EMJ

December 2019 Vol 4.4 ISSN: 2397-6764

+ ONCOLOGY

Boosting Cancer Immunotherapies with Optical Biosensor Nanotechnologies

+ NEUROLOGY

A Case of Lance-Adams Syndrome Post Life-Threatening Bronchial Asthma

+ CARDIOLOGY

Cardiovascular Complications in Pre-eclampsia: Can they be Predicted Electrocardiographically?

THE EUROPEAN MEDICAL JOURNAL

+ EDITOR'S PICK

Identifying Shared Features and Addressing Common Challenges in Clinical Trials for Chronic Inflammatory Diseases: An Overview

SHARED RESULTS

SHARED RELIEF

NOW ALSO APPROVED FOR PATIENTS WITH MODERATE-TO-SEVERE ATOPIC DERMATITIS AGED 12–17

- First and only therapy that **specifically targets IL-4 and IL-13**, key drivers of persistent underlying Type 2 inflammation[1,2]
- **Rapid and sustained improvement** in lesion extent and severity, pruritus intensity and quality-of-life measures[1,3–5]
- Demonstrated a **consistent safety profile** in adults and adolescents[1]
  - — No monitoring for organ toxicities required[1]
  - Most common adverse reactions were injection site reactions, conjunctivitis, blepharitis, and oral herpes[1]

DUPIXENT is indicated for the treatment of moderate-to-severe atopic dermatitis in adults and adolescents 12 years and older who are candidates for systemic therapy.

Abbreviated Prescribing Information can be found here.

▼ This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspectedadverse reactions.

References: 1. DUPIXENT summary of product characteristics. 2019. 2. Gandhi NA et al. Nature Rev Drug Disc 2016; 15: 35–50. 3. Blauvelt A et al. Lancet 2017; 389: 2287–2303. 4. de Bruin-Weller M et al. Presentation at 27th EADV Congress; 2018; September 12-16; Paris, France. 5. Simpson EL et al. JAMA Dermatol 2019. [Epub ahead of print].

SANOFI GENZYME REGENERON

▼DUPIXENT® (dupilumab)

kyntheum®▼

Brodalumab

CONFIDENCE STARTS WITH CLEARANCE

Kyntheum® (brodalumab) is indicated for the treatment of moderate to severe plaque psoriasis in adult patients who are candidates for systemic therapy.[1] Kyntheum® is a fully human monoclonal antibody and the only biologic that selectively targets the IL-17 receptor subunit A.[1,2]

