that further evidence on this medicinal product is awaited. The EMA will review new information on this medicinal product at least every year and guidance will be updated as necessary.

The benefit of VITRAKVI has been established in single arm trials encompassing a relatively small sample of patients whose tumours exhibit *NTRK* gene fusions. Favourable effects of VITRAKVI have been shown on the basis of overall response rate and response duration in a limited number of tumour types. The effect may be quantitatively different depending on tumour type, as well as on concomitant genetic alterations. For these reasons, VITRAKVI should only be used if there are no treatment options for which clinical benefit has been established, or where such treatment options have been exhausted (i.e., no satisfactory treatment options).[1]

## VITRAKVI▼ DEMONSTRATED A 67% OVERALL RESPONSE RATE (95% CI: 57%, 76%; n=68) ACROSS THREE CLINICAL STUDIES[1]

# In solid tumours with *NTRK* gene fusion, consider treating with VITRAKVI.

**The indication for VITRAKVI is limited to patients with TRK-fusion cancer who have a disease that is locally advanced, metastatic or where surgical resection is likely to result in severe morbidity and who have no satisfactory treatment options.[1]**

## PRESCRIBING INFORMATION

**VITRAKVI®▼ (Larotrectinib) 20 mg/ml oral solution**
**Prescribing Information**
(Refer to full Summary of Product Characteristics (SmPC) before prescribing)
**Presentation:** One bottle of 100 mL oral solution. Each mL of oral solution contains larotrectinib sulfate equivalent to 20 mg of larotrectinib. **Indication(s):** Larotrectinib as monotherapy is indicated for the treatment of adult and paediatric patients with solid tumours that display a Neurotrophic Tyrosine Receptor Kinase *(NTRK)* gene fusion, - who have a disease that is locally advanced, metastatic or where surgical resection is likely to result in severe morbidity, and - who have no satisfactory treatment options. VITRAKVI® has been authorised under a conditional approval scheme. **Posology & method of administration:** The presence of an *NTRK* gene fusion in a tumour specimen should be confirmed by a validated test prior to initiation of treatment with larotrectinib. For oral use. The oral solution should be administered by mouth using an oral syringe of 1 mL or 5 mL volume or enterally by using a nasogastric feeding tube. Do not mix with feeding formulas. Do not take with grapefruit or grapefruit juice. ***Adults:*** 100 mg larotrectinib twice daily, until disease progression or until unacceptable toxicity occurs. ***Children & adolescents:*** Dosing is based on body surface area (BSA). The recommended dose in paediatric patients is 100 mg/m$^2$ larotrectinib twice daily with a maximum of 100 mg per dose until disease progression or until unacceptable toxicity occurs. ***Hepatic impairment:*** The starting dose of larotrectinib should be reduced by 50% in patients with moderate (Child-Pugh B) to severe (Child-Pugh C) hepatic impairment. No dose adjustment is recommended for patients with mild hepatic impairment (Child-Pugh A). ***Renal impairment:*** No dose adjustment is required. ***Elderly:*** No dose adjustment is recommended. ***Co-administration with strong CYP3A4 inhibitors:*** Reduce larotrectinib dose by 50%, refer to SmPC. **Contra-indications:** Hypersensitivity to the active substance or to any of the excipients. **Warnings & precautions:** Larotrectinib should only be used if there are no treatment options for which clinical benefit has been established, or where such treatment options have been exhausted (i.e., no satisfactory treatment options). Neurologic reactions including dizziness, gait disturbance and paraesthesia were reported in patients receiving larotrectinib. Withholding, reducing, or discontinuing larotrectinib dosing should be considered, depending on the severity and persistence of these symptoms. ALT and AST increase have been observed, therefore liver function, including ALT and AST assessments, should be monitored before the first dose, and monthly for the first 3 months of treatment, then periodically during treatment, with more frequent testing in patients who develop transaminase elevations. Withhold or permanently discontinue larotrectinib based on severity. If withheld, the larotrectinib dose should be modified when resumed. *Avoid co-administration of strong or moderate CYP3A4/P-gp inducers with larotrectinib due to a risk of decreased exposure.* Women of childbearing potential must use highly effective contraception while taking larotrectinib and for at least one month after stopping treatment. Males of reproductive potential with a non-pregnant woman partner of child bearing potential should be advised to use highly effective contraception during treatment with larotrectinib and for at least one month after the final dose. VITRAKVI® 20 mg/mL oral solution contains excipients with known effects: sucrose, sorbitol, propylene glycol, methyl parahydroxybenzoate. Essentially sodium free (<1 mmol/5 ml). **Interactions:** For the effects of other agents on the action of larotrectinib (e.g. CYP3A, P-gp and BCRP inhibitors; and CYP3A and P-gp inducers) and the action of larotrectinib on other agents (CYP3A substrates, CYP2B6 substrates, other transporter substrates and PXR regulated enzymes) refer to the SmPC. It is unknown if larotrectinib interacts with hormonal contraceptives and, therefore, it is advised that an additional barrier method is used and continued for 1 month after final dose. **Pregnancy & lactation:** Avoid the use of larotrectinib during pregnancy. 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Prescribers should consult the SmPC in relation to other side effects. **Overdose:** In the event of overdose, physicians should follow general supportive measures and treat symptomatically. **Special Precautions for Storage:** Store in a refrigerator (2 °C - 8 °C). Do not freeze. **Legal Category:** POM. **Package Quantities & Basic NHS Costs:** 100ml glass bottle £5,000. **MA Number(s):** EU/1/19/1385/003. **Further information available from:** Bayer plc, 400 South Oak Way, Reading RG2 6AD, United Kingdom. Telephone: 0118 206 3000. **Date of preparation: September 2019.**



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# Pulmonary Rehabilitation is Improved by In-Shoe Foot Orthosis Intervention

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## Abstract

Some studies have found that patients with asthma have bilateral foot dorsal flexion limitations, contributing to impaired quality of life. The authors hypothesised that foot misalignments could also occur in patients with chronic obstructive pulmonary disease (COPD), and that foot orthoses could increase the motor benefits of their pulmonary rehabilitation (PR).

Presented herein are the results from a nonrandomised controlled study in 40 patients with COPD. Twenty patients had foot misalignment (Group 1) and wore foot orthoses for a 1-month PR period. Their data were compared to those obtained in 20 other patients with COPD who had no foot misalignment and did not wear foot orthoses (Group 2). Bodily fatigue, 6-minute walk test (6MWT) distance, peak plantar flexion force (PFF), and oscillations of the centre of pressure (CoP) were measured. Measurements were performed prior to and following completion of PR (Groups 1 and 2), immediately after wearing the foot orthosis (Group 1), and after completion of PR plus foot orthoses (Group 1).

In Group 2, PR increased the 6MWT distance, but did not increase PFF nor reduce CoP oscillations and fatigue scale. Wearing the foot orthosis for the first time significantly increased the 6MWT distance (+98+12 m). Following PR with foot orthoses (Group 1), a further increase in 6MWT distance occurred (+120+13 m), bodily fatigue was reduced, PFF increased, and CoP oscillations decreased.

In patients with COPD and foot misalignment, foot orthoses enhanced the functional capacity and improved the postural control.

## INTRODUCTION

Pulmonary rehabilitation (PR) is currently the most cost-effective approach designed to improve exercise tolerance, peripheral muscle function, and quality of life in patients with chronic obstructive pulmonary disease (COPD).[1,2] The clinical management of patients with COPD and peripheral muscle dysfunction, based on improving the functional capacity of skeletal muscles, is widely documented,[3,4] and the 6-minute walk test (6MWT) is commonly used to evaluate the benefits of exercise training.[5]

Despite data not being available in a COPD setting, some studies have reported that patients with asthma have increased fall rates as a result of bilateral foot dorsal flexion limitations of the ankle,[6] contributing to impaired quality of life.[7] These observations suggest that foot orthoses intervention could improve the ambulatory performance and thus quality of life of patients with respiratory disorders. It has in fact been shown that foot orthoses improve the ambulatory capacities and posture of normal weight and obese patients,[8] venous return,[9] and foot sole mechanosensitivity,[10] an observation also confirmed in patients with asthma.[11] The in-shoe foot orthoses are designed to reduce rear foot misalignment and hold the foot close to its subtalar neutral position in order to restore normal alignment of the entire lower limb.

In the present study, podiatrists diagnosed a major foot misalignment in the authors' COPD population (Group 1). Based on previous findings,[8-11] it was hypothesised that a foot orthosis intervention supplementing regular PR could induce additional functional benefits to these patients, improving their ambulatory performance and reducing the posture sway. The authors also examined the benefits of regular PR alone in another group of patients with COPD and no foot misalignment (Group 2), who did not wear the foot orthosis.

## METHODS

### Study Design

This was a nonrandomised, controlled study in patients with COPD comparing the consequences of PR alone (Group 1; n=20) or associated with foot orthoses intervention (Group 2; n=20) on their capacity to walk and the postural changes. The French institutional review boards for human studies (Agence Nationale de Sécurité du Médicament et des Produits de Santé [ANSM] and Comités de Protection des Personnes [CPP]) approved the study protocol, which followed the principles outlined in the code of ethics of the World Medical Association (WMA) (Declaration of Helsinki). Written informed consent was obtained from all patients. The characteristics of patients are shown in Table 1. All patients were undergoing treatment of their chronic respiratory disease but did not receive oxygen supplementation. Their practitioners had good control of their symptoms. Exclusion criteria was the diagnosis of diabetes, neuromuscular disorders, leg trauma, and spine diseases.

In both groups, PR lasted 4 weeks. All data were measured prior to and after each period of PR. In both groups, the foot configuration was determined by podiatrists based on: 1) the measurement of the distance between the rear foot and forefoot surface plans; 2) the diagnosis of the plantar deformity; and 3) the analyses of the plantar footprint. Group 1 patients had major foot misalignment (navicular drop: 6; high-arch foot: 4; low-arch foot: 5; static disorders due to anisomelia that is a leg length inequality: 5). Assessment prior to PR included pulmonary function tests, cardiopulmonary exercise testing, 6MWT, maximal plantar flexion force (PFF), bodily fatigue scale, and measurements of oscillations of the centre of pressure (CoP). For patients in Group 1, the acute effects of foot orthoses were also assessed prior to PR with foot orthoses.

### Pulmonary Function and Gas Exchange

Forced vital capacity and forced expiratory volume in 1 second were measured spirometrically (MasterLab©, Levallois-Perret, France). The reference values were those proposed by Quanjer.[12] Arterial blood gas tensions ($PaO_2$ and $PaCO_2$) and pH (pHa) were measured by a blood gas analyser (Corning Chiron model 860©, Chiron Technologies, Villeuneuve-La-Garenne, France). Cardiopulmonary exercise testing was performed on a cycle ergometer, included in the PR programme to determine the ventilatory threshold and the corresponding heart rate.

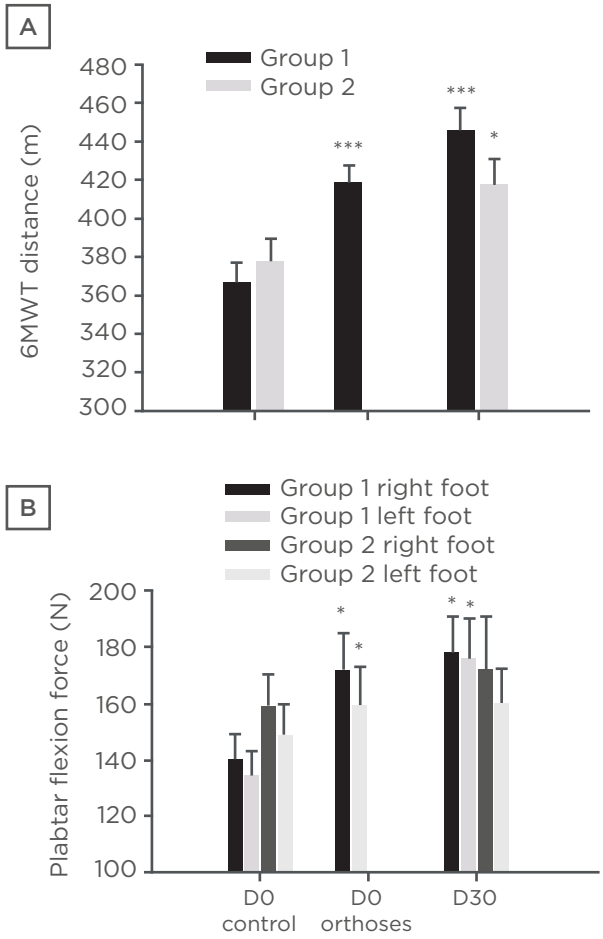


**Figure 1: (A) Benefits of foot orthoses intervention on the ambulatory capacities. (B) Peak plantar flexion force.**

The 6MWT distance significantly increased in Group 1 patients who wore their foot orthoses for the first time to begin pulmonary rehabilitation (D0 orthoses) (**p<0.01). After completion of pulmonary rehabilitation (D30), this effect increased in Group 1 (***p<0.001) and Group 2 (**p<0.01). In Group 1 patients, the foot orthoses significantly increased the peak plantar flexion force when they were worn for the first time and also after completion of pulmonary rehabilitation with orthoses (*p<0.05). No benefits of pulmonary rehabilitation on plantar flexion force were noted in Group 2.

D: day; 6MWT: 6-minute walk test.

## Pre and Post-PR Measurements

### Maximal Plantar Flexion Force

Maximal peak PFF was measured under isometric conditions using a custom-built device previously described in detail.[8,13] Three 5-s PFF maneuvers were executed by the subject using each leg to determine the maximal force value. The best values were considered.

### Pedobarographic Measurements

A recent review validated the use of a pedobarographic platform in postural assessment.[14] Subjects were bare-footed when standing on the platform for 30 seconds (WinPOD Medicapteurs SA©, Balma, France). Patients were positioned standing on the platform, with lowered arms, heels 2 cm apart, barefoot, and feet at 30° so that the centroid of the sustentation polygon was located on the sagittal axis of the platform. Postural oscillations were recorded for 30 seconds. The authors measured postural variables: the peak and mean foot pressures, the surface covered by displacements of the CoP, and its total length of displacement. Measurements of PFF and postural variables were repeated twice.

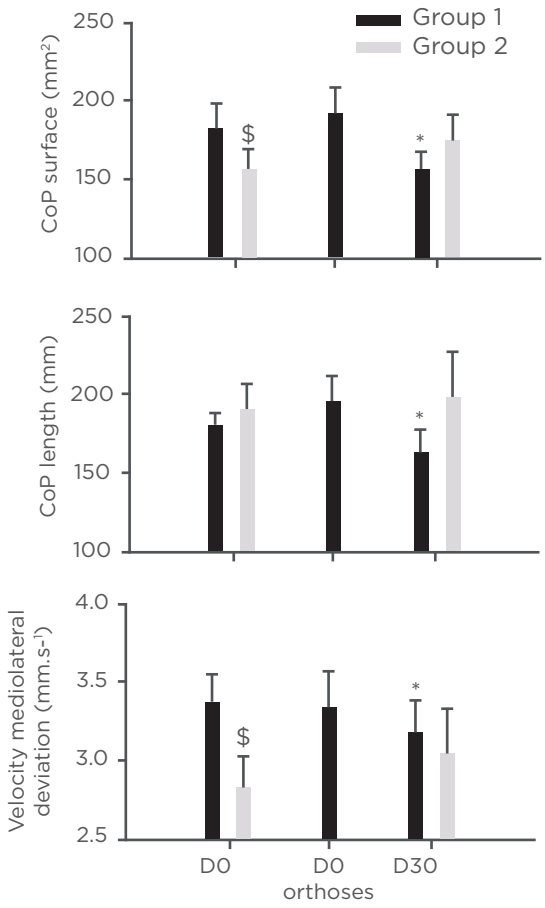


**Figure 2:** Posturographic indices (surface and length of the CoP and velocity of mediolateral CoP deviation) measured before PR (D0), when wearing foot orthoses before beginning PR (D0 orthoses) in Group 1, and after completion of the whole PR programme (D30) in both groups. Wearing foot orthoses during the PR programme significantly reduced the three variables in Group 1. No effects were measured in Group 2 patients.

CoP: centre of pressure; D: day; PR: pulmonary rehabilitation.

### 6-Minute Walk Test

As recommended by the American Thoracic Society (ATS),[5] 1 hour after the first 6MWT, a second test was performed during which all the measurements were considered.

### Sensations of Bodily Fatigue

The patients addressed the questions of the Pichot bodily fatigue scale (validated for French subjects).[15]

### Pulmonary Rehabilitation Programme

All patients participated in a comprehensive 4-week PR programme based on the updated definition proposed by the ATS–European Respiratory Society (ERS) group consensus.[2] The training programme included endurance exercise of both interval and continuous modalities (cycling and walking), at a training intensity corresponding to a heart rate of 80% of that measured at the ventilatory threshold during a maximal cardiopulmonary exercise test. PR also included peripheral muscle strength training of upper and lower limbs, and upper extremity dynamic exercises on an arm cycle ergometer three to five times per week for 60 minutes each time.

## Custom-Molded Foot Orthoses

Thermoformed pads were built and middle density (20–30 Shore) materials were placed on selected sole locations to correct the foot misalignment. Depending on the type of foot abnormality, retro capital bars and/or foot arch reinforcement were added.

## Statistical Analyses

Based on mean and standard error of the mean data from previous studies that explored the consequence of foot orthoses intervention,[8] and the 6MWT data[13] on the postural control and PFF values in normal and excess weight subjects, the authors estimated that 18 patients were necessary for each group. A two-way repeated measures ANOVA test allowed depiction of significant changes of the variables across the different time points between the two PR conditions (PR alone versus PR plus foot orthoses). When the normality test failed, the pairwise multiple comparison procedure (Holm–Sidak method) was used. With the numbers available, no significant difference could be detected when $p>0.05$.

# RESULTS

## Intergroup Differences at Baseline

At study entry, pulmonary function (forced expiratory volume in 1 second and $PaO_2$ values) and exercise tolerance (maximal $O_2$ uptake) did not differ between groups (Table 1). On the contrary, compared to Group 2, the bodily fatigue scale was significantly higher in Group 1 patients (13.7±2.2 versus 8.0±3.0; $p<0.001$), and their 6MWT distance (Figure 1A) and PFF (Figure 1B) were lower. Moreover, the postural control of Group 1 patients was less efficient than that of Group 2 (Figure 2), with higher CoP surface and velocity of mediolateral CoP deviation.

**Table 1: Patient characteristics.**

| | Group 1 | | Group 2 | |
|---|---|---|---|---|
| | PRE | POST | PRE | POST |
| n | 20 | | 20 | |
| Sex ratio | 7/20 | | 5/20 | |
| Age, years | 64±2 | | 67±2 | |
| Weight (kg) | 77±4 | | 68±5 | |
| Height (cm) | 169±1 | | 166±2 | |
| BMI | 27.0±1.4 | | 24.6±1.6 | |
| $FEV_1$, BTPS | 1.43±0.14 | 1.57±0.14 | 1.27±0.11 | 1.34±0.12 |
| | (53±4) | (57±4) | (49±4) | (53±5) |
| FVC, BTPS | 2.66±0.15 | 2.97±0.16 | 2.40±0.15 | 2.51±0.19 |
| | (77±4) | (80±4) | (70±4) | (74±6) |
| FEV1.0/VC (%) | 54±3 | 56±3 | 56±2 | 56±3 |
| $PaO_2$ (mmHg) | 77±2 | 79±2 | 78±2 | 75±2 |
| $PaCO_2$ (mmHg) | 40±1 | 39±1 | 36±1 | 37±1 |
| pHa | 7.40±0.01 | 7.42±0.001 | 7.42±0.01 | 7.43±0.01 |
| $VO_2$/body weight ($ml.min^{-1}.kg^{-1}$) | 19.5±1.1 | | 18.5±1.4 | |
| HR threshold beats ($min^{-1}$) | 116±4 | | 115±5 | |

Variables measured at inclusion in the study (PRE) and after completion of the 8-week rehabilitation program (POST).

Peak oxygen uptake ($VO_2$/body weight) and heart rate measured at the ventilatory threshold. Values are the mean ± standard error of mean. Values in parentheses are the percentage of predicted FEV1 and FVC.

BTPS: body temperature pressure saturated; FVC: forced vital capacity; $FEV_1$: forced expiratory volume measured at 1.0 s; HR: heart rate; $PaO_2$ and $PaCO_2$: partial pressures of oxygen and carbon dioxide in arterial blood; VC: vital capacity.

## Foot Orthoses Intervention and Bodily Fatigue Sensation

At the end of the PR programme, compared to data at Day 0, a significant reduction of bodily fatigue scale was measured in Group 1 (9.2±2.4 versus 13.7±2.2), whereas there was only a trend to a reduction in Group 2 (7.2±2.8 versus 8.0±3.0).

## Foot Orthoses Intervention and the 6-Minute Walk Test Distance

In Group 1, wearing the foot orthosis for the first time prior to beginning PR significantly increased the 6MWT distance (+55±12 m) (Figure 1A). Following PR completion with foot orthoses, a further increase in 6MWT distance was noted (+95±13 m), however the changes were not significant. PR alone also significantly increased 6MWT distance in Group 2 but to a lesser extent (+40±8 m).

## Foot Orthoses Intervention and Plantar Flexion Force

The benefits of the foot orthosis on PFF values occurred soon after the Group 1 patients wore their foot orthoses for the first time. At the end of PR with foot orthoses, PFF of both feet continued to be increased but no further changes were measured. No significant PPP variations occurred in Group 2 (Figure 1B).

## Foot Orthoses Intervention and Postural Control

No significant postural changes were noted in Group 1 patients after they wore their foot orthoses for the first time. On the other hand, after completion of the 4-week PR programme, the CoP surface and length, and also the velocity of mediolateral CoP deviation, were significantly reduced. No significant variation of the CoP characteristics was noted after PR completion in Group 2 (Figure 2).

# DISCUSSION

The authors found ergonomic differences between the two groups at inclusion in the study. In patients who presented foot misalignments at study entry, the fatigue scale was significantly higher, the PFF values significantly lower, and their postural control less efficient, with higher CoP surface and higher velocity of mediolateral CoP deviation. Thus, foot misalignment reduced the ambulatory performance of Group 1 patients with COPD. These data confirm the observations of reduced ambulatory capacities in patients with asthma.[6,7]

The present study shows in Group 1 patients with COPD experiencing foot abnormalities that foot orthoses reduced bodily fatigue, markedly increased the 6MWT distance and the PFF, and improved postural control. PR alone in the Group 2 patients increased the 6MWT distance to a lesser extent but did not increase PFF nor reduce the CoP oscillations and the fatigue scale.

Several studies in healthy subjects and in patients with neuromuscular disorders support the hypothesis that foot orthoses intervention may help walking. In healthy subjects, foot orthoses improve the biomechanical capacities of the ankle,[8,16,17] increase the tactile sensitivity of the foot sole,[10] and improve the postural control.[8,18] Based on these observations, wearing foot orthoses has been proposed as a support of the functional rehabilitation of patients with chronic muscle dystrophy,[19] spinal muscular atrophy,[20] or chronic stroke.[21]

Most activities of daily living are performed at submaximal levels of exertion and this is reproduced during a PR programme. Moreover, the 6MWT reflects the functional capacity for daily physical activities.[5] Several possible causes for the improvement of ambulatory capacities by foot orthoses have been previously identified. Firstly, foot orthoses were suggested to increase the strength of the foot muscles participating in plantar flexion, the benefits of which are still presenting in this study. This effect was already reported,[22] showing that medially posted insoles consistently influenced the foot pronation. The authors recently confirmed these observations showing that the addition of 3 mm heel pads significantly increased the peak PFF, probably through an increased lever arm exerted by the rearfoot on the forefoot.[8] Secondly, the present study in patients with COPD confirmed the previous observation in healthy subjects that foot orthoses improved posture control; this effect could have contributed to the increased 6MWT distance.[8] This improved postural control may

also result from an increased mechanosensitivity of the foot sole, induced by the foot orthoses.[10] The cutaneous mechanoreceptors of the foot sole detect changes in the application of mechanical loads on the plantar surface during gait and standing, and contribute to controlling the standing balance and postural reflexes in healthy subjects.[18]

## CONCLUSION

One message of this study is that foot orthoses markedly improve ambulatory capacities. Patients with COPD wearing foot orthoses benefitted by ergonomic improvements during PR, including increased leg muscle force and improved postural control. In a future study it would be interesting to compare a case-control group on the use of plantar support. Regardless, the present data strongly recommend that clinicians should routinely examine their patients that have COPD for foot abnormalities. However, the benefits of foot orthoses on rehabilitation is almost certainly not limited to a COPD population. It is likely that in any population with significant foot misalignment, treatment targeting the foot disorder would improve the walking distance and postural control.

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# Epigenetics of Diabetic Nephropathy: From Biology to Therapeutics

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## Abstract

Diabetic nephropathy (DN) is a lethal microvascular complication associated with Type 1 and Type 2 diabetes mellitus, and is the leading single cause of end-stage renal disease. Although genetic influences are important, epigenetic mechanisms have been implicated in several aspects of the disease. The current therapeutic methods to treat DN are limited to slowing disease progression without repair and regeneration of the damaged nephrons. Replacing dying or diseased kidney cells with new nephrons is an attractive strategy. This review considers the genetic and epigenetic control of nephrogenesis, together with the epigenetic mechanisms that accompany kidney development and recent advances in induced reprogramming and kidney cell regeneration in the context of DN.