PSORIASIS

Is PASI 100 worth fighting for?*

Abbreviated Prescribing Information for Kyntheum® 210mg solution for injection in pre-filled syringe Please refer to the full Summary of Product Characteristics (SmPC) approved in your country before prescribing. ▼This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions. **Indication:** Treatment of moderate to severe plaque psoriasis in adult patients who are candidates for systemic therapy. **Active ingredient:** Each pre-filled syringe contains 210mg brodalumab in 1.5ml solution. 1ml solution contains 140mg brodalumab. **Dosage and administration:** *Posology: Adults:* The recommended dose is 210mg administered by subcutaneous injection at weeks 0, 1, and 2 followed by 210mg every 2 weeks. Consideration should be given to discontinuing treatment in patients who have shown no response after 12-16 weeks of treatment. Some patients with initial partial response may subsequently improve with continued treatment beyond 16 weeks. Each pre-filled syringe is for single use only. *Elderly:* No dose adjustment recommended. *Hepatic and renal impairment:* No dose recommendations can be made. *Children and adolescents below the age of 18 years:* Safety and efficacy of Kyntheum have not been established. *Method of administration:* Subcutaneous (SC) injection. Kyntheum should not be injected into areas where the skin is tender, bruised, red, hard, thick, scaly, or affected by psoriasis. The pre-filled syringe must not be shaken. After proper training in SC injection technique, patients may self-inject Kyntheum when deemed appropriate by a physician. Patients should be instructed to inject the full amount of Kyntheum according to the instructions provided in the package leaflet. **Contraindications:** Hypersensitivity to the active substance or to any of the excipients. Active Crohn's disease. Clinically important active infections (e.g. active tuberculosis). **Precautions and warnings:** *Crohn's disease:* Exercise caution when prescribing Kyntheum to patients with a history of Crohn's disease. They should be followed for signs and symptoms of active Crohn's disease. If patients develop active Crohn's disease, treatment should be discontinued permanently. *Suicidal ideation and behaviour:* Suicidal ideation and behaviour, including completed suicide, have been reported in patients treated with Kyntheum. The majority of patients with suicidal behaviour had a history of depression and/or suicidal ideation or behaviour. A causal association between treatment with Kyntheum and increased risk of suicidal ideation and behaviour has not been established. Carefully weigh the risk and benefit of treatment with Kyntheum for patients with a history of depression and/or suicidal ideation or behaviour, or patients who develop such symptoms. Patients, caregivers and families should be advised of the need to be alert for the emergence or worsening of depression, suicidal ideation, anxiety, or other mood changes, and they should contact their healthcare provider if such events occur. If a patient suffers from new or worsening symptoms of depression and/or suicidal ideation or behaviour is identified, it is recommended to discontinue treatment with Kyntheum. *Infections:* Kyntheum may increase the risk of infections. Caution should be exercised when considering the use of Kyntheum in patients with a chronic infection or a history of recurrent infection. Patients should be instructed to seek medical advice if signs or symptoms suggestive of an infection occur. If a patient develops a serious infection, they should be closely monitored and Kyntheum should not be administered until the infection resolves. Kyntheum should not be given to patients with active tuberculosis. Anti-tuberculosis therapy should be considered prior to initiation of Kyntheum in patients with latent tuberculosis. *Reduced absolute neutrophil count:* A decrease in absolute neutrophil count, generally transient and reversible, has been observed in 5.6% of patients receiving Kyntheum. *Vaccinations:* It is recommended that patients be brought up-to-date with all immunisations in accordance with local immunisation guidelines prior to initiation of treatment with Kyntheum. Live vaccines should not be given concurrently with Kyntheum. The safety and efficacy of Kyntheum in combination with immunosuppressants, including biologics, or phototherapy have not been evaluated. **Drug interactions:** Live vaccines should not be given concurrently with Kyntheum. **Fertility, pregnancy and lactation:** *Women of childbearing potential:* Use an effective method of contraception during treatment and for at least 12 weeks after treatment. *Pregnancy:* There are no or limited amount of data from the use of brodalumab in pregnant women. As a precautionary measure, it is preferable to avoid the use of Kyntheum in pregnancy. Benefit risk for exposure of the infant to live vaccines following third trimester exposure to Kyntheum should be discussed with a physician. *Breast-feeding:* It is unknown whether brodalumab is excreted in human milk. A risk to the newborns/infants cannot be excluded. Whether to discontinue breast-feeding or discontinue Kyntheum therapy should be decided, taking into account the benefit of breast-feeding for the child and the benefit of therapy for the woman. *Fertility:* No data are available on the effect of brodalumab on human fertility. **Adverse reactions:** *Common (≥1/100 to <1/10):* Influenza, tinea infections (including tinea pedis, tinea versicolor, tinea cruris), neutropenia, headache, oropharyngeal pain, diarrhoea, nausea, arthralgia, myalgia, fatigue, injection site reactions (including injection site erythema, pain, pruritus, bruising, haemorrhage). *Uncommon (≥1/1,000 to <1/100):* Candida infections (including oral, genital and oesophageal infections), conjunctivitis. **See SmPC for a full list of adverse reactions. Precautions for storage:** Store in a refrigerator (2°C-8°C). Do not freeze. Keep the pre-filled syringe in the outer carton in order to protect from light. Kyntheum may be stored at room temperature (up to 25°C) once, in the outer carton, for a maximum single period of 14 days. Once Kyntheum has been removed from the refrigerator and has reached room temperature (up to 25°C) it must either be used within 14 days or discarded. **Marketing authorisation number and holder:** EU/1/16/1155/001, LEO Pharma A/S, Ballerup, Denmark. **Last revised:** November 2018

Reporting of Suspected Adverse Reactions
Adverse reactions should be reported according to local guidelines.

® Registered trademark

References:
1. Kyntheum® (brodalumab) Summary of Product Characteristics. English version, November 2018.
2. Campa M, et al. *Dermatol Ther* 2016;6:1–12.
3. Lebwohl M, et al. *N Engl J Med* 2015;373:1318–28.

*PASI 100 at 12 weeks with Kyntheum®: 44% in AMAGINE-2 and 37% in AMAGINE-3.[3]

November 2019
MAT-21979

LEO

Contents

"EMJ 4.4 is a celebration of triumphant advancements throughout an outstanding year."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

Aims and Scope

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

EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

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

EMJ is published quarterly and comprises review articles, case reports, practice guides, theoretical discussions, and original research.

EMJ also publishes 16 therapeutic area journals, which provide concise coverage of salient developments at the leading European congresses. These are published annually, approximately 6 weeks after the relevant congress. Further details can be found on our website: www.europeanmedical-journal.com

Editorial Expertise

EMJ is supported by various levels of expertise:

- Guidance from an Editorial Board consisting of leading authorities from a wide variety of disciplines.
- Invited contributors are recognised authorities from their respective fields.
- Peer review, which is conducted by EMJ's Peer Review Panel as well as other experts appointed due to their knowledge of a specific topic.
- An experienced team of editors and technical editors.