## INTRODUCTION

Diabetic nephropathy (DN) is one of the major microvascular complications associated with diabetes in terms of increased healthcare costs, high morbidity, and premature mortality.[1] More than 50% of patients with Type 2 diabetes mellitus (T2DM) and 30% of those with Type 1 diabetes mellitus (T1DM) develop kidney disease, and a considerable number of cases can progress to end-stage renal disease (ESRD).

In addition, diabetic patients with ESRD are more likely to develop adverse macrovascular complications such as hypertension, atherosclerosis, and peripheral and cerebrovascular disease leading to an increased mortality rate. DN is clinically characterised by progressive albuminuria, decreased glomerular filtration rates (GFR), and a constant decreased kidney function. It is histologically defined by renal glomerular hypertrophy, expansion of mesangial and tubular compartments, accumulation of mesangial

extracellular matrix proteins, and podocytopenia associated with foot process effacement.[2]

DN is a complex multifactorial disease caused by multiple genetic and environmental factors. Genetics alone cannot fully explain the variability in the incidence of nephropathy and the uneven distribution and graveness of complications in diabetic patients.[3] More recent studies have shown that epigenetic mechanisms are involved in the pathogenesis of DN. These processes influence gene expression patterns or cellular phenotypes and disease states with no underlying change in DNA sequence.[4] Acute hyperglycaemia leads to chronic metabolic and haemodynamic derangements,[5] which trigger chromatin structural changes, transcription factor activation, and gene expression.[6] These changes persist even after returning to normoglycaemia:[7] a phenomenon referred to as 'metabolic memory' or 'legacy effect'.[8] Thus, environmentally-induced epigenetic events compounded by genetic predisposition play significant roles in diabetes and its related complications (Figure 1).[6]

Identifying novel approaches for the prevention and treatment of DN relies on an improved understanding of the molecular mechanisms driving DN.[9] The current review examines the emerging evidence for epigenetic mechanisms and pathways in DN. In addition, the authors also review future strategies in DN treatment such as transcriptional reprogramming of mature adult kidney cells into uncommitted induced pluripotent stem cells for renal repair and therapeutics. Key transcription factors involved in DN that can be targeted to halt disease progression are also described. A comprehensive understanding of the different molecular mechanisms driving DN is crucial to identify new therapeutic targets and potential biomarkers.[9]

## PATHOBIOLOGY AND EPIGENETICS OF DIABETIC NEPHROPATHY

DN is a lethal microvascular complication associated with T1DM and T2DM and is the leading single cause of ESRD. DN is defined by a progressive increase in the urinary albumin excretion rate accompanied with an increased blood pressure and decline in GFR, with end-stage renal failure as the final endpoint. DN affects one-third of patients with T1DM and rarely develops before 10 years of diabetes duration, whereas in T2DM the prevalence ranges from 25% in patients younger than 65 years old to almost 50% in individuals older than 65 years.[10] The first clinical sign of DN is an increased urinary albumin excretion rate in the range of ≥20 to <200 µg/min, or ≥30 to <300 mg/24 hours, termed microalbuminuria. Overt DN or macroalbuminuria is classified if the albumin excretion rate is ≥200 µg/min or ≥300 mg/24 hours, and is typically followed by a decline in kidney function, renal impairment, and ultimately ESRD (Table 1).

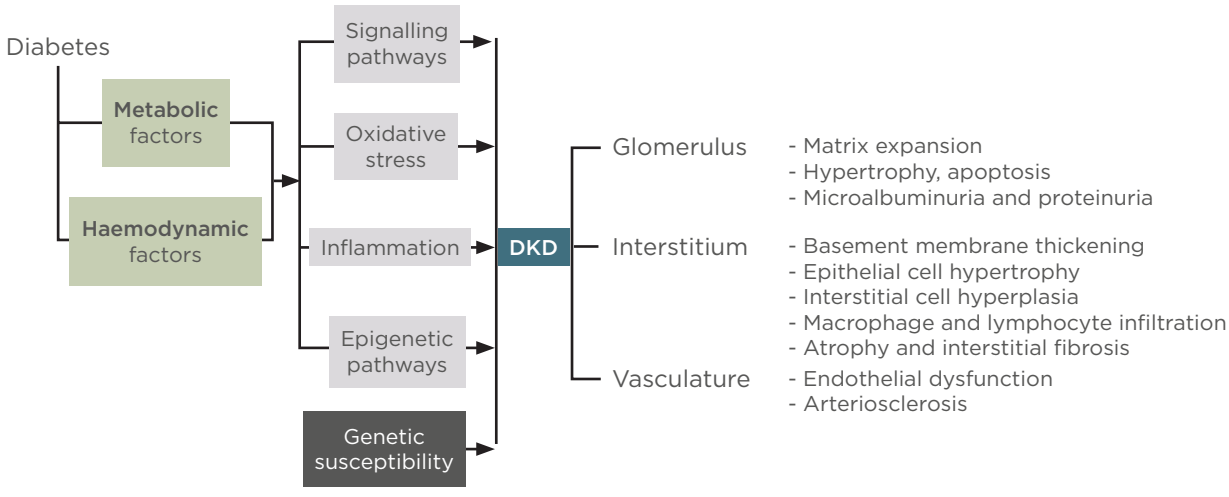


**Figure 1: Pathobiology of diabetic nephropathy.**

**DKD: diabetic kidney disease**

**Table 1: Classification of diabetic nephropathy.**

| | Designation | Characteristics | GFR (mL/min/1.73 m²) | Albumin excretion | Chronology |
|---|---|---|---|---|---|
| **Stage 1** | Hyperfunction and hyperfiltration | Glomerular hyperfiltration | ≥90 | <30 | Present at time of diagnosis |
| **Stage 2** | Silent stage | Thickened basement membrane, hypertrophy of mesangium | 60–89 | <30–300 | First 5 years |
| **Stage 3** | Incipient stage | Microalbuminuria | 30–59 | 30–300 | 10–15 years |
| **Stage 4** | Overt diabetic nephropathy | Macroalbuminuria | 15–29 | >300 | 15–25 years |
| **Stage 5** | Uraemic | End-stage renal disease/kidney failure | <15 | >1,000 | 25–30 years |

*Table adapted from Haneda et al.*[11]

**GFR: glomerular filtration rate.**

Chronic DN is the most common cause of renal replacement therapy in Western society.[12]

It is characterised by a decline in renal function, measured directly by serum creatinine, calculated creatinine clearance, or GFR. Renal function is classified based on GFR: normal renal function is considered as GFR ≥90, mild decrease in renal function is 60–89, moderate decrease in renal function is 30–59, severe decrease in renal function is 15–29, and renal failure <15 mL/min/1.73 m².[13]

DN is associated with abnormalities in renal cell types including tubular and glomerular cells. Morphological changes occur in the glomeruli in individuals with DN. Among these changes, dysfunction of glomerular podocytes is critical for the subsequent development of glomerulosclerosis and nephron dropout.[14]

With the expectation of somatic mutation events, an individual's DNA sequence is identical across different cell types; however, each has its own unique phenotype attributed to changes in gene expression. Epigenetics refers to the covalent modification of DNA and sequence-specific targeting of mRNA to control a cell's phenotype via changes in gene expression. Epigenetic DNA modifications are heritable, reversible, and do not change the DNA sequence, but rather alter DNA structure and stability, replication, and transcription. The true genome-wide assessment of epigenetic modifications utilising next-generation technology is referred to as epigenomics.

## Epigenetic Model for Human Disease or the Epigenetic State

The mechanism by which the epigenetic state of a normal cell is established can be defined and divided into three broad stages or mechanistic signals.[15] The epigenetic state involves the interplay of an epigenator, which can be an environmental cue or trigger for the cell that precedes any modification to that cell's epigenome. Next, an epigenetic initiator, which includes DNA-binding proteins and noncoding RNA, translates and coordinates specific responses conferred by the epigenator. The epigenetic initiator may not dissipate after its action, but rather persists with the maintainer. Lastly, the epigenetic maintainer, i.e., persistent marks such as DNA methylation and post-translational histone modifications, enable influence on structure and function of the genome, including when and where genes are transcriptionally activated or deactivated.

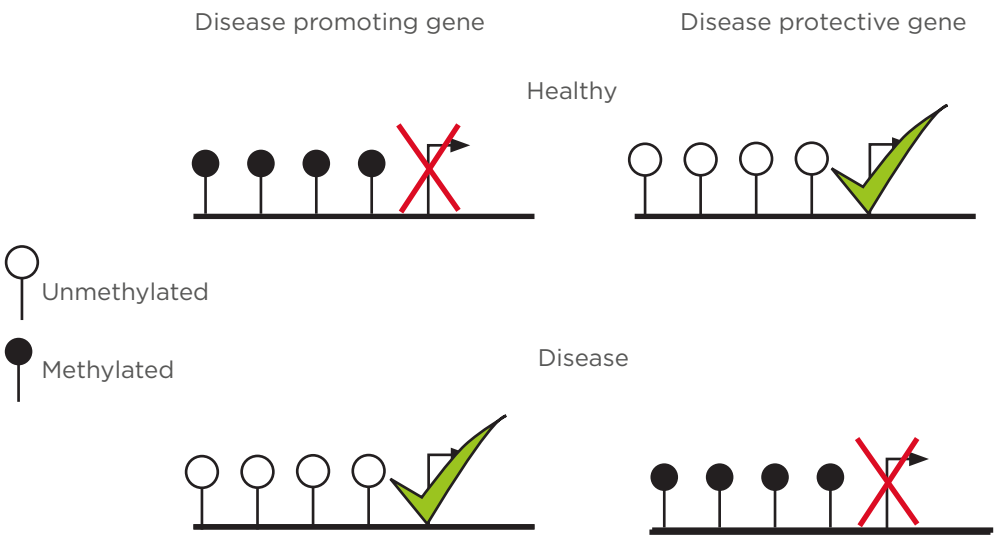


**Figure 2: DNA methylation at disease promoting and protective genes.**

Depiction of gene regulation mediated by DNA methylation at gene promoters.

Maintainers may function by carrying an epigenator signal through the cell cycle or could maintain epigenetic landscapes in terminally differentiated cell types.[15]

Aberrations at any stage of this system can potentially lead to subtle phenotype variations and molecular variations associated with a diverse range of human pathologies, including cancer, cardiovascular disease, and diabetes.[16]

The best characterised maintainer of the epigenetic state is DNA methylation. Considered a stable epigenetic mark, DNA methylation regulates gene expression and genome organisation and is transmissible from cell to cell, and in some instances is hereditary.[17] In mammals, it involves the covalent addition of a methyl group to cytosine-phosphate-guanine (CpG) dinucleotides from the methyl donor S-adenosylmethionine and catalysis by DNA methyltransferases. The regions with higher CpG density, termed CpG islands, are often found around gene promoters and are usually methylated in a tissue-specific manner.[18] Generally, a low methylation status of CpG islands at promoter sites is associated with gene activation, while transcription is repressed under a high DNA methylation status.[19] Gene regulation is critically important for normal functioning of the genome. To this end, even genes that carry no mutations or disease-predisposing polymorphisms can be considered harmful if they are not expressed at the appropriate level in the correct type of cell at the right time. Aberrant DNA methylation patterns in key regulatory elements of the gene, such as promoters and enhancers, regulate gene expression.[19] Classically, increased levels of gene methylation result in transcriptional repression[21] and changes in DNA methylation have been found in human diseases, including allergies,[22] cancer,[23] T1DM,[24] metabolic diseases,[25] and cardiovascular diseases.[26] Several models have been proposed for the molecular mechanisms of DNA methylation in gene regulation, in which one model proposes that aberrant DNA methylation at promoter regions can alter transcription of disease promoting and protective genes coordinated by methyl-CpG-binding domain proteins and transcription factor binding (Figure 2).

## DNA Methylation and Type 1 Diabetes Mellitus

In the last decade, experimental evidence has exemplified the importance of DNA methylation as a key mechanism by which the environment influences and interacts with genetic factors in the development of T1DM. For example, prospective analysis was performed on a cohort of nondiabetic monozygotic twins of patients with T1DM.[27] In this analysis, a median discordance time of 4.2 years was observed in the 47 twins (25%) who had become concordant

on follow-up.[27] These results imply that genetic and nongenetic factors could contribute to the development of T1DM. Several studies in twin-pairs have measured changes in DNA methylation at CpG sites in immune effector cells, including monocytes and peripheral lymphocytes, as well as whole blood samples with an aim of identifying nongenetic and underlying epigenetic mechanisms influencing the development of T1DM. Rakyan et al.[28] identified 132 differentially methylated CpG sites in T1DM-affected co-twins, of which 74 were hypomethylated and 58 were hypermethylated genes including *GAD2* and *HLA-DQB1*, previously described in T1DM. Interestingly, T1DM-associated methylation was also detected in islet autoantibody genes *GAD65* and *IA-2*. A similar study of monozygotic twins by Stefan et al.[29] assessed changes in DNA methylation in DNA isolated from lymphoblast cell lines from three pairs of monozygotic twins discordant for T1DM and six pairs of monozygotic twins concordant for T1DM. They identified 88 CpG sites with significant changes in DNA methylation between all T1DM-discordant monozygotic twin pairs.[29] Functional analysis suggested that differentially methylated genes were clustered in the immune response and defence response pathways.

A more recent twin study, using the Infinium® HumanMethylation450 BeadChip (Illumina, San Diego, California, USA), profiled changes in whole blood DNA methylation in twin pairs discordant for T1DM.[30] The authors reported modest DNA methylation differences (range: 2.2–5.0%) for the major histocompatibility complex region and T1DM-associated CpG sites in *BACH2*, *INS-IGF2*, and *CLEC16A*. Other genes reported to have differential DNA methylation were *MAGI2*, *FANCC*, and *PCDHB16*. These findings are indicative of global DNA hypomethylation within gene promoter regions which may contribute to T1DM; however, the results do not show large DNA methylation differences at CpG sites between T1DM-affected and unaffected twins. More recently, a more comprehensive epigenome-wide association study across 450,000 CpG in 52 monozygotic twin pairs discordant for T1DM in three immune effector cell types showed substantial enrichment of differentially variable CpG positions (DVP) in T1DM twins when compared with their healthy co-twins and with healthy, unrelated individuals.[31] A total of 10,548 DVP were identified in B cells, 4,314 in T cells, and 6,508 in monocytes. DNA methylation differences between the T1DM twin and their healthy co-twin were found to be comparatively large in many cases.[31] Functional annotation and integration with cell type-specific gene regulatory circuits highlight pathways involved in immune cell metabolism and the cell cycle, including mTOR signalling. Interestingly, in the same study T1DM-associated DVP identified in T1DM-twins were measured in the cord blood of newborns who progressed to overt T1DM. This comparison revealed no statistically significant overlap between cord blood and T1DM-twins, but the results suggest that changes in DNA methylation likely emerge after birth.

DNA methylation patterns have also been suggested to be affected by hyperglycaemia. In a study using zebrafish as a model of hyperglycaemic memory, the authors demonstrated DNA hypomethylation was heritable.[32] Hyperglycaemia was induced in adult zebrafish before entering a recovery phase. However, hyperglycaemia-induced global DNA hypomethylation was seen in the daughter cell tissue that did not have prior exposure to hyperglycaemia.

In patient populations, the large-scale prospective Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) and follow up studies[33,34] have signified that the establishment of tight glycaemic control is critical for long-term control of T1DM diabetes. The findings also emphasise that poor glycaemic control, even when followed by intensive therapy, can mediate sub-inflammatory conditions and vascular complications. Recently, DNA methylation was studied at specific loci over two different time points in individuals from the DCCT/EDIC T1DM cohort.[35] Chen et al.[36] measured DNA methylation of approximately 450,000 CpG sites in genomic DNA of whole blood isolated at EDIC Study baseline from 32 cases (DCCT conventional therapy group subjects showing retinopathy or albuminuria progression by EDIC Study Year 10) versus 31 controls (DCCT intensive therapy group subjects without complication progression by EDIC Study Year 10). DNA methylation was also profiled in blood monocytes of the same patients obtained during EDIC Study Years 16–17. Comparing DNA methylation

profiles of whole blood from the cases versus control, 53 regions showed hypomethylation and 225 hypermethylation, whereas in monocytes, 155 regions were hypomethylated and 247 hypermethylated. Notably, only 12 differentially methylated regions were common to both cell populations, including thioredoxin-interacting protein, known to be associated with hyperglycaemia and related complications.[35]

The authors also found a set of differentially methylated regions represented similar trends of associations with prior HbA1c in both whole blood monocytes. Follow-up experiments in monocytes showed that high glucose induced similar persistent region-specific hypomethylation at the thioredoxin-interacting protein region and this was inversely correlated with gene expression. This study provides evidence that hyperglycaemia can mediate changes in DNA methylation which persist for several years. In addition to the work described, the authors have also shown that hyperglycaemia regulates genome-wide DNA methylation signatures in primary vascular cells.[36] These studies highlight the importance of glucose exposure in DNA methylation and that consideration should be made while analysing methylation data from patients with diabetes.

## DNA Methylation and Diabetic Microvascular Complications

T1DM is associated with multiple macrovascular and microvascular complications leading to increased morbidity and mortality. While studies have shown that DNA methylation is associated with the pathogenesis of diabetes, there is emerging evidence that it also may contribute to the development of diabetic complications in peripheral organs such as the kidneys, retina, and peripheral nerves.

Increasing evidence suggests that changes in DNA methylation are involved in DN.[37] Epigenetic mechanisms have been proposed by which protection occurs in some individuals with diabetes, or through which some individuals with diabetes seem predisposed to progressive chronic kidney disease. One clinical study showed that whole blood genomic DNA from T1DM patients with DN exhibited differential DNA methylation patterns at 19 genes including *UNC13B*, relative to those without nephropathy.[38] A large case-control association study undertaken in T1DM individuals with or without DN employing the 450,000 and 27,000 methylation arrays identified 54 differentially methylated probes across 51 unique genes in blood-derived DNA. A sub-analysis, assessing DNA methylation in individuals with ESRD (versus without DN) revealed the detection of 755 differentially methylated probes in 374 genes. Of interest, 43 of the top-ranked genes for DN were also identified in the subgroup of patients with ESRD.[39] Pathway analysis of top-ranked genes revealed an association with metabolic pathways and mitochondrial function implicated in DN. DNA methylation profiles in proximal tubules obtained from db/db mice uncovered differentially methylated gene targets implicated in glucose metabolism and transport, leading to a resistance to the effects of pioglitazone.[40]

## CONTROL OF CELL IDENTITY IN KIDNEY DEVELOPMENT AND THERAPEUTICS

The adult mammalian kidney cannot sufficiently regenerate or replace damaged kidney tissue with new nephrons after injury.[41] Given the drastic shortage of donor kidneys for transplantation, this calls for urgent development of novel regenerative therapies to reverse the damage caused by T1DM on the kidney.

The latest discoveries in the fields of developmental nephrology hold great promise for kidney regenerative medicine, enabling researchers to design novel therapeutic tools and approaches to regenerate nephrons for DN. To advance kidney therapeutics further, it is mandatory to gain a deeper understanding of the key cellular and molecular programmes involved in nephrogenesis and kidney regeneration. All cells in the body arise from embryonic precursors through the coordinated activity of trans-acting transcriptional regulators and cis-acting modifications in DNA. These transcription factors act at multiple stages of kidney development, and adult kidney function or repair. Many of these transitions governing cell identity involve changes in gene expression. This in turn is regulated by epigenetic processes, including DNA methylation and histone modifications.

However, the therapeutic targeting of DNA methylation and transcriptional control in DN

are understudied; therefore, a focus on histone modification has become the preferred option.

## HISTONE MODIFICATIONS AS A THERAPEUTIC MODALITY IN DIABETIC NEPHROPATHY

The human kidney has a complex internal organ system and is composed of highly diverse cell types, including epithelial, stromal, and endothelial cells.[42] All of these must be assembled into discrete anatomic and functional structures at the earliest embryonic stages. The human kidney arises from an embryonic structure known as the metanephros, the last of the three excretory organs (pronephros, mesonephros, and metanephros) to develop from the intermediate mesoderm around embryonic Day 10.5, namely the metanephric mesenchyme (MM) and the ureteric bud (UB).[43] On entering the metanephric mesenchyme, cell interactions between the UB and adjacent mesenchyme drive the assembly of the functional kidney. At the same time, the UB induces the condensation of MM cells to form cap mesenchyme. Cap MM cells contain the progenitors/stem cells of the nephrons identified by their expression of *Six2*.[43] While the UB gives rise to renal collecting ducts, the condensed cap mesenchyme gives rise to a population of stem/progenitor cells that undergo mesenchymal–epithelial transition originating nephrons.[43] At 34 weeks of gestation in humans, nephron progenitors cease propagation and are terminally differentiated with no cell renewal/replication capability, and thus no nephron formation occurs in the adult kidney, underlying the irreversible nature of DN.[44] Histone modifications are closely linked to nephron differentiation. Nephron progenitors feature equally enriched active and repressive marks (*H3K4me3*, *H3K9me3*, and *H3K27me*).[45]

Over a decade ago, the seminal work by Takahashi and Yamanaka[46] showed that ectopic expression of key transcription factors Oct4, Sox2, Klf4, and c-Myc (OSKM cocktail) could reprogram differentiated cells to pluripotency.[46] Induced pluripotency is a process characterised by gradual changes in the epigenetic landscape.[47] Understanding how the reprogramming factors alter the cell epigenome to reset cell identity represents an important aim for the regenerative medicine field. Successful reprogramming to induce pluripotency is largely dependent on faithful remodelling of the cell's epigenetic states to silence gene expression and activate the transcriptional machinery characteristic of pluripotent cells. Trans-differentiation, also known as lineage reprogramming, is a process in which one somatic cell transforms into another mature somatic cell bypassing the pluripotent state. A number of mammalian programming strategies have recently been described, i.e., Al-Hasani et al.[48] have demonstrated that the paired box (Pax) 4 protein as well as γ-aminobutyric acid convert adult α-cells (glucagon) in pancreatic islets into functional β-like insulin producing cells *in vivo*.[49] Epigenetic modification with the DNA methyltransferase inhibitor 5-aza-2'-deoxycitidine or the histone deacetylase (HDAC) inhibitor trichostatin A was shown to contribute directly to reprogramming of nonosteoblasts into functional osteoblasts.[50,51] Cardiomyocytes were also reprogrammed epigenetically using a combination of epigenetic drugs.[52] Fibroblasts were able to convert into cardiomyocytes using cardiac-specific transcription factors (Gata4, Mef2c, and Tbx5) and epigenetic remodelling proteins.[53-55] Several recent reports have demonstrated the differentiation of human pluripotent stem cells into populations of nephron progenitor cells, specifically cells of the intermediate mesoderm and the metanephric mesenchyme using the transcription factors Osr1, Pax2, and Lhx1.[56] Recently, Hendry et al.[57] provided the first evidence of direct transcriptional reprogramming in the kidney, whereby human proximal tubule-derived renal epithelial cells (HK2) were reprogrammed back to an embryonic nonprogenitor-like state through forced expression of six factors: Six1, Six2, Osr1, Eya1, Hoxa11, and Snai2. A more efficient approach was recently developed by Vanslambrouck et al.[58] using a novel inducible piggyBac transposon system, harbouring three reprogramming factors (Six1, Eya1, and Snai2), to induce reprogramming of adult kidney cells to nephron progenitor-like cells that possess differentiation capacity. Another study by Papadimou et al.[59] converted human bone marrow stromal cells into renal tubule-like cells using cell-free extracts, and were shown to improve renal function in mice following kidney injury. More recently, another group described successful reprogramming of mouse and human fibroblasts into renal tubular epithelial-like

cells utilising four transcription factors: Emx2, Hnf1b, Hnf4a, and Pax8.[60] These induced renal epithelial cells were shown to take up albumin by endocytosis. The administration of mesenchymal stem cells (MSC) may also constitute a future form of treatment for DN. In a streptozotocin (STZ)-mouse diabetic model, the administration of MSC improved renal function in a Type 1 DN rat model as well as podocyte damage.[61,62] Numerous genetic and epigenetic factors regulating kidney morphogenesis, differentiation, and maturation have been identified through decades of progress in developmental nephrology. Although nephrogenic transcription factors have been extensively studied, the mechanisms by which chromatin remodellers modulate activation or repression of transcriptional networks are not well understood. Given that the kidney has limited regeneration capacity, further investigation will be required to elucidate the roles of kidney epigenetic factors for a better understanding of the process of nephrogenesis, as well as directed differentiation or reprogramming. Several studies have also reported the use of HDAC inhibitors in rats and mice with diabetes, showing them to have renoprotective benefits. In one study, investigators reported that the broad-spectrum HDAC inhibitor trichostatin A attenuated the upregulation of both smooth muscle actin and fibronectin, and downregulation of E-cadherin.[63] In another study, vorinostat was tested in STZ-diabetic rats and was found to decrease tubule cell proliferation, glomerular hypertrophy, and renal enlargement.[64] Another major class of HDAC inhibitor, valproate, when injected into STZ-diabetic rats, attenuated renal fibrosis and tubule cell injury.[65] Kidney organoid technology combined with CRISPR/Cas9 is providing a novel experimental platform for mechanistic studies of kidney gene function at an epigenetic level. Liu et al.[66] have adapted the CRISPR/Cas9 gene-editing technology to edit DNA methylation, correlating specific modifications with chronic kidney disease. In summary, genetic factors as well as epigenetic factors play a significant role in DN.[67-70]

## CONCLUSION

This review highlights the regulatory effect that epigenetic modifications exert in DN. Defining epigenetic signatures through the stages of kidney disease could provide novel strategies to develop cutting edge therapeutic interventions for curing the disease. It is precisely these epigenetic marks that are the obstacles for cell fate conversion. Major challenges include overcoming these epigenetic hurdles which will only be resolved once a greater understanding of the epigenome is achieved. Given that the mammalian kidney has very limited regenerative capacity, direct reprogramming together with HDAC inhibitors have emerged as promising approaches for ameliorating the disease state.

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# Managing Heart Disease in Pregnancy

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## Abstract

Cardiac disease is an important cause of mortality in pregnancy. It has the potential to remain undiagnosed and may present with cardiovascular decompensation during pregnancy, at the time of delivery, or immediately postpartum. It can have long-term implications to the life of the affected women and their families. This review summarises the current knowledge of the incidence, prevalence, and management of pregnancy-related cardiovascular disease in women presenting preconceptionally or during pregnancy.

## INTRODUCTION

Cardiovascular diseases complicate approximately 0.2–4.0% of pregnancies.[1] Their incidence is increasing, and they are already the most common cause of maternal death in the UK.[2] These patients are at higher risk of mortality as well as increased morbidity and thus need special care during the period of pregnancy.

## PREGNANCY CHANGES IN MAJOR BODY ORGAN-SYSTEMS

Several changes during pregnancy in a normal patient can occur:

- Central nervous system changes: decreases in minimum alveolar concentration[3] and local anaesthetic requirement during neuraxial blocks.[4]
- Respiratory system changes: development of upper airway oedema, decreases in functional residual capacity, and increases in ventilation.[5]
- Cardiovascular system changes: increases in blood volume, cardiac output (CO), and development of supine hypotension syndrome.[6,7]
- Gastrointestinal system changes: altered gastric emptying, increased gastrin secretion, and increased likelihood of gastroesophageal reflux.[8]
- Renal and hepatic changes: increased glomerular filtration rate and raised levels of liver enzymes.[9]

These changes are usually well-tolerated in normal patients; however, in patients with pre-existing disorders of body systems these changes can cause acute decompensation of disease status and can be catastrophic.

In this review the management of pregnant patients with cardiac disease is discussed, beginning with the discussion of the cardiovascular system changes in normal pregnancy followed by management of specific cardiac diseases during pregnancy.

## LITERATURE SEARCH AND SELECTION

For the literature search, an electronic search in Google Scholar, PubMed, and Cochrane Databases for original and review articles on cardiac disease in pregnancy until March 2019 was performed. Only full text articles were included.

## CARDIOVASCULAR CHANGES IN NORMAL PREGNANCY

The changes in the cardiovascular system evolve as a pregnancy advances.

### Changes in the Left Ventricle

The left ventricular (LV) mass increases during pregnancy.[10] The LV diastolic function increases during the first two trimesters and falls in the third trimester.[10] Pre-eclampsia patients and patients with multiple gestation show a greater increase in LV mass.[11,12]

### Changes in Cardiac Output

Increased levels of the hormone progesterone cause peripheral vasodilation leading to a decrease in systemic vascular resistance. As a response to decreased systemic vascular resistance, CO increases progressively as the duration of pregnancy increases. Although an increase in both heart rate and stroke volume contribute to increased CO, the increase is predominantly a result of augmented stroke volume.[6] By 8 weeks, CO has increased by 20% and then up to 50% by 20 weeks.[13] The CO further increases by 15% during the first stage of labour.[14,15] After delivery of the baby, autotransfusion of approximately 300–500 mL of blood from uterine circulation to maternal circulation occurs. This can lead to an increase in CO of up to 60–80% during the second stage of labour.[16] In case of twin pregnancies, there is an additional 10-20% increase in CO.[17]

### Changes in Blood Pressure

Systolic, diastolic, and mean blood pressure decrease during mid-pregnancy and return toward baseline as the pregnancy approaches term.[18] The mean (±2 standard deviations) systolic blood pressure and diastolic blood pressure in women who have never given birth at 12 weeks have been described to be 112.1 mmHg (88.6–135.5 mmHg) and 65.4 mmHg (48.9–81.9 mmHg), respectively.[19] It has been suggested that a raised mid-trimester mean arterial BP is predictive of subsequent development of pregnancy-induced hypertension/pre-eclampsia.[20]

### Total Blood Volume Changes

Pregnancy is also characterised by an increase in total blood volume. This increase begins as early as 6 weeks of pregnancy and is rapid during the first half of pregnancy, after which the increase in blood volume progresses at a slower rate.[21,22] There is also an increase in red blood cell mass during pregnancy; however, this increase is to a lesser extent compared with the increase in blood volume. Hence, there is haemodilution and resultant development of 'physiological anaemia of pregnancy'.

### ECG Changes

ECG changes during pregnancy consist of nonspecific ST-segment and T-wave changes. Repolarisation abnormalities are absent in normal pregnancies. LV mass increases during normal pregnancy.[10]

## ASSESSMENT OF PREGNANCY RISK

All female cardiac patients should undergo a preconception counselling, which should include a detailed discussion of the risk of pregnancy. There are numerous important issues to be considered during this counselling:

- Evaluation of present cardiac status using history, clinical exam, and relevant investigations.
- Optimisation of cardiac status.
- Changes in medication regimen, e.g., replacing teratogenic drugs with nonteratogenic drugs.

- Discussion about maternal life expectancy and long-term effects of pregnancy on their heart.
- Genetic screening in patients with inherited disorders.

## CARPREG RISK SCORE

Siu et al.[23] had proposed the Cardiac Disease in Pregnancy (CARPREG) Risk Score to estimate a woman's cardiac risk during pregnancy. During the calculation of the total score, one point is assigned for each of the four risk factors:

1. A history of cardiac event or arrhythmia,

2. New York Heart Association (NYHA) functional class greater than II or cyanosis,

3. left-heart obstruction (mitral valve area <2.0 $cm^2$, aortic valve area <1.5 $cm^2$, or LV outflow tract gradient >30.0 mmHg), and

4. LV ejection fraction (LVEF) <0.40.

A score of 0 points confers a 5% risk of cardiac complications, whereas scores of 1 or 2 points denote a 27% and 75% risk, respectively. Post CARPREG, many such risk stratification models have been suggested by other bodies, prominent among them being World Health Organization (WHO) classification, ZAHARA risk score, European Society of Cardiology (ESC) guidelines, plus more. To determine the risk of pregnancy in cardiac patients, WHO has classified patients into four pregnancy risk classes (Classes I–IV) as determined by their medical condition (Box 1).

## MANAGEMENT OF CONGENITAL HEART DISEASES

### Atrial Septal Defect

Patients with unrepaired atrial septal defect (ASD) should have their right ventricular function assessed. It has been suggested that pregnant women with an ASD are more likely to develop supraventricular and ventricular arrhythmias than nonpregnant women.[24] In patients with unrepaired ASD the risk of paradoxical air embolism is present. Patients with repaired ASD can be managed as normal patients; however, ASD (both repaired or unrepaired) patients with pulmonary arterial pressure >40 mmHg are considered as high-risk patients and preload should be maintained.

### Ventricular Septal Defects

Pregnancy is usually well-tolerated in patients with ventricular septal defects. The only risk from a small haemodynamically insignificant ventricular septal defect is of endocarditis, and antibiotics should be administered at the time of instrumental or complicated deliveries. However, patients with raised pulmonary arterial pressures (>40 mmHg) are considered high risk. Additionally, women with either unoperated ventricular septal defects or with late repair may have associated pulmonary vascular disease.

### Patent Ductus Arteriosus

Usually pregnancy is well-tolerated in patent ductus arteriosus patients with complications being rare.[25] However, in patients who have developed reversal of shunt (Eisenmenger syndrome), pregnancy is not recommended because of the risk of death reaching as high as 40–50%.[26]

### Transposition of the Great Arteries

There are two types of transposition of the great arteries (TGA): D-TGA, in which only origins of aorta and pulmonary trunk are transposed; and L-TGA, in which the morphological left and right ventricles with their corresponding atrioventricular valves are also transposed, in addition to aorta and pulmonary trunk. D-TGA patients undergo either arterial (Jatene or Rastelli) or atrial switch (Senning or Mustard) operations. The patients who have undergone arterial switch operation are predisposed to develop myocardial ischaemia (because the coronary arteries are reimplanted during these procedures), while patients with atrial switch can be afflicted by pulmonary hypertension, atrial arrhythmias (as a result of atrial scarring), tricuspid regurgitation, plus more.[27] In these patients, a cardiac evaluation and ECG/MRI are recommended prior to planned pregnancy. Pregnancy is usually well-tolerated in NYHA Class I–II patients post-Mustard procedure.[28] Patients with L-TGA usually have uneventful pregnancies.

### Ebstein's Anomaly

Ebstein's anomaly is a congenital heart defect in which the septal and posterior leaflets of the tricuspid valve are displaced towards the apex of the right ventricle of the heart.

**Box 1: Modified World Health Organization (WHO) classification of maternal cardiovascular risk.**

| | |
|---|---|
| Class I<br><br>No detectable increased risk of maternal mortality and no, or mild, increased risk of morbidity. | 1. Uncomplicated small or mild pulmonary stenosis, patent ductus arteriosus, mitral valve prolapse.<br>2. Successfully repaired simple lesions (ASD, VSD, PDA, anomalous pulmonary venous drainage).<br>3. Isolated atrial or ventricular ectopic beats. |
| Class II<br><br>Small increased risk of maternal mortality or moderate increase in morbidity. | 1. Unoperated ASD or VSD.<br>2. Repaired tetralogy of Fallot.<br>3. Most arrhythmias. |
| Class II–III<br><br>Significantly increased risk of maternal mortality or severe morbidity. Expert counselling required.<br><br>If pregnancy is decided upon, intensive specialist cardiac and obstetric monitoring needed throughout pregnancy, childbirth, and the puerperium. | 1. Mild left ventricular impairment.<br>2. Hypertrophic cardiomyopathy.<br>3. Native or tissue valvular heart disease not considered WHO I or IV.<br>4. Marfan syndrome without aortic dilatation or aorta <45 mm in aortic disease associated with bicuspid aortic valve.<br>5. Repaired coarctation. |
| Class III<br><br>Significantly increased risk of maternal mortality or severe morbidity. Expert counselling required.<br><br>If pregnancy is decided upon, intensive specialist cardiac and obstetric monitoring needed throughout pregnancy, childbirth, and the puerperium. | 1. Mechanical valve.<br>2. Systemic right ventricle.<br>3. Fontan circulation.<br>4. Cyanotic heart disease (unrepaired).<br>5. Other complex congenital heart disease.<br>6. Aortic dilatation 40–45 mm in Marfan syndrome.<br>7. Aortic dilatation 45–50 mm in aortic disease associated with bicuspid aortic valve. |
| Class IV<br><br>(Pregnancy contraindicated)<br><br>Extremely high risk of maternal mortality or severe morbidity; pregnancy contraindicated.<br><br>If pregnancy occurs termination should be discussed. If pregnancy continues, care as for Class III. | 1. Pulmonary artery hypertension of any cause.<br>2. Severe systemic ventricular dysfunction (LVEF <30%, NYHA III–IV).<br>3. Previous peripartum cardiomyopathy with any residual impairment of left ventricular function.<br>4. Severe mitral stenosis, severe symptomatic aortic stenosis.<br>5. Marfan syndrome with aorta dilated >45 mm.<br>6. Aortic dilatation >50 mm in aortic disease associated with bicuspid aortic valve.<br>7. Native severe coarctation. |

ASD: atrial septal defect; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; PDA: patent ductus arteriosus; VSD: ventricular septal defect; WHO: World Health Organization.

Patients with preserved ventricular function tolerate pregnancy well, whereas those with associated ASD and cyanosis have an increased risk for fetal loss.[29] Additionally, arrhythmias are very common in these patients. Severe Ebstein's anomaly should be repaired prior to pregnancy.

## Tetralogy of Fallot

It is rare to have tetralogy of Fallot patients survive to adulthood without corrective surgery. Women with repaired tetralogy of Fallot and well compensated haemodynamic function tolerate pregnancy well, though they remain at risk for atrial and ventricular arrhythmias. However, the presence of pulmonary hypertension, right ventricular dysfunction, right ventricular dilation, and pulmonic regurgitation predisposes these patients to adverse peripartum complications such as arrhythmias and right-sided heart failure.

## Fontan Circulation

In Fontan surgery, the right ventricle is bypassed and venous blood flows directly from vena cavae to pulmonary arteries. Fontan operation is performed for tricuspid or pulmonic atresia, as well as other anomalies with a single ventricle. These patients are predisposed to the development of thrombus formation (due to slow flow of venous blood) and arrhythmias (as a result of surgical scar tissue in the atrium).[30,31]

## MANAGEMENT OF ACUTE CORONARY SYNDROME

Acute coronary syndrome (ACS) includes ST elevation myocardial infarction (STEMI), non-STEMI, and unstable angina. ACS during pregnancy is rare, with an incidence of 1 in 16,000 deliveries.[32] The classical presentation of ACS presenting as crushing chest pain with radiation to neck and arm is uncommon, and even in those patients who present with these classical features, often these symptoms are attributed to dyspepsia and changes of normal pregnancy. Hence diagnosis may be difficult.

STEMI should be a clinical diagnosis based on the presence of pain with typical ECG changes (either a 1 mm of ST elevation in contiguous leads corresponding to an arterial territory or new left bundle branch block). All patients with a history of pain that may be a result of cardiac ischaemia should have a prompt 12-lead ECG.

Diagnosing non-STEMI will be based on the presence of elevated cardiac troponin levels (or a documented rise and fall) in the setting of pain compatible with cardiac ischaemia. Unstable angina may present similarly to non-STEMI but without an elevated troponin level. Often these patients are still at high risk of future events or development of more extensive ACS if not managed appropriately.

In patients who are presenting with stable symptoms (symptoms or exertion), noninvasive investigations of cardiac ischaemia are the preferred management. Exercise testing is safe in pregnancy provided the patient is not having obstetric complications such as per vaginal bleeding or significant placenta praevia. The disadvantage of this test, however, is the high false-positive rate in the nonpregnant woman.

### In patients with STEMI

- Oxygen supplementation.
- Aspirin (300 mg) and clopidogrel (300 mg).
- Appropriate intervention in the form of coronary angiography, emergency coronary intervention, and thrombolysis should not be withheld in the pregnant or puerperal woman.[33] The first choice for treatment of ACS in pregnant women is percutaneous coronary intervention (balloon angioplasty with or without a stent).[34]

### In patients with Non-STEMI/Unstable Angina

Low-risk patients should be managed medically (aspirin, clopidogrel, low-molecular-weight heparin, other antianginal agents), while high-risk patients should preferably undergo angiography and, if needed, coronary stenting.

## MANAGEMENT OF VALVULAR HEART DISEASES

### Mitral Stenosis

Even patients with mild mitral stenosis can develop arrythmias and pulmonary oedema during pregnancy. When possible, preconception treatment of symptomatic moderate or severe mitral stenosis is preferred, percutaneous balloon mitral valvuloplasty being the procedure of choice.[35] For patients who require percutaneous valvuloplasty during pregnancy, the procedure is ideally performed after 12–14 weeks gestation in order to minimise fetal radiation exposure during the period of organogenesis. If the patient can be stabilised with medical management, delaying the procedure to 26–30 weeks gestation will help reduce the risk for preterm birth. Open surgical mitral valve commissurotomy is another treatment option but is associated with higher rates of fetal mortality than percutaneous valvuloplasty (38% versus 5%, respectively).

Medical management includes administration of β-blockers, aimed at slowing the heart rate and thereby lengthening the diastolic filling period. Atrial fibrillation and atrial flutter should be treated promptly with rate control, and early cardioversion should be considered. Furthermore, systemic anticoagulation is recommended for the duration of pregnancy and postpartum along with diuretics and bed rest.

### Mitral Regurgitation

Mitral regurgitation is usually well-tolerated during pregnancy, but ECG is recommended because chronic mitral regurgitation may be associated with LV dysfunction. If valve intervention is indicated in women of childbearing age who have

severe mitral regurgitation, valve repair should be offered when possible.

## Aortic Stenosis

Mild and moderate aortic stenosis are associated with favourable pregnancy outcomes.[36,37] Severe aortic stenosis patients are more predisposed to develop cardiac complications as well as the need for cardiac intervention. Balloon valvuloplasty is preferred if technically possible because it is reported to have a smaller risk of fetal loss, and even if the benefit is short lasting, it may be sufficient to allow successful completion of the pregnancy.

## Aortic Regurgitation

Chronic, moderate, or even severe aortic regurgitation is usually well-tolerated if LV function is preserved; nevertheless, women with severe aortic regurgitation are at a risk of developing pulmonary oedema and arrhythmias during pregnancy. Valve replacement during pregnancy for treatment of aortic regurgitation is rarely required.

## Pregnant Patients with Prosthetic Valves

These patients have increased chances of morbidity and mortality.[38] In the subset of 134 women with bioprosthetic valves in the Registry of Pregnancy and Cardiac Disease (ROPAC) study, heart failure complicated 8.2% of pregnancies in women with bioprosthetic valves, endocarditis and thrombotic complications in <1.0%, and haemorrhagic complications in 5.1% of pregnancies.[38] Prosthetic heart valves can either be bioprosthetic or mechanical; the former are often recommended in young females because of a lower risk of thromboembolism and anticoagulation. However, bioprosthetic valves tend to deteriorate early, predisposing to repeated surgeries.

In patients with mechanical prosthetic heart valves, the current American Heart Association (AHA)/American College of Cardiology (ACC) guidelines suggest continuing warfarin in the first trimester if the daily warfarin dose is ≤5 mg, after patient information and consent. In women whose daily warfarin dose is >5 mg, and for those who consent against taking warfarin in the first trimester, it may be discontinued between Weeks 6 and 12 and replaced with either weight-adjusted twice-daily low-molecular-weight heparin or an intravenous infusion of unfractionated heparin. Warfarin is restarted along with aspirin 75 mg in the second and third trimester until 36 weeks of gestation. Overall, pregnancy in women with mechanical prosthetic heart valves is high risk, and the safest option is not to become pregnant at all.

## Infective Endocarditis in Pregnancy

Infective endocarditis is a rare, potentially life-threatening complication. Maternal and fetal mortality rates are both high.[39] The AHA guidelines on the prevention of endocarditis and the ESC guidelines on the management of cardiovascular diseases during pregnancy do not recommend antibiotic prophylaxis at the time of delivery. If infective endocarditis is diagnosed, antibiotic treatment should be guided by blood culture results and antibiotic sensitivities.

## Cardiomyopathy

Cardiomyopathy is a congenital or acquired disease of heart muscle. Congenital cardiomyopathy can be inherited in an autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive, or mitochondrial manner. Various types of cardiomyopathies exist:

### Hypertrophic Cardiomyopathy

In hypertrophic cardiomyopathy, abnormal thickening of heart muscles occurs, rendering the heart muscle stiff and noncompliant. These patients are unable to sufficiently increase their stroke volume as a result of outflow obstruction in conjunction with the small stroke volume due to a small LV cavity size. Patients may be asymptomatic or present with palpitations, breathlessness, or arrhythmias.

Patients with hypertrophic cardiomyopathy are at risk from atrial and ventricular arrhythmia, pulmonary oedema, and increasing outflow tract obstruction. Atrial arrhythmia, especially atrial fibrillation from left atrial dilatation, is common and can cause thromboembolism. Normally, atria contribute around 10–30% of ventricular filling, but in hypertrophic cardiomyopathy patients it may increase to 50% of total ventricular filling. Hence restoring sinus rhythm promptly is imperative.

### Dilated Cardiomyopathy

Dilated cardiomyopathy can be primary or secondary to myocarditis, alcohol or other toxins, endocrine and autoimmune disorders, and nutritional factors. Breathlessness, fatigue, exercise intolerance, and fluid retention are common symptoms. Pregnancy in women with dilated cardiomyopathy is associated with adverse outcomes, especially in those with significantly impaired LV function (moderate or severe LV systolic dysfunction, EF <45% on ECG). Assessment of LV function and exercise tolerance is important in these patients preconceptionally. If the patient is being treated with heart failure medication known to have teratogenic effects, such as angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, they should be stopped prior to conception. If LV function then deteriorates even before pregnancy, the patient should be advised against pregnancy. Thereafter also, ECG should be performed at regular intervals to determine if LV function declines as a consequence of their withdrawal. β-blockers should be continued during pregnancy and postpartum. Other drugs used are diuretics (to manage fluid status) and nitrates or hydralazine (to reduce preload). Pregnancy may have to be terminated at any duration of gestation if LV function deteriorates.

### Peripartum Cardiomyopathy

The Working Group on Peripartum Cardiomyopathy (PPCM) of the ESC recently updated the operational definition of PPCM, defining PPCM as cardiomyopathy with reduced EF, usually <45%, presenting toward the end of pregnancy or in the months after delivery in a woman without previously known structural heart disease.[40] PPCM is associated with significant morbidity and mortality.[41] The exact aetiology of PPCM remains unknown; however, fetal microchimerism, increased cardiomyocyte apoptosis, hormonal insults, genetics, autoimmune inflammation and myocarditis with and without viral triggers, and a familial association have been proposed.[42] Most cases present in the first week following delivery.[43] Increasing age, multiple gestations, race, and pre-eclampsia have been found to be associated with development of PPCM.[44] ECG usually shows dilatation of LV, LV systolic dysfunction, right ventricular and biatrial enlargement, mitral and tricuspid regurgitation, and pulmonary hypertension.[44,45]

Digitalis is used to augment systolic function and is safe, although its role in the management of systolic heart failure has been questioned.[46] Anticoagulation may be added and continued postpartum if the LVEF remains <30% for prevention of thromboembolic complications. Angiotensin-converting enzyme inhibitors should be started as soon as possible after delivery, and are safe for breastfeeding. Bromocriptine, a dopamine receptor agonist and prolactin inhibitor, has showed improvements in LV recovery.[47] Cardiac assist devices and implantable cardiac defibrillators have been used in patients with severe depression of LV function and persistent arrhythmias.[48,49]

### Restrictive Cardiomyopathy

Restrictive cardiomyopathy can affect both sides of the heart and is characterised by small, stiff ventricles with abnormal relaxation. As a result, diastolic filling is impaired, which in turn causes small stroke volumes and a low CO, despite an often preserved systolic function.

## Pulmonary Hypertension

Pulmonary hypertension (PAH) is defined as an increase in mean pulmonary arterial pressure ≥25 mmHg at rest.[50] In patients with congenital heart disease, PAH most commonly occurs as a result of long-term left-to-right shunting, leading to increased pulmonary flow that eventually causes high PVR, resulting in reversed or bidirectional shunts, which is referred to as Eisenmenger syndrome.

Women with PAH should be strongly counselled against pregnancy at the time the diagnosis of PAH, and advice on appropriate contraception should be provided. Pulmonary vasodilators, such as iloprost, inhaled nitric oxide, endothelin receptor antagonists, and phosphodiesterase inhibitors, have been used in these patients and have improved outcomes to some extent.[51]

## Aortic Dissection

Aortic dissection is a particular risk in women with Marfan syndrome. β-blockers should be continued or started in pregnant patients with Marfan syndrome who have aortic dilatation or

hypertension because they have reduce the rate of aortic dilatation.[52] Monitoring during pregnancy will normally include regular (e.g., every 4–8 weeks) transthoracic ECG to assess aortic root diameter. The timing of delivery will be dependent on the root diameter and the rate of dilatation, as well as any other complicating factors.

## COMMON ARRYTHMIAS DURING PREGNANCY

Cardiovascular changes during pregnancy predispose the development of new onset arrhythmias as well as recurrence in patients with a history of arrhythmias.[53] Ectopic beats are said to be common during pregnancy and usually do not require any treatment.[54]

Patients without any history of heart disease usually present with atrioventricular nodal reentrant tachycardia.[55] Direct current cardioversion has been found to be safe in all stages of pregnancy; however, fetal monitoring is advised during cardioversion.[56] In haemodynamically stable patients, intravenous adenosine is commonly used for termination of atrioventricular node dependent supraventricular tachycardia. Catheter ablation therapy is generally contraindicated due to high radiation exposure. Patients presenting with symptomatic bradycardia and a heart rate <50 beats per minute are candidates for permanent pacemaker insertion.[57]

## RADIATION EXPOSURE DURING PREGNANCY

The effects of *in utero* radiation exposure include intrauterine growth retardation, childhood cancers, mental retardation, and fetal death. The gestational age, radiation dose, and repair mechanisms determine the final effect of the radiation on the fetus. A dose <0.05 Gy does not cause any malignancy related health issues.[58]

## CONCLUSION

The spectrum of cardiac disease has changed over time, with rheumatic disease becoming uncommon while congenital heart disease becomes more common. As newer and better treatment modalities for the management of cardiac diseases emerge, the number of cardiac disease patients becoming pregnant also continues to increase.