Peer Review

On submission, all articles are assessed by the editorial team to determine their suitability for the journal and appropriateness for peer review.

Editorial staff, following consultation with either a member of the Editorial Board or the author(s) if necessary, identify three appropriate reviewers, who are selected based on their specialist knowledge in the relevant area.

All peer review is double blind.

Following review, papers are either accepted without modification, returned to the author(s) to incorporate required changes, or rejected.

Editorial staff have final discretion over any proposed amendments.

Submissions

We welcome contributions from professionals, consultants, academics, and industry leaders on relevant and topical subjects.

We seek papers with the most current, interesting, and relevant information in each therapeutic area and accept original research, review articles, case reports, and features.

We are always keen to hear from healthcare professionals wishing to discuss potential submissions, please email: editorial.assistant@emjreviews.com

To submit a paper, use our online submission site: www.editorialmanager.com/e-m-j

Submission details can be found through our website: www.europeanmedical-journal.com/contributors/authors

Reprints

All articles included in EMJ are available as reprints (minimum order 1,000). Please contact hello@europeanmedical-journal.com if you would like to order reprints.

Distribution and Readership

EMJ is distributed through controlled circulation to healthcare professionals in the relevant fields across Europe.

Indexing and Availability

EMJ is indexed on DOAJ, the Royal Society of Medicine, and Google Scholar®; selected articles are indexed in PubMed Central®.

EMJ is available through the websites of our leading partners and collaborating societies.

EMJ journals are all available via our website: www.europeanmedical-journal.com

Open Access

Congress Notice

Staff members attend medical congresses as reporters when required.

This Publication

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

EMJ 4.4

EMJ 4.3 2019

In this edition you will find a selection of peer-reviewed articles covering the latest developments across therapeutic areas including rheumatology, hepatology, plus more.

VIEW ALL JOURNALS ←

Welcome

The succession of publications in 2019 here at EMJ fittingly culminates in our final flagship journal of the year. EMJ 4.4 is a selection box of assorted scientific morsels for readers with a hunger for academic excellence. EMJ is expanding at an exponential rate and we expect no less in the coming year; in light of this, we invite you to join us in any capacity as a reader, writer, author, researcher, or student for your opinion and contribution as we continue go from strength to strength.

The festive period is upon us and there is plenty to be merry about in the following journal. Here you can find peer-reviewed articles on neurology, hepatology, respiratory, and much more. Authors of the Editor's pick in EMJ 4.4, La Noce and Ernst, investigate clinical trials for the treatment of chronic inflammatory diseases. Challenges which arise when identifying biologic drugs for multiple chronic inflammatory diseases with similar manifestations are tackled, as well as consideration of how successful trials for these can be executed. Kim has penned a case report detailing the rare Lance-Adams syndrome in a patient who experienced cardiorespiratory collapse after life-threatening bronchial asthma and an analytical study by Raharjo et al. investigated the role of ECG as a means of predicting cardiovascular complications in pre-eclampsia.

It's the most wonderful time of the year to enjoy breaking news stories on cutting-edge research in dermatology, haematology, and urology. Discover the use of rapamycin to decrease the rate of ageing in human skin and the physical effect of its topical delivery; the prognostic value of difficult-to-detect circulating clonal plasma cells in multiple myeloma as a noninvasive method for early detection or monitoring of progression; and, as telehealth continues to rise in prevalence, read about the use of a recently U.S. Food and Drug Administration (FDA)-approved mobile app that will allow patients to carry out urinary tract infection testing at home.