Pregnancy may predispose cardiac disease patients to many complications, some of which may be life threatening. While the risk may be so high in certain disease states that pregnancy should be altogether avoided, successful outcomes are possible in other cases provided close co-ordination and collaboration is maintained between all the members of the treating team.


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# Personalising Exercise and Nutrition Behaviours in Diabetes Lifestyle Prevention

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## Abstract

The alarming increase in global diabetes, especially Type 2 diabetes mellitus (T2DM), is affecting diverse populations and leading to consequent burdens of morbidities, mortalities, and healthcare costs. Physical activity and nutritional approaches form the cornerstones of lifestyle T2DM prevention. Advances in understanding an individual's behaviour and biological responses to different exercise conditions are concurrent with new personalised exercise and nutritional and behavioural tools effective for preventing T2DM and associated chronic diseases. Targeted exercise and nutrition interventions can be personalised across diverse population groups and different settings. Such approaches can benefit from evolving technologies embedding genomics, metabolomics, proteomics, and transcriptomics, together with behavioural reduction strategies such as addressing sedentary behaviour. This narrative review focusses on describing personalised lifestyle prevention approaches, which address different population needs and environmental settings. These methods can be better directed towards translating T2DM interventions and laboratory trials into sustainable, healthy behaviours, and help form personalised lifestyle T2DM prevention guidelines.

## INTRODUCTION

The prevalence of Type 2 diabetes mellitus (T2DM) is rising globally, in some regions more than others, and overall diabetes rates are expected to reach one in 10 adults within the next decade.[1,2] Societal and healthcare costs are some of the many burdens related to the increase in T2DM prevalence, which is also a risk factor of cardiovascular disease and premature mortality.[2] Major T2DM risk factors include obesity, sedentary lifestyle, physical inactivity, poor nutrition, older age, and family history of diabetes.[3]

Lifestyle prevention is the major preventive measure of T2DM, as evidenced by major diabetes prevention studies across the globe.[4-7] The risk reduction has been shown as an overall 58% decrease in incidences of those with prediabetes and impaired glucose tolerance across different population groups from the Diabetes Prevention Program (DPP) and the Finnish Diabetes Prevention Study (DPS).[4,5] It has also appeared lower (e.g., 28% in the Di Qing study) and higher (e.g., 70% in a subcohort of the Finnish DPS).[6-8] These lifestyle interventions have targeted high-risk individuals with various behavioural, multicomponent lifestyle prevention approaches

(e.g., structured and unstructured exercise, nutrition, education, and counselling). For example, an average of 2.8 years follow up after implementation of a lifestyle modification programme, involving increased physical activity and weight loss, produced a 58% reduction in the incidence of T2DM in those with prediabetes, compared to a 31% reduction after taking metformin (an insulin-sensitising drug).[5] Other dietary-only interventions such as the Mediterranean diet (MD) have retrospectively showed an association with reversing prediabetes into remission state after 5 years of adherence.[9-11]

Since the risks of diabetes and associated chronic diseases can be reversed by lifestyle interventions, it is important to personalise the intervention approaches that help form personalised behavioural guidelines instead of using a 'one size fits all' approach. Recent technological approaches (e.g., proteomics, metabolomics, genomics, and pharmacogenetics) have been publicised for personalising diabetes drug development.[12,13] However, personalising complex lifestyle approaches in T2DM prevention, especially physical activity and nutrition behaviours, has received little attention. This narrative review translates key debates about lifestyle behaviours into specific T2DM recommendations and discusses how the most recent behavioural approaches, including physical activity and exercise intensity, sedentary lifestyle, and nutrition, can be individualised when targeted for T2DM prevention.

## PERSONALISED EFFECTIVENESS OF LIFESTYLE INTERVENTION (EXERCISE AND NUTRITION) AND THE PREVENTION PHASE

Components of lifestyle include exercise, nutrition, and sleep, the first two of which have gained significant attention over the past three decades. Sleep research has also gathered recent research interest in T2DM prevention,[14] though is beyond the scope of this review. Although selecting components for a lifestyle intervention varies across different T2DM programmes (single components such as structured or unstructured exercise, nutrition, unstructured or unsupervised education and counselling, or multicomponents), the timing of the intervention determines its component effectiveness. T2DM interventions can be classified into two timings or phases: a late phase (e.g., targeting T2DM risks such as prediabetes and obesity) or an early phase targeting the whole population (e.g., targeting all risk factors). Interventions with the early-phase interventions target all risk factors and engage multilevel stakeholders and policy makers (e.g., health, education, and sport authorities and nutrition providers).[15] Late-phase interventions, which target sedentary, older, and postmenopausal individuals, and those with prediabetes with high cardiovascular disease (CVD) risk (elevated fasting blood glucose [FBG] and postprandial glucose [OGTT]), are likely to induce better effectiveness compared with those who are at reduced risk. Therefore, personalised preventive measures are most effective for those at high risk for T2DM in the late phase (Figure 1).

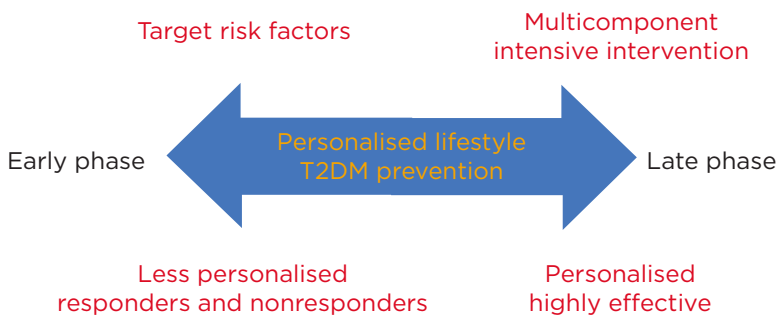


**Figure 1: Schematic of personalised Type 2 diabetes mellitus prevention based on the intervention phase.**

T2DM: Type 2 diabetes mellitus.

However, it is important to consider that early-phase targeting of T2DM risks can be irrespective of chronological age prior to developing T2DM, since T2DM is becoming increasingly prevalent among younger adults, teenagers, and even children.[16]

Evidence suggests that intraindividual variations in the response to a lifestyle prevention programme can vary by >20%. For example, variations in achieving physical activity T2DM prevention outcomes has been observed among a subset of older age groups with impaired glucose tolerance showing higher risk reduction (70%), compared with the average response (58%) following the same lifestyle prevention.[4,8] This implies that achieving a better physical activity response is more easily achieved when personalised according to the higher risk groups in the late phase (e.g., older age groups with prediabetes).

On the other hand, effectiveness of recommended nutrient intake can also be influenced by the phase of the intervention, especially because nutrient deficiency is now a common symptom in individuals with obesity and diabetes.[17,18] For those diagnosed with T2DM, preventing complications is an essential prevention approach which can be personalised based on their nutritional and lifestyle needs. Decreased levels of vitamins A, C, and E, which are associated with antioxidation, were observed in those with diabetes. This is possibly because of an increased need to control the excessive oxidative stress produced by abnormalities in glucose metabolism.[17] The B group vitamins thiamine (B1), pyridoxine (B6), and biotin (B7) are decreased in T2DM individuals.[18] Vitamin D and K modulatory effects on diabetes mechanisms have also been reported.[17] For example, vitamin D can improve insulin sensitivity and promote pancreatic β-cell survival by modulating the effects of cytokines and nuclear transcription factors such as NF-κB.[17] However, such T2DM protective mechanisms are not necessarily achieved by vitamin D supplementation. Supplementation of vitamin D longitudinally (4,000 IU/day for 24 months in 2,423 adults with obesity and prediabetes) did not reduce T2DM incidence, despite increasing serum levels.[19] Vitamin C and Vitamin E supplementation have been shown to be effective in the short-term,[20,21] while other micronutrients such as magnesium have also been suggested to enhance insulin sensitivity[22,23] with promising effectiveness that is most likely dependent on the severity of the deficiency (Table 1).[17,19,21-25]

Postulated antioxidation and anti-inflammatory effects of vitamins and minerals promote their benefits in the late-intervention phase for those with diagnosed T2DM with complications or those with multiple morbidities (Table 1).[17,19,21-25] However, the evidence on the supplementation effectiveness of vitamins, minerals, and other antioxidants for preventing T2DM remains inconclusive, especially in the absence of well-designed, longitudinal studies which combine supplementation with other functional diet and lifestyle approaches. Personalised T2DM prevention should consider the augmented effects of other lifestyle behaviours, especially physical activity strategies,[3] to determine the effectiveness of nutrient supplementation.

## PERSONALISED COMPONENT CONTRIBUTION WITHIN LIFESTYLE TYPE 2 DIABETES MELLITUS INTERVENTIONS

Current epidemiological evidence suggests that nutrition and physical activity guidelines are far from being upheld, with only 3% of Americans reported to have met T2DM risk-reduction guidelines.[26] Meta-analyses have reported that similar barriers to implementing and scaling T2DM guidelines at real-world primary and secondary settings also exist in the UK.[27] Implementing T2DM guidelines on an individual level is difficult, possibly attributable to multifaceted and confusing lifestyle recommendations, especially those related to physical activity and nutrition, together with a complex range of social, financial, behavioural, and organisational barriers.[28] Furthermore, the relative contribution and effectiveness of each lifestyle component within an intervention (exercise behaviours, nutrition, and education) is also difficult to quantify.

**Table 1: Common micronutrient supplementation, postulated effects, and recommendations in Type 2 diabetes mellitus.**

| Micronutrient | Metabolic function | Supplementation effectiveness | Related recommendation for T2DM prevention |
|---|---|---|---|
| Vitamin B group | Involved in the synthesis of methionine, pyrimidine, and purine bases. Its deficiency due to DNA damage or faulty repair is involved in cancer, vascular diseases, and some birth defects, while a consequent hyperhomocysteinemia is also related to folic acid deficiency. They are also a risk factor for hypertension and atherosclerosis.[17] | Folic acid, pyridoxine, and B12 or placebo produced no difference in T2DM incidence following 7-year supplementation in women with or without cardiovascular disease risk factors.[24] | Supplementation is not necessary. Dietary intake from dietary sources (e.g., green leafy vegetables) is recommended. |
| Vitamin C | Inversely correlated to glycosylated haemoglobin and fasting and postprandial blood glucose and oxidative stress.[17] Acts as a potent antioxidant, in collagen, neuropeptide, and carnitine synthesis, increasing iron absorption, inhibiting histamine release, and stimulating the immune system.[17,21] | Associated with antioxidative enzymes in T2DM patients.[21] 3-month supplementation of vitamins C and E decreased hypertension and blood glucose while increasing superoxide dismutase and glutathione levels.[22] | Supplementation when oxidative stress is high is useful, and in the short term in reducing cardiovascular disease risk, particularly hypertension in individuals with T2DM. |
| Vitamin D | Can improve insulin sensitivity and promote pancreatic β-cell survival by modulating the effects of cytokines and nuclear transcription factors such as NF-κB.[17] | A 24-month vitamin D supplementation (a dose of 4,000 IU/day) in 2,423 adults with obesity and prediabetes (criteria of: HbA1c 5.7–6.4%; FPG 100–125 mg/dL; 2h-OGTT 140–199 mg/dL) did not reduce T2DM incidence.[19] | Supplementation may be effective in those with prior vitamin D deficiency. |
| $Mg^{2+}$ | Regulates electrical activity and insulin secretion in pancreatic β cells. Intracellular $Mg^{2+}$ helps the phosphorylation of the insulin receptor and other downstream signal kinases of the target cells. Low $Mg^{2+}$ levels result in defective tyrosine kinase activity, postreceptor impairment in insulin action, and altered cellular glucose transport and utilisation, which promotes peripheral insulin resistance in T2DM.[22] | Cross-sectional associations showed $Mg^{2+}$ levels correlated negatively with fasting insulin levels, and positively with the lipid profile. There is a 20% T2DM risk reduction for each 1 mg/dL increase of circulating $Mg^{2+}$ in those with hypomagnesaemia <0.5 mM (healthy circulatory threshold is 1 mM).[24] | $Mg^{2+}$ supplementation could ameliorate insulin sensitivity, reducing the risk of developing T2DM in those with hypomagnesaemia. |
| Multivitamins and minerals | Can enhance antioxidative capacity in T2DM patients.[17,25] | Has been shown to reduce the incidence of infections in T2DM patients with subclinical micronutrient deficiency in older adults.[23] | Supplementation may be effective in advanced states and in older T2DM adults. |

2h-OGTT: 2-hour oral glucose tolerance test; FPG: fasting plasma glucose; $Mg^{2+}$: magnesium ion; T2DM: Type 2 diabetes mellitus.

Meta-analysis of 23 lifestyle interventions that were beneficial in reducing T2DM risks (e.g., glycaemic control [FBG, 2-h OGTT, HbA1c], lipid profile [low-density lipoprotein, high-density lipoprotein, total cholesterol, triglycerides], blood pressure, and waist size) have poorly reported the relative contribution specific to either physical activity or dietary components.[29] Understanding the contribution and effectiveness of exercise in T2DM risk-reduction interventions is essential for forming meaningful and scalable guidelines.

Individuals within the Finnish DPS who reported an increase in their exercise levels (moderate-to-vigorous, low-to-moderate, or strenuous) were 63–65% less likely to develop T2DM, which fell to 51% after adjustments for diet and body weight.[8] This suggests a significant physical activity contribution and a dose-response relationship for T2DM prevention. Slentz et al.[30] have applied the same model used within the first 6 months of the USA DPP to quantify the exercise components in 195 individuals with prediabetes. They assessed to what extent the amount of exercise is a contributor to improving glucose control outcomes of FBG, OGTT, and glucose area under the curve, and whether moderate or vigorous-intensity exercise is better for improving glucose homeostasis. They randomised sedentary older individuals with elevated FBG of 5.28–6.94 mmol/L, no CVD, and uncontrolled hypertension or diabetes into four groups during a 6-month intervention, based on the energy expended during physical activity interventions: 1) low levels of moderate intensity exercise (42 kJ/kg of body weight/week [kkw]) equivalent to walking exercise (approximately 16.0 km/week at 50% of their $O_2$ uptake [$\dot{V}O_2$]$_{peak}$); 2) high levels of moderate intensity exercise (67 kkw) equivalent to walking exercise (approximately 22.3 km/week at 50% $\dot{V}O_{2peak}$); 3) high levels of vigorous intensity exercise (67 kkw) equivalent to Group 2 (approximately 22.3 km/week at 75% $\dot{V}O_{2peak}$); and 4) moderate intensity exercise (42 kkw) equivalent to Group 1 walking exercise (approximately 16.0 km at 50% $\dot{V}O_{2peak}$) combined with diet and weight loss (7%) to mimic the first 6 months of the DPP. Results showed significantly better glycaemic control improvement (OGTT and glucose area under the curve) in the two groups who followed moderate exercise (effect size: 0.60 and 0.73) than the vigorous exercise group (effect size: 0.21), and that the combined approach (Group 4) further induced a decrease in FBG (effect size: 0.71 versus approximately 0.17 in the remaining groups). The results suggested that moderate-intensity exercise in the form of 'easy to follow walking' was more effective than the same amount of vigorous-intensity exercise for T2DM glycaemic outcomes.

## ISSUES WITH EXERCISE INTENSITY FOR PERSONALISED TYPE 2 DIABETES MELLITUS PREVENTION

Levels of physical activity are known to be associated with reduced metabolic and cardiovascular risks including T2DM and reduced all-cause mortality in a dose-response relationship.[31] Exercise prescriptions for T2DM have often involved significantly different volumes and durations of exercise types: low intensity exercise (e.g., spending less time sitting), moderate-to-vigorous intensity exercise (150–300 min/week), heavy or vigorous intensity exercise (75 min/week), or severe intensity exercise (20 min/week), combined with strength training exercises (2/week).[2,32] This places a significant burden and confusion to those at risk or with T2DM to meet almost 300 min/week of exercise, especially considering the recent contradictory recommendation of performing shorter bouts of high intensity exercise.[2,33]

The recently proposed high intensity interval exercise training (HIIT) was based on promoting time efficiency because this method can take less time to achieve comparable outcomes than lower intensity exercise.[34,35] For example, six HIIT sessions (10 × 60 sec cycling bouts eliciting 90% maximal heart rate, interspersed with 60 sec rest) have been attributed to modulate postprandial and 24-hour glucose control, and muscle biopsy mitochondrial capacity biomarkers.[35] The Homeostatic Model of Insulin Resistance (HOMA-IR) and OGTT response were also changed following a single training session at this intensity in selected T2DM patients.[36] However, the short-term nature of the studies (only 2 weeks intervention, early T2DM diagnosis, strict laboratory supervision, lack of long-term adherence), makes it difficult to translate their recommendation into real-life settings for T2DM prevention. Other high-risk

groups such as overweight women, who followed a HIIT programme over 12 weeks, showed no improvement in insulin sensitivity despite reduced total and abdominal body fat percentage.[37] Furthermore, simply changing the time of day that the exercise is performed at could negate HIIT effectiveness in T2DM patients, and it has been shown that an afternoon HIIT is superior to a morning HIIT in enhancing T2DM outcomes.[38] Concerns have also been raised about HIIT safety and feasibility as a public health strategy.[39] A recent meta-analysis involving 411 heart failure patients concluded that HIIT is not superior to other types of lower intensity exercise when isocaloric exercise protocols were compared.[40] Furthermore, T2DM risk reduction among 48,000 individuals who engaged in either vigorous running exercise or walking behaviours was almost the same (12.1% versus 12.3% for running versus walking, respectively).[41]

Promoting HIIT had originally been associated with athletic performance for different endurance sports.[42] As such, HIIT was defined as repeated short-to-moderate duration exercise sessions lasting 10 sec to 5 min at a range of high intensities, provided they are higher than anaerobic threshold (based on lactate or ventilatory threshold). Such sessions were separated by either brief periods of low-intensity work or inactivity that allow a partial, but often not a full, recovery.[43] There was an intensity range flexibility in the original definition, which was based on stressing the physiological systems that are used during a specific endurance-type exercise to a higher level than that usually required during the activity.[42] Since HIIT is an acknowledged heavy exercise intensity physiological domain which determines a plethora of cardiovascular, respiratory, and metabolic responses, its training effectiveness can be attributed to a dose-response relationship. Exercise intensity domains, including those of HIIT and associated physiological responses (e.g., blood lactate concentration, oxygen uptake, and respiratory quotient), have been well known since the turn of the 20th century.[44]

Undoubtedly, a higher dose of intensity exercise, such as those within HIIT heavy-domain or strength type exercises, induce an elevated physiological response compared to lower-intensity exercise. This has been demonstrated in medium-term training effects reported on selected proteomics and transcriptomics protein translational machinery following either HIIT or strength training, compared with lower-intensity, moderate exercise in younger and older adults.[45] However, the effectiveness of each type (HIIT versus strength) varied significantly amongst the group, with older adults benefiting more from strength training given the age-related muscle atrophy risks.[45] Whether, and how, effects of HIIT can be found longitudinally in those with prediabetes or T2DM is as yet unknown. Interventions in real-life settings where long-term adherence, self-referral, injury, and risk reduction matter, reported higher injury prevalence (resulting in lower adherence rate) in sedentary individuals with T2DM who trained using HIIT compared to that of moderate-intensity exercise.[46] Long-term effectiveness of using heavy types of exercise training with a specific definition is needed to determine whether HIIT can be implemented at an individual level. Perhaps under continual specialist supervision, HIIT-type interventions in combination with appropriate behavioural lifestyle support can be effective in high-risk individuals. However, population scalability for T2DM prevention favours lower intensity types of physical activity.[47]

## COMBINING PERSONALISED BEHAVIOURAL NUTRITION, EDUCATION, AND PHYSICAL ACTIVITY APPROACHES

The suggestion is that an easier to follow intervention design may be better for individuals' adherence and primary T2DM outcomes than complex interventions, especially if a simplified approach is personalised by using or developing technology-based tools for both diet and exercise. For example, recently reported physical activity accelerometer-based interventions have been shown to increase physical activity levels, concurrent with improved diabetes and CVD outcomes in sedentary T2DM patients in the ADDITION-Plus study after a 4-year follow up.[48] T2DM prevention counselling, behavioural or individualised 'toolboxes of adherence strategies', materials to address ethnic diversity, training, feedback, and clinical support have been discussed in details elsewhere as part of large T2DM prevention programmes.[4,5] For example,

improvement in T2DM outcomes and related quality of life have been reported in people with T2DM attending a behavioural programme (DESMOND),[49] whilst other types of diabetes have also benefitted from similar diet and physical activity counselling programmes including Type 1 diabetes mellitus (DAFNE) and gestational diabetes.[50] Determining the relative contribution and effectiveness of counselling and behavioural tools within a multi-component T2DM requires further research.

Nutritional education and dietary counselling are essential within primary, secondary, and tertiary T2DM clinical care settings, and are used in screening and evaluating the dietary behaviour of people with diabetes. It helps individuals to understand food and beverage consumption behaviours, and develop skills needed to better manage their diabetes and prevent the development of related comorbidities.[51] Although nutritional education is a personalised method for preventing T2DM, individual counselling is laborious and cost-intensive; however, the use of technology such as mobile phone apps could be an excellent adjuvant method in personalising T2DM lifestyle prevention. Recent systematic reviews and meta-analyses have concluded that diabetes management mobile phone apps can be effective in improving self-efficacy, disease knowledge, physician–patient communication, and reducing diabetes incidence through delivering information, education, self-management, therapeutic advice, and drug guidance.[52,53] However, despite the availability of >120 downloadable apps for personal devices, only a few (approximately 11) appear to provide specific feedback for nutrition and exercise behaviours related specifically to T2DM or prediabetes. Additionally, these are limited to measuring HbA1c as a primary T2DM outcome while secondary outcomes are only addressed through a focus on persuasive weight management and behavioural lifestyle changes, which may explain their benefits.[52]

Overall, whether facilitated face-to-face or through a phone app, dietary counselling still heavily relies on teaching caloric and carbohydrate counting (e.g., identifying sources of carbohydrate in reported foods, portion estimates using measuring cups and smart food scales based on food composition tables, mobile apps, and websites).[51,54] Nonetheless, technologies provide great promise in providing personalised behavioural approaches to prevent T2DM.

In the context of forming personalised lifestyle recommendations, it is important to note that caloric restriction for weight loss may not be an effective T2DM prevention strategy. Improvements in T2DM or associated cardiovascular outcomes were not often matched by weight loss, especially when caloric restriction was used. For example, the MD, which is associated with reduced T2DM, CVD, and mortality risk, is not a caloric restrictive diet.[10,11] Interventions with a MD that reduced other cardiometabolic risks in high-risk individuals have also reported no change in body weight.[55,56] Large T2DM longitudinal, multicomponent interventions that used components of caloric restriction for weight loss have reported minimal changes (e.g., 2 kg),[30] or no body weight change.[48] Results from a large caloric restriction-induced weight-loss intensive intervention (combined with physical activity and dietary counselling) in sedentary T2DM individuals demonstrated an improved HbA1c but no reduction in cardiovascular mortality, which suggests that intensive weight loss may not be a sufficient outcome for cardiovascular events in those with T2DM.[57] Therefore, T2DM dietary recommendations should distinguish between qualitative versus quantitative composition of diet (e.g., a healthy nutritional plan versus plain caloric restriction). On the other hand, in terms of exercise recommendations that sustain behavioural change, the evidence from comparing large-scale interventions in individuals with prediabetes or diagnosed T2DM suggests that long-term adherence can be achieved by simple lifestyle modifications when they are targeted towards specific T2DM outcomes (Table 2).[30,48,57]

Recently, there has been growing evidence on the association between sedentary behaviour and several chronic diseases, including diabetes.[58] Therefore, it is important to understand the specific determinants of sedentary-related risks and their association with diabetes incidence and factors across different populations and environmental settings in order to devise effective targeted and personalised intervention strategies. Sedentary behaviour (e.g., 1 hour of watching TV) is associated with increased T2DM risk and all-cause mortality, independent of physical activity levels.[59-61]

**Table 2: Recommendations for physical activity and exercise for those with Type 2 diabetes mellitus based on longitudinal interventions.**

| Lifestyle T2DM interventions | Intervention method | T2DM intervention outcomes | T2DM recommendation |
|---|---|---|---|
| Randomised intervention trial with 195 individuals with prediabetes (Slentz et al.,[30] 2016). | T2DM outcomes (FBG, OGTT) in four groups:<br><br>**1)** Mod-LV (42 kkw, approximately 16 km/week at 50% $\dot{V}O_{2peak}$).<br><br>**2)** Mod-HV (67 kkw, approximately 22 km/week at 50% $\dot{V}O_{2peak}$).<br><br>**3)** Vig-HV (67 kkw at 75% $\dot{V}O_{2peak}$).<br><br>**4)** Diet/weight loss and LV (42 kkw at 50% $\dot{V}O_{2peak}$). | Diet and exercise induced better FBG.<br><br>Only moderate exercise enhanced OGTT.<br><br>Moderate exercise was more effective than the same volume of vigorous exercise, with an 82% adherence rate. | Moderate exercise (e.g., simple intervention of walking with counselling) is as effective for primary T2DM outcomes as vigorous exercise with intensive treatment rate, with better adherence rates. |
| Prospective 328 T2DM cohort ADDITTION-Plus followed for 4 years (Lamb et al.,[48] 2016). | HR-based accelerometer-measured PAEE to reduce WC, BP, and CVD risk associated with sedentary lifestyle and low PA. | PA accelerometer intervention increased PAEE and CRF and reduced sedentary related CVD risks in T2DM patients. | Simply increasing PA by accelerometery interventions can be effective in reducing CVD risk in individuals with T2DM. |
| Intensive intervention to prevent CVD mortality. Look AHEAD cohort with 5,145 overweight T2DM patients (Wing et al.,[57] 2013). | 1 year (within 5 years) with 15 years follow-up.<br><br>Intensive calorie restriction weight loss and increased PA versus counselling. CVD-mortality (MI/stroke, angina). | Weight loss (8.6 versus 0.7% at Year 1; 6.0% versus 3.5% at Year 5) initially improved CRF and HbA1c.<br><br>It did not reduce CVD-mortality (403 versus 418). | Specify the targeted T2DM outcomes. Intensive intervention that is good for reducing T2DM risks may not be good for CVD mortality. Weight-loss caloric restriction may not be a sufficient T2DM prevention strategy. |

BP: blood pressure; CRF: cardiovascular fitness; CVD: cardiovascular disease; FBG: fasting blood glucose; HbA1c: haemoglobin A1c test; HR: heart rate; kkw: kJ per kg of body weight per week; MI: myocardial infarction; Mod-HV: moderate high-volume physical activity; Mod-LV: moderate low-volume physical activity; OGTT: oral glucose tolerance test; PA: physical activity; PAEE: physical activity energy expenditure; T2DM: Type 2 diabetes mellitus; Vig-HV: vigorous high-volume physical activity; $\dot{V}O_{2peak}$: peak oxygen uptake; WC: waste circumference.

Interestingly, this risk can be in both leisure-based and workplace-based sedentariness.[62] Therefore, individually targeted workplace-specific interventions to reduce sedentary-related T2DM risks can be effective. For example, it has been shown that a 10-week exercise intervention in 54 sedentary university employees (aged 45–55 years old) enhanced cardiorespiratory outcomes (e.g., ventilatory threshold, cardiorespiratory capacity) irrespective of their job role, but not sex, for which women responded better across administrative and academic job roles.[56,63]

Intervention in this high-risk group was based on increasing physical activity levels during working hours (e.g., lunch time) to a tolerable level, such as using individual's perceived effort of exertion and using a self-selected intensity lasting <1 hour and falling within an effective training range (individualised ventilatory threshold), which was long enough to induce significant risk-reduction and CVD benefits. However, younger sedentary university workplace groups such as university students can tolerate and adhere to a more vigorous physical activity intensity.

A 10-week intervention involving 158 students relied on a variety of sports games to reduce cardiovascular risk factors such as blood pressure and cardiorespiratory capacity, as well as weight and fat-loss outcomes.[64] Other high-risk groups included postmenopausal women with T2DM, in which exercise training induced a significant vascular endothelial function improvement when exercise was combined with a nutritional approach based on the MD.[56] Therefore, sedentary-associated risks, which are carefully evaluated across different populations and settings, can be directly reduced using an individualised approach targeted at specific sedentary related risks.

Individuals with T2DM or its complications showed higher sedentary behaviour levels than their nondiabetic counterparts.[65] A dose-response relationship has been reported between the volume of uninterrupted sitting time and poor metabolic health.[66] Poor glycaemic control (reduced insulin sensitivity, FBG, and OGTT) has been associated with sitting times and patterns in individuals with T2DM.[67] Recent personalised assessments of sedentary behaviour using accelerometers have also shown a prevalence of concurrent sedentariness (sitting >9 hours/day) and insufficient physical activity (<30 min/day of moderate activity) in those with T2DM and obesity,[68] and amongst women at high risk of gestational diabetes.[69]

Similar sedentary associations with poor glycaemic control and reduced insulin sensitivity were observed in a 1-year study following and tracking physical activity levels using concurrent wrist-worn accelerometery and continuous glucose monitoring sensors in those with T2DM.[70] Such objective assessment tools help to provide an individualised intervention for sedentary individuals with T2DM. Sedentary behaviour-related diabetes risks should be targeted with personalised interventions based on their specific lifestyle determinants and associations (e.g., sex, age, occupation, workplace, and environmental risk factors).[68-71]

## CONCLUSIONS

Preventing T2DM relies primarily on changing lifestyle behavioural components, especially physical activity and nutrition. A personalised behavioural approach can support both early- and late-phase interventions, targeted physical activity, and nutritional guidelines for specific high-risk populations for different settings and risk factors. Quantifying the exercise components within large-scale interventions can inform how much intensity, volume, and mode is required to maximise T2DM risk reduction benefits. The recent high intensity exercise recommendations for T2DM require more personalisation, especially given the concerns about their scalability and long-term adherence. Mobile technology advances offer objective assessment, intervention exercise, nutrition, and counselling tools which can target high-risk individuals, especially when integrated with other direct medical approaches and targeted at different settings such as workplaces. Personalising lifestyle behaviours offers a holistic understanding which could enhance T2DM prevention guidelines.

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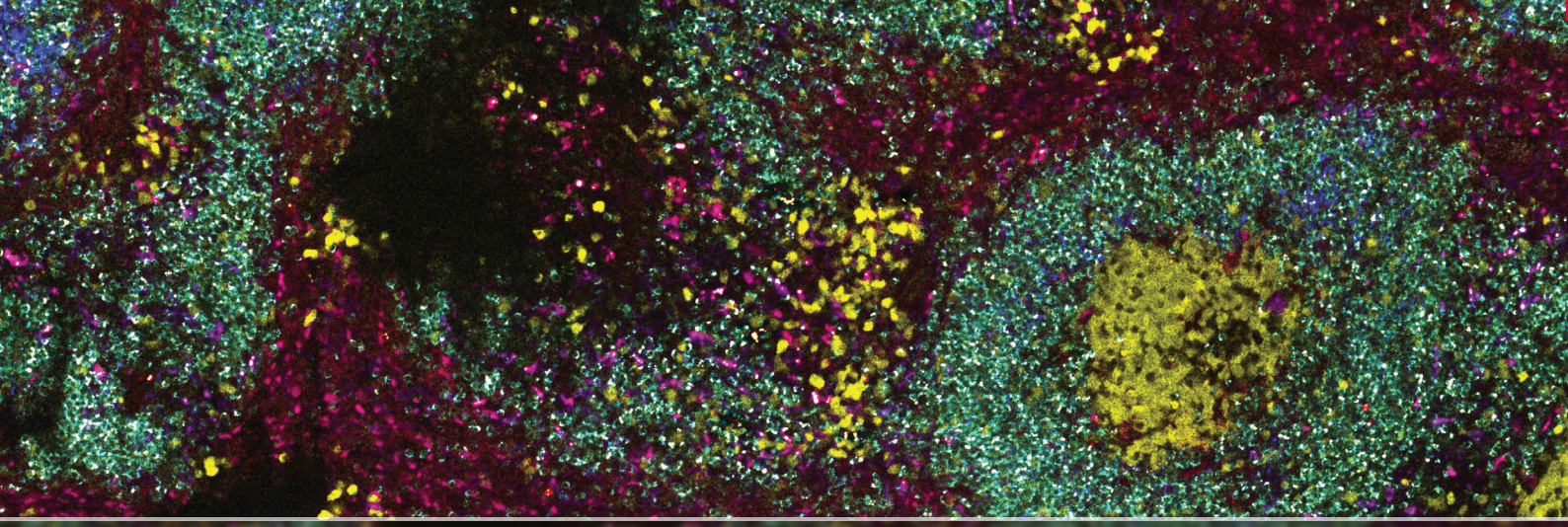

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# Clinical Profile of Patients with Psoriasis and their Attitude Towards the Illness

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## Abstract

**Introduction**: Psoriasis is a chronic inflammatory hyperproliferative disease of the skin and affects 0.6–4.8% of the world's population. In addition to the skin and joint manifestations, psoriasis impairs many aspects of individual wellbeing, including emotional, physical, sexual, and financial status. Skin diseases such as psoriasis can profoundly influence a patient's self-image, self-esteem, and sense of wellbeing. This study aimed to assess the clinical profile of patients with psoriasis and their attitude towards living with the disease.

**Methods**: A cross-sectional descriptive design was used. Two hundred subjects diagnosed with psoriasis were recruited through purposive sampling. A structured proforma was used to assess the clinical profile and attitude was assessed by Psoriasis Attitude Assessment Questionnaire (PSAQ).

**Results**: The most common type of psoriasis was chronic stable psoriasis (86.5%). A family history of psoriasis was present in 30 (15.0%) subjects. Stress was attributed to the exacerbation of disease in 90 (45.0%) subjects. Itching was reported by 124 (62.0%) subjects, and sleep disturbance was reported by 85 (42.5%) of the subjects. The majority of individuals (n: 187; 93.5%) had a moderately favourable attitude towards living with psoriasis.

**Conclusion**: Living with psoriasis can be difficult for patients because of the inconvenience caused by the disease in their daily life. Knowledge about the pathogenesis and treatment of psoriasis may increase the patient's perception of control and attention to aggravating factors may increase patient's compliance with treatment and positive lifestyle habits.

## INTRODUCTION

Skin diseases such as psoriasis can profoundly influence a patient's self-image, self-esteem, and sense of wellbeing. The chronic nature of the disease, as well as the fact that it cannot be cured and requires continuous treatment, generates frustration and disappointment in affected patients. The disturbances in body image perception contribute to the overall morbidity in psoriasis. Psoriasis requires lifetime treatment, but this is often difficult and

unsatisfactory. Patients with psoriasis directly experience the ineffectiveness of treatments by the inherent visibility of any recurrence. Findings from the National Psoriasis Foundation (NPF)[1] and European Federation of Psoriasis Patients Associations (EUROPSO)[2] surveys showed that respondents felt psoriasis had a negative impact on their lives. Embarrassment, shame, impaired self-image, low self-esteem, self-consciousness, and stigmatisation were more prominent among psoriasis patients compared to the general population.[3] Despair and feelings of stigmatisation may lead to noncompliance with treatment, possibly worsening the disease.[4]

The literature reviewed by Rabin et al.[5] revealed that patients with psoriasis felt shame and embarrassment over their appearance. According to Linder et al.,[6] the disease was often seen by patients as incomprehensible, incurable, and uncontrollable.[6] The visibility of the skin lesions and the social stigma associated with psoriasis results in anger, inconvenience, and frustration among patients with psoriasis. Hence, this study aimed to investigate the clinical profile of patients with psoriasis and their attitude towards the disease, each of whom attended dermatologic outpatient care services of a tertiary care hospital.

## METHODS

Before commencing the study, approval was obtained from the Institute Ethics Committee (IEC) for human studies at the Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER), Pudhucherry, south India, a tertiary care government hospital where the study was conducted. Participants signed the written informed consent after the risks and benefits of the study were explained. Privacy was provided and confidentiality was maintained throughout the study. The study was based on self-report, after providing confidentiality of the disclosure.

A cross-sectional, descriptive design was used that included 200 subjects diagnosed with psoriasis attending the outpatient department of dermatology at JIPMER. Adult subjects of either sex aged between 18 and 65 years, and with body surface area (BSA) involvement >10%, were recruited as samples by purposive sampling technique. Data collection was done through face-to-face interview.

### Brief Description of Tools Used

The clinical profile consisted of details such as duration of illness, presenting complaints, medical history, previous hospitalisation, family history, personal history, and clinical diagnosis. A Psoriasis Attitude Assessment Questionnaire (PSAQ) was developed for the study, which consisted of 20 items. Content validity of the tool was established in consultation with the experts from the dermatology, psychiatry, and psychology departments of the JIPMER and other reputed institutions. The final instrument was translated into Tamil, and the Tamil translation was retranslated to English for validity of the translated version. The attitude was assessed by five-point Likert scaling method, with the responses ranging from 'strongly disagree' to 'strongly agree'. Test-retest methodology was used to assess the reliability of this instrument. The 'r' value was 0.70 and found to be reliable.

## RESULTS

### Clinical Profile of the Study Subjects

The disease was chronic in 157 (78.5%) subjects and exacerbated in 32 (16.0%) subjects. Among the 200 subjects, 92 (46.0%) were treated as inpatients up to six times. Disease onset most commonly occurred when subjects were aged between 21 and 30 years (n: 55; 27.5%), followed by 31–40 years in 52 (26.0%) subjects. In respect to the duration of the current episode of illness, 153 (76.5%) subjects reported it to be <3 months. The details of the clinical profile of the subjects are documented in Table 1.

The distribution of the subjects based on other clinical variables is documented in Table 2. Out of 200 subjects, 123 (61.5%) had the illness for <10 years, 71 (35.5%) subjects had the illness ranging from 10 to 30 years, and six (3.0%) subjects had psoriasis for >30 years. One hundred and forty-five (72.5%) subjects had flare-ups of the disease <10 times and 13 (6.5%) experienced them ranging from 11 to 20 times. Clinical severity, which was based on BSA involvement, showed that 122 (61.0%) subjects had 20–40% BSA involvement, whereas 60 (30.0%) subjects had >40% BSA involvement.

**Table 1: The clinical profile of the subjects.**

| Variables | Categories | Number of subjects (N: 200) | Percentage (%) |
|---|---|---|---|
| Nature of illness | Chronic | 157 | 78.5 |
| | Exacerbation | 32 | 16.0 |
| | Acute | 6 | 23.0 |
| | Relapsing | 5 | 2.5 |
| Age of onset of illness | 10–20 years | 40 | 20.0 |
| | 21–30 years | 55 | 27.5 |
| | 31–40 years | 52 | 26.0 |
| | 41–50 years | 32 | 16.0 |
| | 51–60 years | 17 | 8.5 |
| | 61–65 years | 4 | 2.0 |
| Duration of present episode | 1–3 months | 153 | 76.5 |
| | >3 months | 47 | 23.5 |
| Type of medical services received | Outpatient | 104 | 52.0 |
| | Hospitalised 1–6 times | 92 | 46.0 |
| | Hospitalised >6 times | 4 | 2.0 |

**Table 2: The clinical profile of subjects based on other clinical variables.**

| Clinical variables | Categories | Number of subjects (N=200) | Percentage (%) |
|---|---|---|---|
| Total duration of illness | 1–10 years | 123 | 61.5 |
| | 11–30 years | 71 | 35.5 |
| | >30 years | 6 | 3.0 |
| Previous flare ups | No flare ups | 42 | 21.0 |
| | 1–10 times | 145 | 72.5 |
| | 11–20 times | 13 | 6.5 |
| Clinical severity as assessed by body surface area | 10–20% | 18 | 9.0 |
| | 21–40% | 122 | 61.0 |
| | >40% | 60 | 30.0 |
| Precipitating factor | Stress | 90 | 45.0 |
| | Unknown | 46 | 23.0 |
| | Infection | 39 | 19.5 |
| | Drugs | 22 | 11.0 |
| | Alternative medicine | 3 | 1.5 |

**Table 3: Item-wise responses for psoriasis attitude assessment questionnaire.**

| | | Strongly disagree | Disagree | Uncertain | Agree | Strongly agree |
|---|---|---|---|---|---|---|
| 1 | Psoriasis does not interfere with my daily activities. | 30 | 121 | 0 | 42 | 7 |
| 2 | Others think that my skin condition is contagious and do not like to touch me. | 10 | 47 | 18 | 118 | 7 |
| 3 | There is a lot which I can do to control my symptoms. | 2 | 13 | 13 | 153 | 19 |
| 4 | In spite of having psoriasis, I am happy. | 17 | 121 | 1 | 60 | 1 |
| 5 | It is difficult to adujst with application of ointments. | 4 | 113 | 5 | 71 | 7 |
| 6 | I would like to talk to my friends about my disease. | 2 | 34 | 1 | 156 | 7 |
| 7 | I feel that I am a person of worth at least equal to others. | 7 | 91 | 4 | 83 | 15 |
| 8 | I am satisfied with the medical care I receive. | 1 | 13 | 5 | 170 | 11 |
| 9 | I feel inferior because of my psoriasis. | 5 | 73 | 1 | 117 | 4 |
| 10 | The medicines I take/ ointments I use can usally control my symptoms. | 0 | 5 | 29 | 157 | 9 |
| 11 | I become physically unattractive when my psoriasis is bad. | 1 | 12 | 14 | 168 | 5 |
| 12 | Living with psoriasis is hard for me. | 4 | 37 | 0 | 139 | 20 |
| 13 | I fear that my child also might get psoriasis. | 14 | 76 | 12 | 81 | 17 |
| 14 | I have difficulty in finding a job because of my skin condition. | 13 | 91 | 5 | 78 | 13 |
| 15 | I prefer to have a physician of the same sex to perform the physical examination. | 3 | 89 | 2 | 97 | 9 |
| 16 | When lesion-free, I live with a constant fear of relapse. | 12 | 51 | 1 | 118 | 18 |
| 17 | My family supports me in all the ways to cope with my illness. | 3 | 10 | 1 | 179 | 7 |
| 18 | My friends/co-workers avoid me because of my psoriasis. | 18 | 116 | 3 | 54 | 9 |
| 19 | I have difficulty in managing financial issues related to psoriasis treatment (travelling, purchasing of medicines). | 7 | 47 | 0 | 124 | 22 |
| 20 | I am uncomfortable to visit other people's houses, when my psoriasis is bad. | 2 | 8 | 1 | 171 | 18 |

Furthermore, the findings showed that 90 (45.0%) subjects attributed the exacerbation of the disease to a preceding episode of a stressful event, 39 (19.5%) of them had exacerbation followed by infection, and 22 (11.0%) subjects had exacerbation as a result of the intake of various pharmaceuticals.

The most common type of psoriasis was chronic stable psoriasis (86.5%). Psoriatic erythroderma was present in 4.0% of the subjects, palmoplantar psoriasis in 3.0%, scalp psoriasis and unstable psoriasis in 2.5%, and pustular psoriasis in a very small number (1.5%). The study results revealed higher mean scores towards negative attitude in patients with pustular psoriasis (43.33±0.58) and unstable psoriasis (42.60±5.51) when compared to other types of psoriasis. In addition, patients also had physical symptoms such as itching (62.0%), joint pain (34.0%), cosmetic disfigurement as a result of the visibility of the disease (50.0%), and photosensitivity (52.5%). Furthermore, sleep was disturbed in 85 (42.5%) subjects, and appetite was reduced in 52 (26.0%) subjects.

Regarding the prevalence of addiction, 40 (20.0%) subjects consumed alcohol, 23 (11.5%) subjects smoked tobacco, and 29 (14.5%) of subjects had both tobacco smoking and alcohol consumption habits. Furthermore, a family history of psoriasis was present in 30 (15.0%) subjects.

## PATIENTS' ATTITUDES TOWARDS LIVING WITH PSORIASIS

The results of the study suggested that the majority (n: 187; 93.5%) of subjects had a moderately favourable attitude towards living with their disease and 11 (5.5%) subjects had a less favourable attitude.

Furthermore, item-wise responses of the attitude questionnaire are documented in Table 3. Of the 200 subjects, 113 (56.5%) said that they had no difficulty in applying ointments; additionally, 170 (85.0%) subjects expressed that they were satisfied with the medical care they received. Conversely, 171 (85.5%) subjects expressed that they felt uncomfortable to visit other people's houses when their psoriasis was severe, with 118 (59.0%) subjects reporting that, because of the unpredictable nature of the disease, they lived with a constant fear of relapse even if there was no lesion present. Furthermore, 118 (59.0%) subjects expressed that others regarded their condition as if it was contagious and avoided to touch them.

## DISCUSSION

Kostyła et al.[7] found that patients who have a higher level of illness acceptance demonstrate an overall better mental condition. The intensity of psychopathological symptoms was affected by the duration of illness, other people's attitude to the skin disease, age, and educational level of the patients.[7]

In the present study, family history of the condition was found in 30 (15.00%) subjects, whereas other studies by Manolache et al.[8] and Ding et al.[9] have reported this as 10.65% and 28.43%, respectively. Other researchers observed that those with early disease onset, i.e., <30 years of age, were more likely to have a positive family history of psoriasis.[10,11]

Itch is a common complaint among patients with psoriasis. Itching and scratching frequently lead to a vicious itch–scratch cycle. Pruritus was reported by 62.0% of the present study subjects. The similar findings were reported by Gupta et al.[12] (67.0%), Fortune et al.[13] (76.0%), and Krueger et al.[1] (79.0%). Disturbed sleep was reported by 85 (42.5%) subjects which was lower than that of study by Gaikwad et al.[14] who had reported it at 67.4%. Many authors found that pruritus interfered with sleep quality by increasing nocturnal awakenings.[15,16] Skin and joint manifestations associated with psoriasis and psoriatic arthritis were assessed in terms of joint pain. Joint pain was present in 68 (34.0%) of the subjects. The present study recorded a higher frequency than that of the study result of 28.8% reported by Valenzuela et al.[11]

The results of the present study revealed that 105 (52.5%) of the subjects had photosensitivity. The majority of patients with psoriasis benefit from ultraviolet radiation exposure, but psoriasis can deteriorate on exposure to sunlight in some people. Nalluri et al.[17] reported a patient with longstanding palmoplantar psoriasis that showed an unusual evolution into photo-aggravated psoriasis. The authors concluded that photosensitivity should be suspected in patients with hand and foot

psoriasis.[17] This present study indicated that 90 (45.0%) subjects attributed the exacerbation of their disease to a preceding episode of a stressful event. In different epidemiological studies, psoriasis was reported to be associated with a stressful life event in 10–90% of cases.[5]

Many researchers have reported that alcohol misuse is common in patients with moderate to severe psoriasis. Drinking alcohol appears to exacerbate pre-existing disease and continued drinking contributes to patient morbidity through treatment resistance.[18-20] Chodorowska and Kwiatek[21] stated that cigarette smoking is an important environmental factor exacerbating the course of the disease and provoking its consecutive relapses. The harmful influence of smoking is connected with the inducing of inflammatory mediators taking part in pathogenic phenomena in the skin of psoriatic patients.

The attitudes among study participants were categorised into three groups based on the scores obtained from the attitude questionnaire. Subjects who scored <50% of the total attitude score of 100 were considered as having a less favourable attitude; those who scored between 51 and 75% were considered as having moderately favourable attitudes, and the subjects who scored >75% were considered as having a favourable attitude towards living with their disease. The study results revealed that only two subjects (1%) had the favourable attitude.

The present study found a significant association between attitude and age. The subjects who were >40 years of age reported more favourable attitudes when compared to the subjects who belonged to the age group of 20–30 years. Similar finding was reported by Lin et al.[22] It is assumed that young adults aged <30 years have important life tasks, such as employment and marriage, and are more conscious about their physical appearance; it is viewed by some patients that psoriasis negatively affects their quality of life in all these areas. No sex difference was found.

In a recent study by Pearl et al.[23] that discussed the stigmatising attitudes toward persons with psoriasis among laypersons and medical students, it is implicated that accurate information is needed to eliminate the myths and misconceptions regarding psoriasis. The authors also suggested that public awareness campaigns and incorporating the importance of educating patients of their illness into the medical curriculum will help in improving the quality of life of people with psoriasis. Moreover, evaluating a patient's perceived stigma towards their illness is also necessary to improve the treatment outcome.[24-26] The present study has explored the attitudes of patients with psoriasis, which may help in planning some interventions. One limitation of the study, however, was that the results lack generalisation because of the sampling technique used being a non-probability sampling technique.

## CONCLUSION

Visible skin symptoms of psoriasis have a severe psychological impact on quality of life. Today, dermatologic treatment of psoriasis has become increasingly effective and can alleviate physical symptoms, but not cure the disease. Many of the difficulties experienced by patients with psoriasis make demands that outstrip the coping measures of patient and their family or social network. They need sufficient education and support from healthcare providers to manage their condition effectively. It is necessary to evaluate the perceptions and beliefs regarding psoriasis from the general public, healthcare providers, as well as a patient's own anticipated and perceived stigma to reduce the negative impact caused by psoriasis among those affected, in addition to its adverse psychological consequences.


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# Attitudes and Perceptions of General Practitioners towards the National Electronic Health Record (NEHR) in Singapore

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## Abstract

**Background**: In Singapore, the National Electronic Health Record (NEHR) was launched in 2011. The central ethos of the initiative was that of "One Patient, One Health Record", as NEHR allows registered doctors to review and upload patient data. However, uptake of the system has been slow in the private sector, with only 27% of doctors with private licenses, including general practitioners (GP) and specialists in the ambulatory care setting, accessing it. A questionnaire-based study was therefore conducted to find out the proportion of GP who used NEHR, and the barriers faced by those who do not.

**Methods**: This study involved a self-administered questionnaire, randomly sampling private GP in Singapore. The questionnaire ascertained the number of GP who used NEHR and gathered their demographic information. A 5-point Likert scale was used to measure the perceived barriers to NEHR use.

**Results**: Of the 315 responses, multinomial logistics regression showed that solo-practising GP who were >40 years old and who had practised for >15 years were less likely to review, or review and upload, data onto NEHR. Doctors who regarded themselves as computer users with lower levels of technical aptitude and those who perceived an inadequate level of support were less likely to use the NEHR. The majority of GP had a positive attitude towards NEHR.

**Conclusions**: This study highlighted key demographics and perceived barriers affecting NEHR use. By raising awareness of these issues to policy makers and working to overcome these barriers, NEHR use may be increased.

## INTRODUCTION

Electronic health records (EHR) are a consolidation of a patient's entire health history and information, including inpatient hospitalisation records, outpatient clinic visits, laboratory results, imaging reports, and records of medication prescribed and dispensed. They allow for improved safety and co-ordination of care, resulting in better

overall care for the patient and improved quality of documentation and administration efficiency.[1] A centralised EHR can allow full interoperability and a seamless transmission of patients' health information across different healthcare settings.[2,3] Other advantages of EHR include enhanced clinical decision-making abilities and cost-effectiveness.[4] There has been significant progress in EHR around the world[5,6,7] with improved usability and increased usage. Yet, these systems have not been fully implemented and effectively utilised.[8]

Many studies have been performed to elucidate the barriers perceived by physicians and their approach to the adoption and usage of EHR.[9-35] Several of these barriers are similar enough to be combined and categorised together to form several distinct domains, namely: organisation's influence and control, adequacy of training, adequacy of support, personal factors, and ethico–legal issues.[33-35] With regard to barriers involving organisation, these include the lack of administrator involvement in the physician's implementation of EHR[22,23] and an organisation's control and supervision undermining a physician's autonomy.[24,33,34] Barriers regarding inadequacy of training include lack of and/or insufficient training[13,17,33,34] and lack of sufficient assessments and feedback to ensure that users are proficient[13] and competent trainers.[14] Adequacy of support can be categorised as either technical or financial support. Specific technical support barriers include a lack of interoperability in supporting and migrating data from previous systems,[9,17-20] unreliable technological support,[14,20] and a lack of infrastructure for technical assistance.[10,13,22] The financial barriers involve mainly the cost of implementation[22-24] and EHR maintenance.[20,21,24,30] Personal factors affecting the use of EHR include barriers pertaining to age,[25,26] practice site,[13,22] computer literacy and technical sophistication,[9,11,21,27,28,35] and perceived negative effects on the patient–doctor relationship.[18,19,33,34] Ethico–legal issues include breaches of security[30-32] and compromised patient confidentiality.[30-33]

In Singapore, the National Electronic Health Record (NEHR) was launched in 2011, owned by the Ministry of Health (MOH) Singapore. The NEHR was developed and managed by Integrated Health Information Systems (IHiS), a technology agency for the public healthcare sector. The central ethos of NEHR was that of "One Patient, One Health Record". The NEHR comprises a consolidated view of a patient's healthcare history, including admission and visit history, hospital inpatient discharge summaries, laboratory results, medication history, history of past operations, allergies and adverse drug reactions, and immunisation history.[36] By allowing a longitudinal view of the patient's medical history, clinicians will be able to make better-informed diagnoses and treatment decisions, thus providing more effective and personalised care for patients and improving outcomes.

Registered physicians are allowed to access, review, and upload patient data. Anyone in Singapore, regardless of citizenship, who has been treated by an authorised healthcare professional from an institution or clinic that contributes data to NEHR will have a record. Singapore's healthcare system consists of both public and private healthcare facilities and providers, with the former offering government subsidies for Singaporean people and permanent residents. As the provision of optimal care for patients requires a multidisciplinary team, including GP, specialists, therapists, and pharmacists, and often spans across public and private healthcare settings, a consolidated healthcare record will allow for holistic, comprehensive, and safe administration of care. Duplicative tests can be prevented, saving patients time and money. The patient experience will also be enhanced by a seamless continuity of care through transitions across different healthcare settings, for example from a GP clinic to a hospital. Sharing of critical patient information such as drug allergies will also significantly improve patient safety as the patient transfers across these various settings. In order to empower patients to take charge of their own health and improve self-maintenance health behaviours, they are also able to access part of their health records captured in the NEHR through a separate programme.[37]

A fund of 20 million Singapore dollars has been set aside to help private practices (including GP, private hospitals, private specialists, and dentists) offset the costs of updating their systems. MOH Singapore also intended to legislate a Healthcare Services Bill in 2018 requiring mandatory data contribution by all healthcare providers.[38] Despite

this, uptake has been slow, with an estimated 27% of private licensees in ambulatory care (GP, specialist, and dentists) accessing and reviewing NEHR and only 3% of them contributing and uploading data to NEHR (estimates from 01.01.2019).

The primary research objective of this study was to find out, amongst the private licensees, the actual proportion of GP who review patient data on NEHR, review and upload patient data to NEHR, and the proportion who have never used NEHR before. The secondary aim was to find out the characteristics of the GP in relation to their usage of NEHR (never use, review only, or review and upload), as well as identify the attitudes and perceptions (perceived barriers and facilitators) affecting their usage of NEHR.

## METHODS

### Study Design

This was an observational, cross-sectional, self-administered survey conducted in Singapore from 01.02.2018–31.07.2018 . A mailed survey was sent to a random sample of 523 GP working in clinics in the private sector, including doctors in both group (a chain of two or more clinics belonging to a single management) and solo practices. The self-administered questionnaire was addressed to the licensee of the clinic. Together with an introductory letter, the questionnaire and a return stamped envelope were mailed to a computer-generated random list of registered private GP clinics in Singapore. The survey was resent at 3 and 7 weeks after the initial mailing to those who had not replied, according to the Dillman protocol.[39] To ensure that the data collected was anonymous to the investigators, an independent team oversaw the receiving and resending of the questionnaires to the nonrespondents. The study was approved by the SingHealth Centralised Institutional Review Board on 17.01.2018.

### Survey Methods and Data Collection

The research instrument was a questionnaire developed using a combination of guidelines on authorship of surveys as well as previous research, review papers, and surveys on attitudes and perceptions of EHR and its perceived barriers.[33-35] This questionnaire had been validated and tested for reliability in prior studies. Local contextual factors relating to Singapore were also taken into account.[40] Questions on the managing organisation's influence over physicians during the EHR preimplementation phase were excluded as these were irrelevant; the initiative had already been implemented nationwide in Singapore, compared to studies in which the EHR systems were still in the preimplementation phase.

This survey consisted of three sections. Section 1 elicited biodemographics of the participants and their self-reported computer literacy, using the terminology as adapted from previous surveys.[33,34] Section 2 collected data regarding five domains, namely:

**1)** Adequate support.

**2)** Adequate training.

**3)** Physician autonomy.

**4)** Ethical and legal concerns.

**5)** Doctor–patient relationship.

A final section provided the respondents with an opportunity to provide comments. All questions, except those in the bio-demographics and comments sections, captured responses via a five-point Likert scale, ranging from "strongly disagree" to "strongly agree". The final survey had been pilot-tested on an expert committee, consisting of senior family physicians as well as doctors within several hospitals' informatics departments, ensuring face and content validity.

### Statistical Analysis

Descriptive summary statistics (frequency and percentages) for the three categories of NEHR use ("never use", "review only", and "review and upload of patients' data"), participant's demographics, and the respective domains were computed. Multinomial logistics regression was performed to quantify the extent to which the NEHR use was affected by the participant's demographics and the respective domains. The statistical analyses were performed using SPSS® Statistics 19 (IBM, Armonk, New York, USA).

## Sample Size

Sample size was based on the assumed prevalence of NEHR use in Singapore, an estimated 50% out of a total number of 1,700 registered GP clinics in Singapore.[41] With a confidence level of 95% and a margin of error of 5%, a sample size of 314 GP clinics was required.[42] With the assumption of a 60% response rate,[43] 523 GP were surveyed.

## RESULTS

There were 315 completed responses to the survey out of a total of 523 surveys sent, indicative of a 60.2% response rate. NEHR use was divided into the categories of "never use", "review only", and "review and upload". In total, 74% of the doctors used NEHR for reviewing patient details, with 18% uploading data into NEHR; 8% of doctors surveyed had not used NEHR before. NEHR use amongst private GP for both review and review and upload of data was higher (74% and 18%, respectively) compared to all the private licensees in ambulatory care, specifically GP, specialists, and dentists (27% and 3%, respectively). Thirty eight percent of the respondents were females and 62% were males. Out of the 315 GP, 57% practised in a group practice and 43% were solo practitioners. The majority of the survey participants were aged 30–49 (30–39: 31.1%; 40–49: 47.9%). Fourteen percent were aged 50–59 and 7.0% were aged ≥60. Most of the GP had at least 11 or more years of experience (11–15 years in practice: 40.6%; >15 years in practice: 35.9%). In terms of personal computer or electronic device use, 92% of GP used their computers or electronic devices to access patients' medical information; 92% used it to access email; 84% used it to access health and clinical resources such as journals; and 97% used the computer to surf the internet. The most frequent task the computer was used to perform in the clinic was patient registration (81%) and electronic documentation of patient consultation (82%), followed by the checking of results (78%), ordering of medications (76%), billing of patients (71%), and ordering of investigations (61%). A total of 47% of survey participants regarded themselves as general computer users, whereby 'general' was defined as 'starting to become well-rounded and knowledgeable (in computers)'; 27% regarded themselves as 'novice' ('a beginner with limited skills and privileges') and 16% regarded themselves as a 'technician' ('an advanced beginner; dabbler; starting to function creatively and assist others, but without significant expertise'). Approximately 10% of the participants considered their computer sophistication as 'advanced' ('experienced, able to assist others independently and critically, and had usually completed formal training in computer science, medical informatics, or a related area'). No-one was an 'expert' ('extra-seasoned; experienced; the most accomplished in the field; will have completed advanced training in both medicine and medical informatics or a related area'). The above points are summarised in Table 1.

Of the GP surveyed, 80.8% were worried that their autonomy would be affected, and that NEHR usage would excessively "increase control and monitoring of their clinical practices and decision-making." Other barriers included concerns over ethical and legal aspects (62.6%), lack of technical support (61.0%), and cost of setting up and maintaining clinic NEHR access (51.0%). Nonetheless, 84.0% of GP felt that all physicians should "learn to use NEHR effectively", and that "implementation of the NEHR technology will support the physician in providing better patient care." GP in group practices were more likely to review and upload to the NEHR compared to GP in solo practices. Males were also more likely to review and upload compared to females, and those aged <40 were more likely to upload compared to those aged ≥50 ($p<0.001$). GP who had practiced for <15 years were more likely to use NEHR to review patient data compared to GP who had practiced for >15 years.

In terms of an individual's self-reported computer sophistication level, doctors who regarded themselves as novice, technician, and general computer users were less likely to review NEHR compared to advanced computer users ($p<0.001$). A doctor who was a novice computer user was also less likely to review and upload data onto NEHR compared to an advanced user ($p<0.001$). There was no association between computer/electronic device usage and NEHR use. The more worried that the GP were of there being no adequate technical and financial support, the less likely they were to review or review and upload onto NEHR ($p<0.001$). The more positive an attitude taken by GP towards NEHR, the more likely they were to use NEHR for review or review and upload.

**Table 1: Summary of results regarding demographics.**

| Demographics | N=315 |
|---|---|
| **Sex** | |
| Male | 62% (195) |
| Female | 38% (120) |
| **Type of practice** | |
| Solo | 43% (135) |
| Group | 57% (180) |
| **Age group** | |
| <30 | 3% (9) |
| 30–39 | 28% (88) |
| 40–49 | 48% (151) |
| 50–59 | 14% (44) |
| ≥60 | 7% (23) |
| **Years in practice** | |
| <5 | 4% (12) |
| 5–10 | 19% (60) |
| 11–15 | 41% (130) |
| >15 | 36% (113) |
| **Level as a computer user*** | |
| Novice | 27% (85) |
| Technician | 16% (50) |
| General | 47% (148) |
| Advanced | 10% (32) |

*Definition of level of a computer user

Novice: beginner with limited skills and privileges; Technician: advanced beginner, dabbler, or starting to function creatively and assist others but without significant expertise; General: starting to become well-rounded and knowledgeable (in computers); Advanced: experienced, able to assist others independently and critically, will usually have completed formal training in computer science, medical informatics, or a related area.

**Table 2: Results of the study.**

| | Multinomial analysis | |
|---|---|---|
| | Review | Review and upload |
| Female versus male | NS | - |
| Solo versus group | - | - |
| Aged <40 | + | NS |
| Practice years<br><10<br>11–15 | <br>+<br>+ | NS |
| Use of computer/electronic device | NS | NS |
| Level as a computer user*<br>Novice<br>Technician<br>General | <br>-<br>-<br>- | - |

**Table 2 continued.**

| | | |
|---|---|---|
| Q9 Adequate support | - | - |
| Q10 Adequate training | NS | NS |
| Q11 Physician autonomy | NS | NS |
| Q12 Ethical and legal concerns | NS | NS |
| Q13 Doctor-Patient Relationship | NS | NS |
| Q14 Attitude | + | + |

Only those p values that were significant were input as '-' or '+', each denoting the respective direction of association with outcome.

*Definition of level of a computer user

Novice: beginner with limited skills and privileges; NS: nonsignificant; Technician: advanced beginner, dabbler, or starting to function creatively and assist others but without significant expertise; General: starting to become well-rounded and knowledgeable (in computers).

For the domains of adequate training, physician autonomy, ethical and legal concerns, and doctor–patient relationship, no significant associations were found (Table 2).

## DISCUSSION

As this study was conducted nationwide across Singapore, the demographic data collected on age, years in practice, and computer experience was distributed across the different categories surveyed. However, it was noted that the majority of doctors surveyed had been practising for >5 years. This was likely secondary to the legislative requirements in Singapore for the setting up of a private medical practice. In Singapore, the MOH requires doctors to be fully registered before they can set up their own practices.[44,45] Doctors who graduate from local universities are also bonded to the public sector for several years.[46]

In this study, GP in group practices were more likely to use the NEHR for review or review and upload of patients' data, compared to solo practitioners. This result was similar to the conclusion reached by Xierali et al.,[22] and may be attributable to better financial and resource support in group compared to solo practices.[22] There may even be incentives and penalties, for usage and nonusage, respectively, of NEHR within the group. IT frameworks are most likely already set up and functional in these group practices. In addition, the doctors may be provided with IT training prior to joining the groups.

More GP who were <40 years old and had practised for <15 years were noted to review or review and upload data onto NEHR. This result was similar to a study by Kuek and Hakkenes,[26] who postulated this difference to be because of education rather than the demographic variable of age. Younger doctors are more likely to have increased exposure to IT and may have received computer training during their school days. This information can be useful for policy makers, who can utilise this information to streamline and target promotion and training of NEHR use to this specific group of doctors, namely those in solo practice, who are >40 years old, and who have >15 years of experience in practice. This will allow better resource allocation.

As for the other barriers, doctors who regarded themselves as 'general' computer users tended to be more likely to use NEHR for review or review and upload of patient data compared to GP who considered themselves as 'novice' computer-users. This is similar to published results from a study by Alasmary et al.[47] Again, this information may be useful for policy makers in conducting targeted computer training for these specific doctors as improving computer literacy and sophistication may improve use of digital information tools such as EHR.[48] On a general level, policy makers can also work towards engaging the relevant IT partners to simplify the NEHR system for users. Doctors who perceived that there is inadequate support, both financially and technically, were also less likely to review or

review and upload onto NEHR, results similar to many of the other studies done.[10,13,20-22,24,30] This is in spite of a significant level of funding being channelled into NEHR implementation by the MOH Singapore.

From a financial perspective, the perceived inadequacy could be due to a lack of knowledge and awareness of the current available resources. Also, the current financial subsidies provided may still be inadequate, with GP requiring more funds to set up and maintain their computer system. From a technical perspective, the perceived inadequacy could include problems with contacting the computer personnel, the lack of support during after office hours, or even unclear instructions given to the doctors by the computer personnel.

This study is not without limitations. When assessing computer sophistication, it is important to note that self-reported scores are always subject to cultural and local influences and may not accurately reflect computer proficiency.[49] Also, even though the questionnaire was addressed to the licensee of the clinic, the survey may have been delegated to another colleague to be answered, leading to response and selection bias. In terms of generalisability, this study was targeted towards GP in the private sector and hence the conclusions may not be applicable to primary care physicians in the public sector. This study explored GP perceptions of NEHR during the time frame indicated. As the emphasis of IT changes over time, with heightened awareness of cybersecurity[50] and sustainable funding, perceptions and priorities may change.

Future research may include extending this study to primary care physicians in the public sectors and comparing both trends. On a wider scale, a recent survey on Singaporean people's perceptions on NEHR[51] revealed that most Singaporean people are generally supportive of NEHR as a tool to facilitate continuity of care as they move through the various healthcare settings. Their main concerns include patient confidentiality and data security. Future research can integrate and compare both the physicians' and patients' perspectives.

## CONCLUSION

This first study of NEHR use in Singapore enumerated the actual proportion of GP who used NEHR. Through a detailed study of these GP, key characteristics associated with a reduced usage of NEHR were highlighted. These include solo GP who are >40 years old, those who have practised for >15 years, and those who regard their level of computer sophistication as novice. Amongst GP who were less likely to use NEHR for the purposes of reviewing or reviewing and uploading patient data, barriers were identified. These included a perceived inadequacy of technical and financial support. In general, the majority of GP were positive towards NEHR use in Singapore. GP with a positive attitude towards NEHR were also more likely to use it for reviewing or reviewing and uploading of patient data. By raising awareness of these issues to policy makers and working on these barriers, NEHR use may be increased.

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# Diabetic Amyotrophy: From the Basics to the Bedside

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## Abstract

Diabetic amyotrophy is a rare complication of diabetes compared to distal symmetric polyneuropathy, but can occasionally be encountered in clinical practice, particularly as the incidence of diabetes increases. The distinctive history of unilateral neuropathic symptoms followed rapidly by atrophy and weakness is typical of the disorder. This complication most commonly occurs in cases of well-controlled Type 2 diabetes mellitus. While the underlying pathophysiology is known to be microvasculitic in nature, the diagnosis is often based on clinical and electrodiagnostic grounds and tissue biopsy is not typically performed. Attempts at corticosteroid administration during immunotherapy should be carefully considered on a patient-by-patient basis. Better recognition of this disorder is likely to result in more rapid diagnosis, counselling, and subspecialty referral.

## INTRODUCTION

Among the most common and costly complications of diabetes is peripheral neuropathy. Most often, this takes the form of a distal symmetric polyneuropathy, manifesting as a length-dependent symmetric neuropathy characterised primarily by distal sensory and motor symptoms. It is important, however, for clinicians to be aware of other phenotypes of neuropathy that may occur in the setting of diabetes. One neuropathic condition of key importance is diabetic amyotrophy, which the authors review in this article.

Diabetic amyotrophy is a subacute, progressive, and often unilateral neurogenic process that occurs in the setting of diabetes. The typical course is one of severe pain, followed soon thereafter by weakness and wasting with weight loss. This condition has been reported in the literature over many decades. An early term for this syndrome was 'Bruns–Garland syndrome'. Bruns described the syndrome in 1890,[1] and in 1955, Garland published a case-series of nine patients whom he assessed to have diabetic amyotrophy.[2] Garland, an English neurologist, astutely observed that the syndrome was characterised by asymmetric, lower extremity symptoms occurring in patients with diabetes who typically had a short and "not severe" course of diabetes.

The terminology surrounding this condition is varied, which may add confusion. More often, 'Bruns–Garland' syndrome is now referred to

as diabetic amyotrophy. Other terms exist in practice and the literature, including femoral neuropathy and proximal motor neuropathy. Diabetic lumbosacral radiculoplexus neuropathy (DLRPN) is a term commonly used and may be useful in drawing similarity to diabetic cervical radiculoplexus neuropathy, a similar syndrome affecting the upper extremities.

## METHODS

The data included in this review was summarised from articles identified on MEDLINE, PubMed, and Google Scholar searches in July 2019, in which citations involving "diabetic amyotrophy", "diabetic lumbosacral radiculoplexus neuropathy", "Bruns-Garland syndrome", or "proximal diabetic neuropathy" were found. The titles and abstracts of the resulting articles were screened to select those with relevance. Namely, we included all articles that appear to describe clinical features of patients diagnosed with diabetic amyotrophy or any of the related terms associated with this condition. The bibliographies of the obtained articles were reviewed to identify additional articles. The search was restricted by language to the extent that English abstracts were required. Individual cases that included information about the symptoms, signs, evaluation, treatment, and course were included. The PRISMA checklist and guidelines were followed, to the extent that they were relevant to the review of observational studies. The review was not designed to capture all the relevant articles but to be highly representative of the existing literature involving diabetic amyotrophy. The search resulted in selection of 20 case report articles[3-22] and 8 observational case-series,[2,23-29] from which data was available for a total of 115 cases.

## CLINICAL FEATURES

The characteristics of 115 cases of diabetic amyotrophy, 95% of whom had Type 2 diabetes mellitus, are summarised in Table 1. The median age was 62 years, the duration of diabetes preceding the onset of diabetic amyotrophy was relatively short (median duration of 3 years), and nearly one-fifth of cases were diagnosed with diabetes during the evaluation of amyotrophy. Overall, the diabetes was well-controlled and nonsevere; although approximately one-third were being treated with insulin. There were few known diabetic complications (neuropathy, retinopathy, or nephropathy) consistent with the relatively brief duration of diabetes. Weight loss beginning coincidentally with the onset of symptoms was common and occurred in up to two-thirds of cases, ranging from 4.5 to 54.5 kg.

Cardinal symptoms included the abrupt onset of severe proximal leg pain involving the thigh, hip, or back, followed by progressive weakness and atrophy within weeks. Pain was unilateral in 70% of cases; when bilateral, it was usually asymmetric. A minority of cases complained of symmetric back and leg pain. The pain itself was characterised as deep and aching as well as tingling with or without burning. Proximal or proximal>distal weakness was the most characteristic sign, accompanied by variable amounts of muscle atrophy. The distribution of weakness and atrophy was unilateral in 25% of cases, and bilateral but asymmetric in >50% of cases. Overall, involvement of the other leg occurred in approximately half of the cases, typically within 6 months. A small number of cases that had an otherwise typical presentation of diabetic amyotrophy then developed diffuse weakness progressing to severe quadriparesis. Sensory loss was common but not particularly useful for diagnostic purposes because examination usually revealed symmetric distal sensory loss. In up to 20% of cases there was evidence of dysautonomia, including orthostatic hypotension. The most common reflex abnormalities included absent knee and ankle reflexes (33%) or asymmetric knee reflexes (27%), and 6% of cases were known to have a polyneuropathy prior to the onset of the diabetic amyotrophy. Nevertheless, >50% of cases had clinical evidence of an underlying diabetic polyneuropathy at initial evaluation.

These results obtained from 115 cases reported individually in the literature are supported by the overall results from several important observational case-series reports. For example, Barohn et al.[23] described 17 patients with diabetic amyotrophy, all of whom had a preceding diagnosis of diabetes (three controlled with insulin, six with oral antihyperglycaemics, and three with diet alone) or were diagnosed at time of presentation.

**Table 1: Characteristics of 115 cases of diabetic amyotrophy at time of initial diagnosis and follow-up evaluations.**

| Characteristics | Number of cases (n) | Yes (%) | Mean | Median | Range | 5–95th percentile |
|---|---|---|---|---|---|---|
| Age (years) | 115 | | 61.0 | 62.0 | 13.0–80.0 | 43.0–76.0 |
| Sex (male) | 115 | 76 (66) | | | | |
| T1DM | 115 | 6 (5) | | | | |
| T2DM | 115 | 109 (95) | | | | |
| Diabetes known pre-DA | 115 | 94 (82) | | | | |
| Diabetes duration (years) | 86 | | 6.0 | 3.0 | 0.1–30.0 | 0.1–20.0 |
| Dx ≤3 months, pre-DA | 13 | 13 (11) | | | | |
| Dx made during evaluation | 115 | 21 (18) | | | | |
| **Diabetes treatment (pre-DA)** | | | | | | |
| Insulin | 115 | 27 (34) | | | | |
| Duration (months) | 9 | | 39.0 | 36.0 | 0.5–120.0 | 1.0–96.0 |
| OHG | 115 | 22 (28) | | | | |
| Duration (months) | 12 | | 59.0 | 36.0 | 0.5–180.0 | 1–180.0 |
| Insulin and OHG | 115 | 4 (4) | | | | |
| Duration (months) | 3 | | 20.0 | 24.0 | 0.5–36.0 | 3.0–35.0 |
| Diet only or no treatment | 115 | 15 (13) | | | | |
| Unknown but not insulin | 115 | 21 (18) | | | | |
| Unknown | 115 | 26 (23) | | | | |
| Start insulin ≤2 months pre-DA | 89 | 2 (2) | | | | |
| **Associated features** | 115 | | | | | |
| Weight loss preceding DA | 115 | 6 (5) | | | | |
| Amount (kg) | 6 | | 16.8 | 14.5 | 6.8–31.8 | 8.6–28.6 |
| Interval (months) | 6 | | 5.0 | 4.0 | 2.0–12.0 | 2.0–11.0 |
| Weight loss coincident DA | 115 | 72 (63) | | | | |
| Amount (kg) | 72 | | 11.4 | 11.8 | 4.5–54.5 | 4.5–18.2 |
| Known diabetic DSPN (polyneuropathy) | 115 | 7 (6) | | | | |
| Clinically evident DSPN | 113 | 58 (51) | | | | |
| Diabetic retinopathy | 115 | 10 (9) | | | | |
| Diabetic nephropathy | 115 | 4 (3) | | | | |
| Illness/surgery | 115 | 0 | | | | |
| Duration of symptoms at Dx (months) | 98 | | 6.0 | 4.0 | 0.3–24.0 | 1.0–12.0 |
| **Symptoms** | | | | | | |
| Pain only | 115 | 4 (3) | | | | |
| Weakness only | 115 | 11 (10) | | | | |
| Pain followed by weakness | 115 | 87 (76) | | | | |
| Intervening interval (weeks) | 41 | | 2.0 | 2.0 | 0.0–10.0 | 0.0–8.0 |
| Weakness followed by pain | 115 | 5 (4) | | | | |
| Pain and weakness coincident | 115 | 8 (7) | | | | |
| Autonomic involvement | 115 | 21 (18) | | | | |
| **Pain distribution** | | | | | | |
| Unilateral | 101 | 71 (70) | | | | |
| Proximal predilection | 71 | 66 (93) | | | | |
| Back and leg | 71 | 4 (6) | | | | |

**Table 1 continued.**

| Characteristics | Number of cases (n) | Yes (%) | Mean | Median | Range | 5–95th percentile |
|---|---|---|---|---|---|---|
| Distal | 71 | 1 (1) | | | | |
| Asymmetric | 101 | 13 (13) | | | | |
| Proximal predilection | 13 | 13 (100) | | | | |
| Symmetric | 101 | 17 (17) | | | | |
| Proximal predilection | 17 | 6 (35) | | | | |
| Back and legs or diffuse legs | 17 | 10 (59) | | | | |
| Distal legs | 17 | 1 (6) | | | | |
| **Signs** | | | | | | |
| Sensory impairment and distribution | 74 | 54 (68) | | | | |
| Unilateral | 55 | 14 (25) | | | | |
| Asymmetric | 55 | 9 (16) | | | | |
| Symmetric (distal symmetric) | 55 | 32 (58) | | | | |
| Weakness/atrophy distribution | 115 | 110 (96) | | | | |
| Unilateral | 110 | 28 (25) | | | | |
| Asymmetric | 110 | 59 (54) | | | | |
| Symmetric | 110 | 23 (21) | | | | |
| Weakest muscle MRC grade | 49 | | 2.3 | 2.0 | 0.0–0.5 | 0.0–0.4 |
| **Weakness/atrophy pattern** | | | | | | |
| Proximal | 110 | 58 (53) | | | | |
| Proximal>distal | 110 | 37 (34) | | | | |
| Distal | 110 | 15 (14) | | | | |
| **Abnormal reflexes** | 78 | 78 (100) | | | | |
| Asymmetric knees | 78 | 21 (27) | | | | |
| Asymmetric knees and ankles | 78 | 4 (5) | | | | |
| Asymmetric knees, absent ankles | 78 | 11 (14) | | | | |
| Absent knees | 78 | 8 (10) | | | | |
| Absent knee/asymmetric ankles | 78 | 1 (1) | | | | |
| Absent knees and ankles | 78 | 26 (33) | | | | |
| Asymmetric ankles | 78 | 3 (4) | | | | |
| Absent ankles | 78 | 4 (5) | | | | |
| Clinically evident underlying DSPN | 113 | 58 (51) | | | | |
| **Laboratory results** | | | | | | |
| HbA1c (%) | 65 | | 8.5 | 8.0 | 5.8–19.8 | 6.0–12.0 |
| Fasting blood glucose (mg%) | 33 | | 200.0 | 170.0 | 81.0–414.0 | 118.0–356.0 |
| Elevated ESR (no [%], mm/hour) | 48 | 20 (42) | 19.0 | 8.0 | 0.0–78.0 | 5.0–36.0 |
| Elevated CSF protein (no [%], mg%) | 64 | 56 (88) | 117.0 | 90.0 | 30.0–1,560.0 | 43.0–212.0 |
| Abnormal needle EMG (denervation) | 40 | 37(93) | | | | |
| Asymmetric proximal>distal | 40 | 25 (63) | | | | |
| Symmetric proximal>distal | 40 | 12 (30) | | | | |
| Paraspinal muscles involved | 37 | 36 (97) | | | | |
| EMG evidence of diabetic DSPN | 34 | 31 (91) | | | | |
| **Treatment** | | | | | | |

**Table 1 continued.**

| Characteristics | Number of cases (n) | Yes (%) | Mean | Median | Range | 5–95th percentile |
|---|---|---|---|---|---|---|
| Intravenous Ig, Solu-medrol®, corticosteroids | 97* | 16 (16) | | | | |
| Improved glycaemic control | 97 | 24 (25) | | | | |
| Started insulin | 97 | 13 (13) | | | | |
| Opioid analgesics | 97 | 28 (76) | | | | |
| None aside from symptomatic | 97 | 26 (26) | | | | |
| **Outcome** | | | | | | |
| Death | 96 | 2 (2)** | | | | |
| Progression/no/minimal improvement | 96 | 3 (3) | | | | |
| Partial improvement (wheelchair) | 96 | 4 (4) | | | | |
| Moderate improvement (cane) | 96 | 40 (42) | | | | |
| Substantial improvement (no aids) | 96 | 35 (36) | | | | |
| Full recovery | 96 | 12 (13) | | | | |
| Time to nadir after Dx (months) | 32 | | 5.0 | 4.0 | 0.5-14.0 | 1.0-12.0 |
| Residual symptoms | 65 | 56 (86) | | | | |
| Weakness/atrophy | 56 | 46 (82) | | | | |
| Pain (although most had improved) | 56 | 19 (34) | | | | |
| Contralateral leg involvement (any time) | 71 | 46 (64) | | | | |
| Arm involvement (any time) | 115 | 21 (18) | | | | |
| Thoracic radiculopathy (any time) | 115 | 15 (13) | | | | |
| Interval (months) | 71 | | 5.0 | 3.0 | 0.0–60.0 | 0.0–11.0 |
| Time of re-evaluation after onset (months) | 74 | | 17.0 | 12.0 | 0.8–42.0 | 3.0–36.0 |

CSF: cerebrospinal fluid; DA: diabetic amyotrophy; DSPN: distal symmetric peripheral neuropathy; Dx: diagnosis; EMG: electromyography; ESR: erythrocyte sedimentation rate; IV: intravenous; MRC: Medical Research Council; OHG: oral hypoglycaemic; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus.

*3 cases treated with intravenous Ig and improved glycaemic control.

**1 case developed acute tubular necrosis and aspiration pneumonia after treatment with intravenous Ig, intravenous cyclophosphamide, and methylprednisolone.

Of the cohort, 14 reported unilateral and three reported bilateral pain at onset, though at time of evaluation pain had become bilateral in all. Latency to involvement of the other leg ranged from 3 days to 8 months. A minority of patients complained of lower extremity paresthesias or numbness, though examination often revealed distal sensory loss consistent with an underlying polyneuropathy. Five patients experienced weight loss, ranging from 13.5 to 36.0 kg. Similarly, Dyck et al.[25] reported 33 patients with diabetic amyotrophy with similarly identified risk factors and initial symptoms.

The median age at onset was 65.0 years with a median duration of diabetes of 4.1 years; all but one had Type 2 diabetes mellitus. Pain was the most common or severe symptom at onset in 27 patients in this group; however, weakness became the more bothersome symptom later in the course of the disease. Progression to bilateral involvement occurred with a median of 3 months and median weight loss associated with the disease was 13.6 kg.

The differential diagnosis for a lumbosacral plexopathy with similar presenting symptoms and signs includes inflammatory (e.g., herpes zoster), ischaemic (e.g., postradiation), and mechanical compression, including neoplastic involvement of the plexus. Additional evaluation may be performed in patients with a known or suspected malignancy. Non-DLRPN has been described as an inherently identical syndrome, also of microvasculitic origin, affecting nondiabetic individuals.

## PATHOPHYSIOLOGY

The subacute time course and painful nature of diabetic amyotrophy are suggestive of a process with vascular or ischaemic causes. This has played out in pathologic studies of DLRPN. Said et al.[29] published their study of 10 diabetic patients with painful, proximal, asymmetric neuropathies who underwent biopsy of the intermediate cutaneous nerve of the thigh. Three biopsies showed apparent changes of ischaemia, including an inflammatory infiltrate or vasculitis in two of the biopsies. In 1999, Dyck et al.[25] published their case-series of 33 patients with diabetic amyotrophy who underwent biopsy of either the sural or superficial peroneal nerve.[25] Of these 33 nerve biopsies, many showed changes indicative of vasculopathy, including focal or multifocal nerve fibre degeneration in 19 and epineurial neovascularisation in 21. Inflammatory changes were found in all nerves, and at a rate significantly more frequent than in patients with typical diabetic polyneuropathy or control nerves. Small arterioles, venules, and capillaries were most frequently involved. Two nerves showed a necrotising vasculitis while 13 others showed changes at least suggestive of a necrotising vasculitis. Similar pathologic changes of ischaemia and microvasculitis have been observed in non-DLRPN, a similar clinical entity in patients without a diagnosis of diabetes.[30] Subsequently, immunostaining has been carried out on nerve biopsies of patients with either diabetic or nondiabetic RPN. These studies have revealed increased ICAM-1 positive cells in blood vessels and increased NF-κB staining in blood vessels. Both findings support an underlying dysimmune vascular basis for these disorders.[31]

These observations regarding the pathophysiology of diabetic amyotrophy have also been supported by additional studies. In 1998, Llewelyn et al.[28] published a case-series of 14 patients who underwent nerve biopsy in the evaluation of proximal diabetic neuropathy.[28] In this cohort, biopsies of the intermediate cutaneous nerve of the thigh were performed. Three out of 14 patients showed inflammation in epineurial vessels, and more mild changes were observed in a fourth. They also included one sural nerve biopsy specimen in their series, which demonstrated inflammatory mononuclear infiltration in an arteriole. Younger et al.[22] published a post-mortem analysis of autopsy data on a 59-year-old man with apparent diabetic amyotrophy who died following treatment with intravenous Ig and cyclophosphamide.[22] Femoral nerve tissue and lumbar plexus tissue showed perivascular epineurial inflammation. Nerve biopsy is not routinely required or performed in the work-up and evaluation of a patient with suspected diabetic amyotrophy.

## CLINICAL EVALUATION

There is no single test that is diagnostic of diabetic amyotrophy. The evaluation should begin with a thorough history check and comprehensive neurological examination. The history typically discloses an acute to subacute, progressive time course. Frequent examination features include atrophy and loss of reflexes in the affected limb or limbs along with motor weakness. Given the presence of diabetes, an underlying distal symmetric polyneuropathy is common. Red flag features that may prompt a broader work-up include a lack of sensory symptoms or the presence of upper motor neuron signs, both of which may imply an alternative disease process including worrisome possibilities such as amyotrophic lateral sclerosis. Involvement beyond the lower extremities is also nontypical in DLRPN and would likely prompt additional evaluation.

Table 1 summarises the application and utility of different diagnostic tests in the evaluation of suspected diabetic amyotrophy. The Table does not include imaging of the lumbosacral spine, although MRI is usually performed to exclude an unsuspected structural explanation and

electromyography (EMG) is also a beneficial test in this setting. The expected findings include active denervation in clinically affected muscles, typically in a pattern suggestive of a lumbar plexopathy. As diabetic amyotrophy is truly a radiculoplexus neuropathy, involvement of the paraspinal muscles is common due to injury at the level of the nerve root and the nerve conduction studies may be impacted by the presence of underlying polyneuropathy. In this current literature review, 37 of 40 patients had abnormal needle EMG studies. The electrodiagnostic findings were asymmetric approximately two-thirds of the time. Of 37 patients, 31 had evidence of underlying diabetic polyneuropathy. EMG features that should prompt consideration of alternative diagnoses include a normal study or a lack of ongoing denervation, neither of which is typical.

Cerebrospinal fluid (CSF) analysis may be considered. The typical finding in diabetic amyotrophy is that of elevated protein. In this review, 56 of 64 patients with available CSF studies had an elevated protein level (Table 1), the median being 90 mg/dL. The presence of a significant pleocytosis is atypical and may prompt additional consideration of mimics of diabetic amyotrophy. It is not the authors' general practice to perform a lumbar puncture on all patients with suspected diabetic amyotrophy. This test is invasive, and an elevated CSF protein is a nonspecific finding, especially in the setting of diabetes in which elevated CSF protein levels are frequently incidental.

With a supportive history and examination findings, the yield of any blood test is limited. If the prior probability of diabetic amyotrophy is high, then serum work-up for other autoimmune conditions, including connective tissue diseases or vasculitides, is unlikely to be impactful. In this review, the sedimentation rate was elevated in 20 of 48 patients in whom it was measured. As the sedimentation rate itself is nonspecific, it should be interpreted with caution. Because diabetic amyotrophy is a complication of underlying diabetes it often prompts some re-evaluation of the patient's underlying diabetic control. This is classically indicated in patients with well-controlled diabetes. In 65 patients reviewed with available HbA1c values, the median level was 8.0% (5.8–19.8%) (Table 1).

In summary, the evaluation of diabetic amyotrophy is typically driven by clinical suspicion, supported by a suggestive history and typical examination findings. EMG testing is a common and reasonable element of this evaluation to support the clinical impression. Other studies, including CSF and blood tests, are unlikely to offer additional insight. Nerve biopsy is usually not needed to reach this diagnosis.

## TREATMENT

The treatment of diabetic amyotrophy remains somewhat controversial with a lack of high-quality evidence from well-designed clinical trials. Given the evidence supporting an underlying microvasculitic basis for the disease process, immunotherapy is certainly of interest. Before approaching this difficult question, clinicians may consider basic supportive measures in the treatment and management of these patients. Although diabetic amyotrophy most often occurs in patients with well-controlled diabetes, presentation with this syndrome merits re-examination of the patient's glycaemic control. Physical therapy is warranted to enhance a patient's mobility. Assistive devices or bracing may be needed depending upon an individual's degree and distribution of weakness.

Corticosteroids are often employed in the treatment of patients with diabetic amyotrophy. A 2006 study by Dyck et al.[32] is often cited but only published in abstract form. In this study, 75 patients were studied, of whom 49 received corticosteroids. The primary endpoint of motor improvement was not significantly different in those who received treatment; however, secondary endpoints, such as neuropathic symptoms, were better in the treatment group.

A 1995 study by Krendel et al.[33] reviewed the response to treatment of 15 patients with proximal, axonal diabetic neuropathies. Twelve of these patients received intravenous Ig showing benefits in strength. Most of these patients also received steroids orally or intravenously. Two patients in these series received intravenous cyclophosphamide, and one was started on azathioprine as a steroid-sparing agent. Pascoe et al.[34] published their series of 12 patients, which included five who

underwent plasma exchange, three who received intravenous Ig, and one who received both treatments.[34] Most patients improved, including four of the five plasma exchange patients and two of the three intravenous Ig treatment patients. Jaradeh et al.[35] studied 15 patients with diabetes and diabetic amyotrophy, including nine who received plasma exchange and six who received intravenous Ig.[35] All exhibited improvements in their pain and neuropathy disability scores.

While these data are exciting and plausible in the setting of the known microvasculitic aetiology for diabetic amyotrophy, some clinicians may be cautious. None of the last three case-series were randomised controlled trials. Further, diabetic amyotrophy is a condition with a natural history that tends towards improvement and resolution of symptoms, making the lack of a control group especially conspicuous. Intravenous Ig and plasma exchange, while frequently used for autoimmune neuromuscular conditions, are invasive and not without potential complications. A recently updated Cochrane review concluded there is currently no evidence from randomised trials to support a positive or negative effect of any immunotherapy in the treatment in diabetic amyotrophy.[36] Steroids are considered, particularly in cases of relatively recent onset, with progressing weakness or with especially severe neuropathic pain.

Although immune-mediated treatments may improve pain during the acute phase, pain is typically treated with agents shown to be effective for painful diabetic neuropathy, including tricyclic antidepressants, the selective serotonin and noradrenaline reuptake inhibitors, anticonvulsants, membrane stabilisers, topical agents such as capsaicin, and opioids.[37] When pain is particularly severe, hospitalisation may be required to achieve pain control.[38]

## PROGNOSIS

Because diabetic amyotrophy is a painful condition accompanied by significant motor weakness, patients are likely to present to their primary physician, endocrinologist, or a neurologist early in the disease course or while their motor weakness is most disabling. After the initial presentation, progression over months to involve the opposite leg, a thoracic nerve root, or even the arm is not uncommon. Nonetheless, the prognosis for eventual improvement in both sensory and motor symptoms is good, and multiple studies have demonstrated that cessation of pain and recovery of normal or near-normal strength are typical.

In 1972, Coppack et al.[39] followed a group of 27 patients for a mean of 61 months. Most patients had excellent recovery at 18 months. Pain recovered first, typically within 1 year, followed by improved strength and, to a lesser extent, recovery of the knee reflex. None of the patients had persistent disabilities, although persistent atrophy or hyporeflexia was common. Also in 1972, Casey and Harrison[24] published their series of 12 patients who were followed for a mean of 12 years. Only two patients had persistent pain, which was mild. A single patient did not report functional improvement, and the examiners also reported that muscle strength had significantly improved in all but one patient. Interestingly, many patients in this study were newly diagnosed with diabetes at the time of presentation. Improvement in the neuromuscular problem was noted to coincide with the initiation of oral medications or improved glycaemic control.

Even in cases with proven vasculitis on tissue pathology, the prognosis is very good. Said[29] reported on four patients with nerve biopsies demonstrating the typical inflammatory or vasculitic features of diabetic amyotrophy. In all four, pain was very severe but remitted soon after the nerve biopsy was performed. Two of the four had complete motor recovery, while the two others had mild residual weakness, which was attributed to a lumbar herniated disc in one case.

Dyck et al.[40] also studied 33 patients with diabetic amyotrophy, for the purpose of comparison to the more seldom studied nondiabetic radiculoplexus neuropathy. Of the diabetic patients, 13 reported severe pain at the time of initial evaluation compared to six at the time of a telephone follow-up. The number of patients reporting significant weakness did not change between the time of initial evaluation and telephone follow-up. While this may imply a somewhat poorer prognosis for motor recovery, it is worth noting that the number of

patients requiring a wheelchair for ambulation declined from 16 to three. Furthermore, the median time to telephone follow-up in these diabetic patients was 25.9 months, less than some of the other studies included in this review.

Diabetic amyotrophy is a painful condition that can be acutely disabling. Even in the absence of immunotherapies, the prognosis for the improvement of both sensory symptoms and for motor recovery is good. Patients are often devastated and can be depressed by the rapid onset of pain and weakness, making it important that they are reassured using guarded optimism that there will be improvement. This favourable natural history should factor into decision making regarding any proposed trial of immunotherapy.

## CONCLUSION AND FUTURE DIRECTIONS

While diabetic amyotrophy is a rare complication of diabetes, it is encountered frequently enough in clinical practice that non-neurologists and neurologists alike are likely to encounter it, especially as the incidence of diabetes increases. It is important for clinicians to carefully consider the clinical history, because the distinctive timeline of unilateral or asymmetric neuropathic symptoms followed by weakness and atrophy is distinct from the typical story of diabetic polyneuropathy. Importantly, this complication most commonly occurs in patients with well-controlled Type 2 diabetes mellitus and may even be the presenting feature of diabetes. While the underlying pathophysiology is known to be microvasculitic in nature, immunotherapy is still controversial. The use of corticosteroids in particular must be carefully weighed in each case. Better recognition of this disorder is likely to result in more rapid diagnosis, counselling, and subspecialty referral. Improved understanding of the underlying pathophysiology of this heterogenous syndrome will direct future treatment protocols.

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# From Traditional Histology to Next-Generation Pathology: A Review of The Workflow for the Characterisation and Molecular Profiling of Non-Small Cell Lung Cancer Samples

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## Abstract

The clinical management of non-small cell lung cancer has shown unprecedented progress into the era of target therapies and immuno-oncology. Despite significant recent achievements in the treatment of these patients, identification of all the clinically actionable alterations required for patient management remains challenging, particularly when dealing with cytological or small bioptic samples. Many investigations have assessed the role of diagnostic tools currently available, including immunohistochemistry and sequencing assays. It is extremely important to be aware of the minimum adequacy criteria for pathology laboratories to ensure correct management of the biological samples in non-small cell lung cancer, including cytological, cell blocks, and histological specimens. In this review, the authors provide a comprehensive overview of the gold standard requirements, processing parameters, and turnaround time for the final integrated report, and additionally outline the values and limitations of the different bioptic strategies.

## INTRODUCTION

In accordance with 'taking care' of the cancer patient, the management of non-small cell lung cancer (NSCLC) is currently carried out by a multidisciplinary team in which the pathologist plays a pivotal role.[1] To allow for patient-tailored testing, appropriate handling of the bioptic material is crucial to allow for the integration of the pathological data with the clinical requests.[2-4] Surgery and subsequent radiation or medical

therapy is the treatment of choice in NSCLC with loco-regional extension.[3] This approach is also possible in selected oligometastatic tumours;[5] however, up to 64% of patients with Stage I/II NSCLC, and approximately 76% of those with Stage IIIA NSCLC, relapse and die within 5 years of initial diagnosis.[6] The vast majority of patients with advanced NSCLC are considered surgically 'unresectable'.[7-10] In Stage IIIB/IV, the effectiveness of various platinum-based drug combinations are similar, with response rates ranging from 30–40%, a duration of <6 months, and median survival of approximately 12 months.[7] In particular, the selection of resistant neoplastic clones by means of somatic evolution and the substantial lack of recognised predictive/prognostic biomarkers are related to treatment failures.[11] Lung cancer has become one of the most challenging fields in pathology and oncology over the past decade, contributing to a revolutionary paradigm-shift in patient management.

Starting from the clinical need for prognostic and predictive biomarkers, pathologists have learned to refine their reports. To date, the analysis of *epidermal growth factor receptor* ([*EGFR*] on locus 7p11.2) gene mutations, *anaplastic lymphoma kinase* ([*ALK*] on locus 2p23.2-p23.1), and *ROS proto-oncogene 1* ([*ROS1*] on locus 6q22.1) rearrangements, and programmed death-ligand 1 (PD-L1) status is capital for clinical decision-making.[12-14] In addition to these biomarkers, other actionable gene alterations are under investigation. *MET proto-oncogene*, *receptor tyrosine kinase* gene (*MET*) exon 14 skipping was identified in approximately 5% of NSCLC patients and showed sensitivity to treatment with crizotinib and cabozantinib.[13]

Activating mutations of *EGFR* can be observed in 10–16% of lung adenocarcinomas in European patients[12-14] and are more frequently observed in young, female, Asian, non-smokers; however, these clinical parameters cannot be used as exclusive selection criteria.[15] Several Phase III studies involving patients with NSCLC with sensitising mutations of *EGFR* showed, compared to standard chemotherapy, a high percentage of approximately 70% of objective responses and a significant increase in progression-free survival of 7–13 months in patients treated with *EGFR* tyrosine kinase inhibitors (TKI).[16-18] The use of *EGFR* TKI in the front line of the population with mutated EGFR is considered a therapeutic standard and allows for routine research in all new cases affected by NSCLC, particularly non-squamous. The *ALK* gene rearrangement is present in approximately 3–5% of all pulmonary adenocarcinomas, especially in those with signet ring cell morphology, and predicts the response to *ALK* inhibitors.[19-23] This rearrangement is infrequent in squamous tumours, although it has been reported in mixed adenosquamous forms, and is generally mutually exclusive to *EGFR* and *RAS* mutations.[24] *ALK* testing is currently considered mandatory and, for practical reasons, it is usually performed with *EGFR* testing as a reflex test by immunohistochemistry (IHC) or fluorescence *in situ* hybridisation (FISH).

The *ROS1* gene codes for a tyrosine kinase receptor belonging to the insulin receptor family.[25] There are several fusion patterns of *ROS1*, the most frequent of which is *CD74*. The translocation of *ROS1* is present in 1–2% of the population affected by NSCLC, and in relatively young patients with pulmonary adenocarcinoma or non-smokers in the remaining population.[26] There is high homology of the tyrosine kinase domain between *ROS1* and *ALK*, and a preclinical study showed that NSCLC cell lines with translocated *ROS1* were sensitive to crizotinib.[27] The U.S. Food and Drug Administration (FDA) has approved crizotinib for the treatment of patients with metastatic *ROS1*-positive NSCLC and is, to date, considered the most appropriate therapy for these patients.[28] The latest biomarker that oncologists have claimed as a potential tool for increasing precision medicine is PD-L1 IHC analysis, due to the immunochemotherapy advent.[29-34] Other biomarkers under investigation are represented by the analysis of the tumour mutational burden (TMB), mismatch repair, and microsatellite instability status for immunotherapy treatment selection, although they do not represent a global standard of care.[35-38] In this scenario, it is extremely important to be aware of the minimum adequacy criteria for molecular pathology laboratories to ensure correct management of the biological samples in NSCLC, including cell blocks (CB), cytological, and histological specimens (Figure 1). Here, the authors illustrate the gold standard requirements, processing parameters, and turnaround time for the final integrated pathology report and outline the values and limitations of the different bioptic strategies.

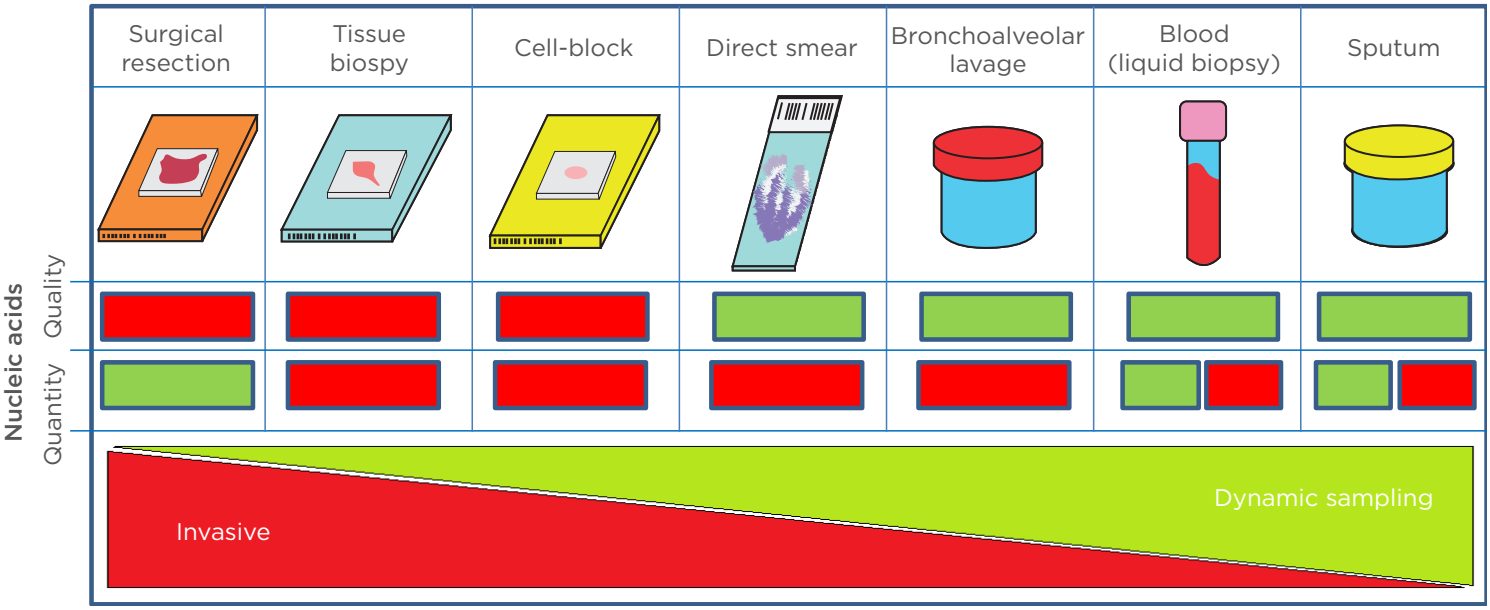


Figure 1: Schematic representation of sample specimens and their quantity and quality of nucleic acid characteristics where green signifies 'good' and red signifies 'bad'. Collection invasiveness decreases dynamic sampling increases from left to right. For blood and sputum, the red box refers to circulating tumour DNA, while the green box refers to genomic DNA.

## SAMPLE TYPES

### General Considerations

The first task of the pathology laboratory is correct management of the biological samples. This is a crucial step for precise diagnosis and subsequent biological characterisation of the neoplasms. Microhistological analyses are considered a good link between traditional histopathology, first-generation molecular biology, and next-generation sequencing (NGS) techniques. In many European countries, fine needle aspiration (FNA) remains the standard tool for the diagnostic approach to NSCLC both in peripheral pulmonary sites and lymph nodes.[39,40] In this setting, the preparation of CB is preferable for molecular testing and requested for the PD-L1 status analysis. For this reason, the rapid on-site evaluation (ROSE) of cytological samples should be performed by a pathologist or a trained cytotechnician, whenever possible. For centrally-located NSCLC, bronchoscopy allows for the direct observation of the lesion to evaluate its characteristics and also to conduct tissue sampling.[41] One of the major drawbacks of the histological and molecular characterisation of NSCLC is that the bioptic samples are usually represented by fragments measuring no more than 2 mm in greatest dimension. Furthermore, the analysis of these small fragments is often hindered by necrosis, spread inflammation, and/or crushing artefacts.

For lesions that cannot be assessed using bronchoscopy, a transthoracic core needle biopsy (CNB) may be performed under imaging guidance, for example C-arm cone-beam CT (CBCT). This technique consists of a flat-panel volume CT in which a cone-beam X-ray tube and a flat-panel detector are integrated with a C-arm gantry, enabling both CT and fluoroscopy guidance.[42,43] The CBCT virtual navigation systems create a virtual needle pathway to the target nodule and navigates the needle into the target after the operator determines the skin entry site and destination based on pre-procedural CBCT data. The use of 18-gauge semi-automatic biopsy needles with a 17-gauge coaxial needle obtains 1.5 cm-long cores in lesions with a diameter >2 cm. The diagnostic performance may reach 90% sensitivity; however, mean core size is crucial for the success or failure of the procedure.[44] Positive predictive value (PPV), negative predictive value (NPV), and overall accuracy in a large series were 100%, 100%, and 87.7%, respectively. Sensitivity was significantly lower for small lesions (57.1%;

p<0.01 for lesions ≤10 mm).[43] For CNB sampling, the radiologist carries out an average of two passes per nodule, with rare exceptions due to technical issues such as the occurrence of a haemorrhage after the first needle passage.

## Transthoracic Core Needle Biopsy Versus Bronchoscopy and Translational Molecular Research

Bioptic fragments from bronchoscopy are immediately formalin fixed and touch preparations to perform ROSE are not reccomended. Fixation should be set in cold (4 °C), 10% neutral formalin for the shortest possible time, typically 6–12 hours for small biopsies. Pathology laboratory technicians should prepare one section for the haematoxylin and eosin (H&E) staining and additional sections for the IHC characterisation of positive cases, using a minimum panel composed of p40, which is more specific than p63, for squamous cell carcinoma, and thyroid transcription factor 1 (TTF1) for adenocarcinoma. Other markers such as synaptophysin, chromogranin, or insulinoma-associated protein 1 (INSM1) should be performed in the presence of neuroendocrine differentiation. In the case of a NSCLC diagnosis favouring adenocarcinoma, subsequent PD-L1, *ALK*, and *ROS1* reflex testing can be completed in 24–48 hours. For CNB, setting up blank slides is encouraged too; the formalin-fixed paraffin-embedded (FFPE) section obtained by CNB can be used to extract DNA for molecular analyses or can be treated to be evaluated by cytogenetic techniques such as FISH. The morphological distinction between neoplastic and benign areas is usually immediate, and necrosis or foci of inflammation can be avoided. The leftover paraffin inclusions should be stored at room temperature. Blank slides should not be used for IHC after a few weeks of storage due to antigenicity loss phenomena; however, they can still be employed for extractive molecular techniques.

The most clinically relevant *EGFR* mutations concern the deletions of exon 19 or the point mutation L858R in exon 21 (80–90% of all mutations), but even rarer mutations in exon 18 and 21 can predict response to the EGFR tyrosine kinase.[45] Different strategies, for either clinical or research purposes, to detect the presence of *EGFR* gene mutations are employed in molecular predictive laboratories according to the available technology. Sanger direct sequencing, massARRAY® (Agena Bioscience, Inc., Hamburg, Germany), and PCR based techniques still represent the 'gold standard' methodologies for molecular testing even if they are not the most sensitive.[46-49] The College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) guidelines recommended that for *EGFR* gene mutations testing, detection of 50% tumour cells is strongly encouraged, but is rarely achieved in routine practice; when using sensitive detection methods, as little as 10% tumour cell detection is acceptable.[50] Currently, for technologies with high sensitivity such as amplification refractory mutation system (ARMS), digital PCR, and NGS applied for gene alterations testing, percentages of tumour cells as low as 1% have been reported.[51]

ARMS was able to identify a single mutant allele in a background of 100–10,000 wild-type alleles.[52] NGS, as demonstrated by Malapelle et al.[53] by using a narrow gene panel, was able to detect one mutant allele in a background of 20,000 wild-types.[53] A similar sensitivity could be obtained by a digital solid PCR approach.[54] In all of these examples, specificity of 100% (no false positive results) were obtained. In order to avoid the risk of false positive and negative results, particularly when considering mutated alleles at low frequencies in paucicellular specimens, careful validation is needed. Secondly, following IHC and instances of *ALK* Score 2 positive adenocarcinomas, the FISH cytogenetic method evaluates translocation involving *EML4* through an intra-chromosomal reversal event. The recommended kit is the Vysis ALK Break Apart FISH Probe Kit (Abbott Laboratories, Abbott Park, Illinois, USA).[55] For testing *ALK* rearrangements the percentages of tumour cells used are not as critical, and areas where tumour cells are not overlapping should be chosen.[56] In this subpopulation of patients, however, the appearance of secondary sequence variants in the kinase domain that favour drug resistance have been noted to favour the future advent of alternative diagnostic strategies such as RT-PCR or NGS.[57]

The test considered to be the gold standard for the determination of translocation of *ROS1* is FISH.[58,59] IHC methods are currently used as screening tests but, in view of the high false positive

rate, tumour samples considered positive in IHC should be tested according to the FISH method.[60] Finally, RT-PCR techniques have also been successfully studied.[61] Gold standard protocol should produce a 24–48 hour turnaround time for the final diagnostic report of histological type and predictive immunohistochemical markers (i.e., *ALK, ROS1,* PD-L1). *EGFR* status should be reported in optimal times of 5–7 days from receipt of the biopsy. For immunochemotherapy, the TMB calculation may be performed using whole exome sequencing (WES) as the standard; however, costs are high, and computational complexity and time make targeted sequencing of pre-customised genes in panels more attractive for routine use. The paraffin block at room temperature is the choice for potential, subsequent NGS profiling. To date, several platforms (SNaPshot Proteomics™ [AVMBioMed™, Limerick, Pennysylvania, USA], MassARRAY MALDI-TOF mass spectrometry, etc.) are available for the massively parallel testing of genetic alterations in NSCLC.[62,63] For poor quality specimens, a manual macro- or microdissection enrichment strategy, which can result in highly purified tumour cells for DNA extractions or repeated biopsies, are requisite.[64] Enrichment strategies, such as laser capture microdissection or flow cytometric sorting for isolation of tumour cells from small biopsies, should be used cautiously because of their typically low yields of DNA.

## Fine-Needle Aspiration and Cell Block

Cytology includes a variety of materials, such as fine-needle aspirations (FNA), bronchial brushing or lavage, sputum, and effusions. The most important parameter is the proportion of tumour cells to non-tumour cells. FNA usually show the highest tumour cell count, in comparison with exfoliative cytology materials.[65,66] All materials need critical pre-analytic evaluation to provide tumour cell enrichment for molecular testing.[67,68] Samples should be immediately fixed for Papanicolaou staining, using alcohol-based spray or liquid 100% ethanol; alternatively, air-dried preparations may be stained with May-Grünwald-Giemsa (MGG). In the case of ROSE, a Diff-Quik Stain Kit (Polysciences, Inc., Warrington, Pennsylvania, USA) is an option. Especially for brushing and bronchial lavage, an acceptable option is the use of liquid-based cytology.[68] Advantages include the elimination of blood in excessively haemorrhagic samples and the possibility to use leftover material for ancillary tests as immunocytochemical staining. Liquid-based preparations may overcome the need for ROSE, reducing artefactual problems related to smear and fixation time. In different studies, *EGFR* mutation analysis on cytological material gave reliable results. High concordance rates between cytology and histology have been demonstrated in the literature.[69] Exfoliative cytological material generally missed a higher proportion of <25% of *EGFR* mutations.

The use of CB is also suggested because most commercially available kits are validated for FFPE materials.[70] The loss of tumour cells and hypocellularity are the two main disadvantages of CB. Molecular tests can be performed on direct smears, liquid-based preparations, and materials stored on filter papers.[71] Both MGG and Pap-stained archived slides may be used but air-dried smears are significantly superior to Pap-stained slides in terms of higher *EGFR* mutation rates. Knoepp and Roh[71] suggested the use of Diff-Quik staining because of better preservation of DNA, whereas Killian et al.[72] demonstrated DNA degradation in archived Pap-stained smears as a result of alcohol fixation. Contrastingly, there are also studies showing superiority or equivalency of Pap- to MGG-stained slides.[73] DNA quality and homogeneity of tumour cells within materials have been shown to be more reliable parameters than quantity.[73] CB represent a 'hybrid preparation' between cytological and histological specimens, due to FFPE of the FNA material.[74] The main advantages of CB include being able to identify the architectural pattern perform ancillary techniques, such as IHC.[75,76] Differing from direct smears, CB do not require particular molecular validation as with histological material.[77] In order to evaluate the possibility of a significant reduction in neoplastic content and assess the neoplastic cellularity, it is good practice to stain first and use CB sections last.[78] This could significantly reduce the risk of false negative results. It is important, particularly in the case of low tumour content, to select neoplastic cells on H&E stained slides to guide tumour cell dissection.[74] On the other hand, adopting different serial sections from CB could allow the molecular analysis on the entire cell nucleus.[78] An important limitation of direct smears is the low quality of nucleic acids.[78-80] To date, a validated cell transfer

technique allows the feasibility of molecular testing on DNA extracted from neoplastic cells derived from routine smears.[81,82]

Another possibility is by liquid-based cytology, in particular, to avoid problems correlated with inadequate triage of aspirated material by untrained clinicians.[83,84] In this preparation, the FNA is completely expelled in an alcohol-based fixative, such as CytoLyt® (Hologic, Marlborough, Massachusetts, USA) or CytoRich™ Red (Thermo Fisher Scientific Inc. UK, Leicestershire, UK) solutions, in order to generate a cell monolayer slide.[85] Several studies have described the feasibility of liquid-based cytology for molecular purposes.[74,85-89] Neoplastic cells are scraped directly into an Eppendorf collecting tube, or obtained by cell lifting with the Pinpoint solution of the Pinpoint Slide DNA Isolation System (Zymo Research, Irvine, California, USA).[90,91] A crucial point for molecular analysis is the coverslip of smears in order to scrape tumour cells by a dedicated blade.[74] Another possibility to reduce the time of preparation is represented by the 'freezer method', in which slides were frozen and a blade was used to remove the coverslip.[92] An important issue was the necessity for neoplastic cell enrichment, particularly when low sensitive techniques were employed.[77,93]

## BIOFLUIDS

### Sputum and Bronchoalveolar Lavage

Sputum is an important source of nucleic acids, proteins, and other analytes that reflect the status of different organs.[94] Different experiences focalised the attention on sputum to investigate *EGFR* status in NSCLC patients. In a large series (N=50), Wu et al.[95] identified a high concordance rate between sputum and tissue samples (74%).[95] Hubers et al.[96] reported a specificity of 100% but low sensitivity of 50%. In addition to predictive purposes, sputum could also be adopted for diagnostic aims and for secondary prevention.[97] Recent evidence suggested the possibility to analyse microRNA (miRNA) in sputum as a non-invasive tool for NSCLC diagnosis.[98] In the experience of Bagheri et al.,[98] the authors identified expression of miR-223 in sputum as a useful diagnostic biomarker to detect NSCLC patients. In order to increase either sensitivity or specificity, Su et al.[99] implemented the analysis of two different biomarkers in sputum (miR-21, miR-31, and miR- 210, and methylation of *RASSF1A, PRDM14*, and *3OST2* genes). Bronchoalveolar lavage (BAL) is a non-invasive procedure useful for diagnostic purposes in different lung diseases.[100] Tuo et al.[101] identified the potential role of *P16INK4a* gene promoter methylation in both BAL and sputum as a diagnostic biomarker for NSCLC, but because of the low sensitivity, it is not suitable as a screening tool. Ren et al.[102] showed that *SHOX2* and *RASSF1A* methylation in BAL can increment the detection rate of lung cancer, with high sensitivity and specificity. Kim et al.[103] showed the high diagnostic role of five miRNA (miR-21, miR-143, miR-155, miR-210, and miR-372) both in sputum and BAL, notably in the early stage of the disease. However, for these biomarkers, careful attention should be paid to possible artefacts, for example FFPE DNA bisulphite conversion. FFPE DNA bisulphite conversion leads to a challenging methylation sequencing data process due to C→T conversion. Bisulphite sequences are not perfectly complementary to the standard reference genome and, in these cases, special alignment tools are necessary.[104]

### Blood

Due to delays in diagnosis, a high percentage (approximately 70%) of NSCLC patients only have small tissue samples (biopsies and cytological specimens) available for either morphological diagnosis or molecular purposes.[105] To avoid inadequate results, the 'liquid biopsy' represents a valid sample to assess the molecular status of *EGFR*.[106] To date, the only analyte approved for *EGFR* molecular assessment is circulating tumour DNA (ctDNA) extracted from plasma in patients either with diagnosis of advanced NSCLC before any treatment and without the availability of tissue (basal setting), or with resistance to treatment with first- or second-generation TKI for the detection of *EGFR* exon 20 p.T790M (progression setting).[106] The most important issues related to ctDNA regard the low concentration, 0.5% of cell-free DNA, short half-life of approximately 15 minutes, and the modification of concentration during the disease.[107,108] For these reasons, the International Association for the Study of Lung Cancer (IASLC) established a statement paper with recommendations for liquid biopsy management.[109] In Phase III randomised clinical trials for gefitinib (IPASS) and afatinib

(LUX-Lung 3), ctDNA was extracted from serum. The analysis using real-time PCR showed a low sensitivity, 43.1% and 28.6%, respectively.[109] An increase in sensitivity, with 100% specificity, was obtained in the Phase IV clinical trial for gefitinib (IFUM) and the Phase III clinical trials for afatinib (LUX-Lung 3 and LUX-Lung 6) in which the analyses were carried out on plasma, 65.7% and 60.5%, respectively.[109] Increased sensitivity was obtained by Reckamp et al.,[110] using a NGS approach. The authors showed sensitivity of 93.0%, 100%, and 87.0%, and a specificity of 94.0%, 100%, and 96.0% for p.T790M, p.L858R, and exon 19 deletions, respectively, compared with matched tissue samples.[110] Malapelle et al.[53] showed specificity of 100% and sensitivity of 90.5% by analysing ctDNA extracted from either plasma or serum from each patient (basal and progression settings) by using an ultradeep NGS approach. The same panel was adopted in the experience by Pisapia et al.[111] who showed 8% *EGFR* mutated cases on plasma samples (n=63) in basal NSCLC patients. All the mutations were confirmed by digital PCR.[111] In the AURA study, Oxnard et al.[112] showed, by using a high sensitivity BEAMing digital PCR (Sysmex Co., Kobe, Japan), different sensitivity and specificity when considering different *EGFR* mutations, the authors particularly focussed on the lower rate for the *EGFR* exon 20 p.T790M. Sensitivity was 82.3%, 86.3%, and 70.3%, and specificity was 97.5%, 96.5%, and 69.0% for p.E746_A750delELREA, p.L858R, and p.T790M, respectively.

## REQUIREMENTS, PERSPECTIVES, AND FUTURE DEVELOPMENTS

Advanced genome sequence technologies have initiated new perspectives in molecular diagnostics. Novel possibilities, including obtaining multiple genome sequences rapidly and at relatively contained costs, are the result of new approaches to next-generation sequencing, especially compared to conventional sequencing techniques. This makes it possible to search for multiple mutations in the same gene, as well as identify mutations of different genes. Currently, application in routine clinical practice is limited by low distribution in companies and by the high expertise necessary for critical interpretation of genetic data. Therefore, only the availability of series with significant numbers and complex case-mixes can serve as candidates for the proposal of pilot projects that aim to validate NGS platform application in international health systems.[113,114] Future projects will investigate NGS methods in pathology, verifying performance indicators such as the efficacy, efficiency, and reproducibility of plasma versus tissue NGS molecular tests. Units of oncology molecular pathology should be able to achieve minimum adequacy criteria for clinical-oncological standards for NSCLC management.

Issues with the biopsy samples (FNA versus transthoracic CNB versus small bronchoscopic biopsy versus CB) may be overcome by interventional contribution of pathologists in radiology or performing a ROSE. Moreover a dedicated technical line to the processing of 'sensitive' samples for molecular profiling should be arranged in every oncology molecular pathology unit, with a 24–48 hour turnaround time for a final diagnostic report of histotype and predictive immunohistochemical markers (*ALK, ROS1*, PD-L1).

The *EGFR* status should optimally be provided 5–7 days from receipt of the biopsy, keeping leftover material in the archive for NGS profiling. In future developments, the complete integration of traditional pathological techniques with those of molecular biology should result in the formulation of a single report, including all the theranostic factors necessary for the medical oncologists. NGS techniques therefore hold great hope for the future, despite current problems related to standardisation, clinical interpretation of data generated, and reimbursement remaining obstacles to implementation in clinical practice.

The latest biomarker claimed as a potential tool in precision medicine for NSCLC is TMB. This analysis, akin to that of mismatch repair protein expression and microsatellite instability, would require tumour-specific protocols and guidelines for the interpretation.[115,116] Larger studies are required to verify whether a high TMB is associated with greater probability of response to immune checkpoint inhibitors and to assess the validity of this new biomarker to represent a real standard of care. Despite the outstanding achievements in the management of lung cancer, and identification of novel biomarkers, the road for precision medicine overlooks a correct

histological diagnosis. Definitive therapy for NSCLC should not be undertaken in the absence of tissue diagnosis. Next-generation pathologists are tasked with providing an accurate, specific, and comprehensive diagnosis to enable the clinician to develop an optimal plan of treatment and, to an extent, estimate prognosis. It is capital to manage the tissue samples with parsimony to allow for broader and more accurate analyses. The goal of precision medicine, however, could be achieved only using a holistic approach, where the analyses performed by a pathologist are integrated with all clinical information. This approach would constitute the highway to be travelled for improving survival in NSCLC patients.

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# What's New

## Preventing Antioxidant Response Suppression as Therapy for Fatty Liver Disease

NONALCOHOLIC fatty liver disease is a major health risk for individuals with obesity and is a condition for which there is currently no medicine available. A novel study has discovered that suppression of a protein that weakens the antioxidant response in immune cells could offer opportunities for therapeutic treatment. The researchers aimed to explore the cellular and molecular biology of excess fat on mouse and human livers, and actions required to restore damage by oxidative stress.

The researchers identified that macrophages counter excess fat by attempting to reduce it in a process that results in the production of large amounts of liver-damaging oxidants. In addition, they found that in the liver of obese mice and humans, the protein NRF2, an antioxidant, was inactive. "This lack of NRF2 protein tells us that obese individuals do not have the ability to properly respond to oxidative stress induced by fat accumulation in the liver," explained Dr Valerio Azzimato, the first author of the study based at Karolinska Institutet, Solna, Sweden.

In the livers of obese mice and patients, an increase in the levels of miR144, a microRNA produced by immune cells and hepatocytes in response to oxidative stress, was measured. This specific microRNA weakens the antioxidant response of NRF2, impacting the *NRF2* gene to decrease its protein levels and reduce the protective effect of the protein from damaging oxidants.

Subduing of miR44 expression was achieved in macrophages using technology to silence genes. This suppression of miR144 caused the amount of oxidants in the whole liver to be lowered, repairing the antioxidant response leading the authors to conclude there may be interaction between macrophages and hepatocytes. The authors thoughts were summarised by the corresponding author, Dr Myriam Aouadi: "Given that using exogenous antioxidants has been associated with long-term side effects in several tissues, we believe that targeting miR144 to increase the endogenous antioxidant response represents a promising therapeutic strategy for the treatment of liver diseases in obese patients."

# What's New

## Combination Approach Demonstrates Better Accuracy in Prostate Cancer Diagnosis

COMBINING systematic and MRI-targeted biopsies could be a more effective approach for the accurate diagnosis of prostate cancer than through using each technique alone. These findings have emerged from new study results published online ahead of print and could hold immense promise for men with prostate lesions visible upon multiparametric MRI.

In this study, 2,103 men with a mean age of 63 years received both systematic and MRI-targeted biopsy in the Trio study (NCT00102544), revealing a 62.4% predominance of prostate cancer based on combined results. The 12-core systematic biopsy was inferior to the MRI-targeted approach in detecting more clinically important cancers defined as Grade III or higher (1.9% versus 8.3%). The MRI-targeted approach also detected significantly fewer indolent Grade I cancers (3.5% versus 7.8%). The combined approach on the other hand led to cancer diagnoses in 9.9% more men (n=208) than through either method alone, as well as to upgrading in 21.8% of the patients (n=458). Of the 19.2% (n=404) who had undergone radical prostatectomy, histopathological findings spurred fewer upgrades to Grade III or higher following combined biopsy as opposed to MRI-targeted or systematic alone (3.5% versus 8.7% versus 16.8%, respectively).

Dr Peter A. Pinto of the National Cancer Institute, Maryland, USA, proclaimed that: "Potentially, these data may usher in a new era of increased confidence in the selection of prostate cancer treatment on the basis of biopsy results." Despite the small net increase in indolent cancer diagnosis, the high predictive value for true pathological grade was perceived to limit the likelihood of misdiagnosis and diagnostic uncertainty. The team did however acknowledge that, in light of the fact the MRI-targeted biopsies were performed right before the systematic biopsies, haemorrhage tracks might have influenced the performance of the latter.

Dr Paolo Dell'Oglio of IRCCS San Raffaele Scientific Institute in Milan, Italy, when considering the case for combination screening, chimed that: "The combination of MRI-targeted biopsy and transrectal ultrasound-guided biopsy should strongly be considered the best available approach to reduce the risk of clinically significant prostate cancer misdiagnosis and to provide the most reliable depiction of prostate cancer multifocality."