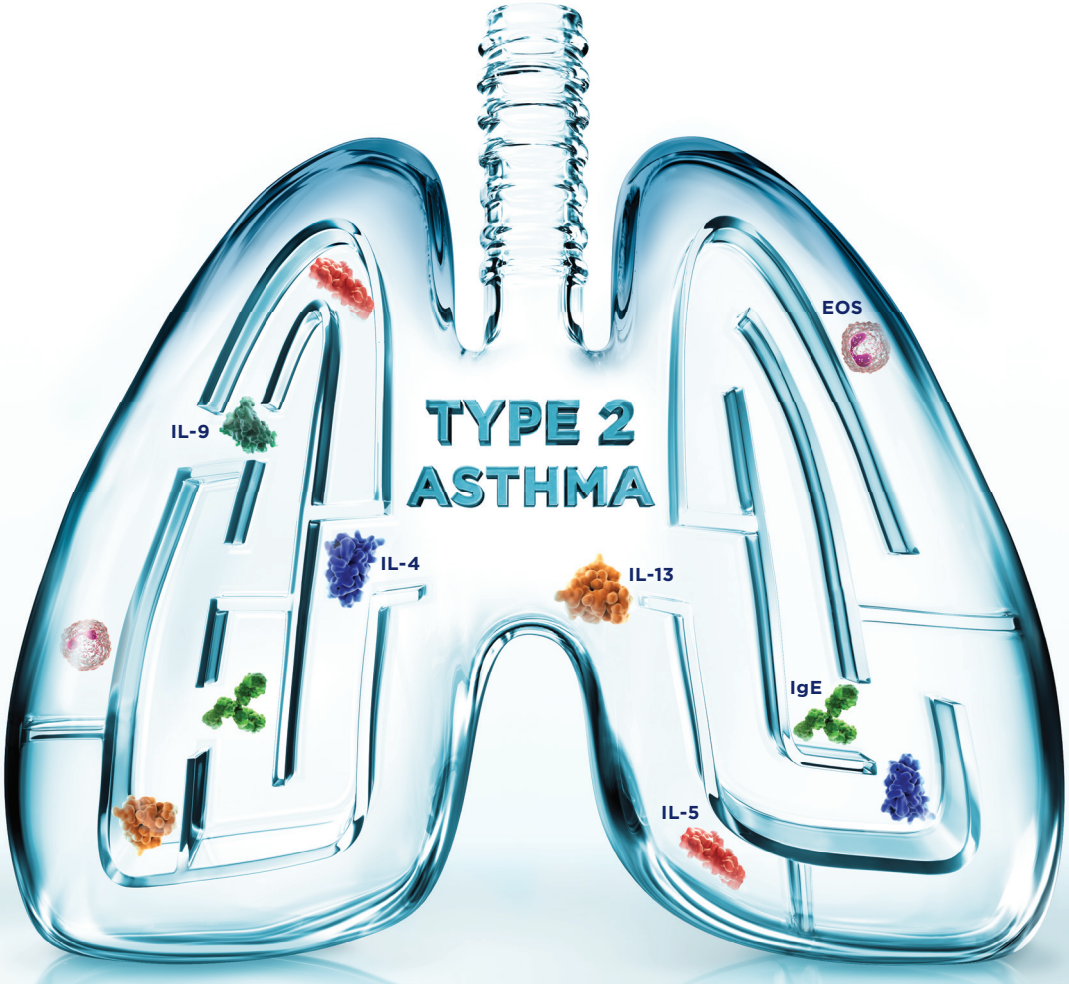
EMJ 4.4 is a celebration of triumphant advancements throughout an outstanding year and our spirited elves have been working hard to ensure the year goes out with a festive bang. We thank you for your continued support, contribution, and readership and we hope you enjoy our latest and last publication of the year. Happy holidays!

Spencer Gore

Chief Executive Officer, European Medical Group

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.

Sanofi Genzyme and Regeneron are committed to providing resources to advance research in areas of unmet medical need among patients with inflammatory and immunologic diseases.

Sanofi Genzyme, AHTC building, Paasheuvelweg 25, 1105 BP, AMSTERDAM, The Netherlands.

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

SANOFI GENZYME

Foreword

Dear Colleagues,

It is my great pleasure to welcome you to *EMJ 4.4*, celebrating the very best of medical advancements and research from across a plethora of fields and specialisms. The papers contained within these papers exemplify the dedication and passion that the EMJ team have for the dissemination of high-quality medical literature; I hope you will be inspired by the messages from these world-class authors.

As we come to reflect on the progress and achievements of 2019, the healthcare field is one in which we can all take great pride. It's been an exciting year: who could forget the exciting news from oncologists that a simple blood test could help us to detect and even predict cancer? Or the results from two clinical trials that showed positive results for a triple-combination drug to treat cystic fibrosis? And of course, CRISPR continues to astound us with the possibilities for treating a wide range of health problems. As healthcare professionals from all walks of life and all medical specialities, I know that each and every one of our contributors and readers are making a great difference to the lives of their patients.

The articles and news stories in this journal have something for everyone: gastroenterology, respiratory, cardiology, and allergy and immunology are all topics that feature amongst many more. The Editor's Pick is an exploration of the common challenges in clinical trial planning and implementation for chronic inflammatory diseases. La Noce and Ernst present this fascinating review alongside their suggestions for overcoming such challenges and getting the most out of the trials that are saving lives.

All that remains is for me to wish all of our contributors and readers a very joyous and peaceful holiday season. I hope that this journal will provide food for thought and inspire debate and discussions among colleagues and friends, and I look forward to seeing what exciting new developments will occur in 2020.

Yours Sincerely,

Professor László Vécsei

University of Szeged, Szeged, Hungary

PSA doubling time of ≤10 months in nmCRPC despite ADT?

ERLEADA® 60 mg film coated tablets ABBREVIATED PRESCRIBING INFORMATION BASED ON THE EU SUMMARY OF PRODUCT CHARACTERISTICS

ACTIVE INGREDIENT: 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 (NM-CRPC) who are at high risk of developing metastatic disease. **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. Medical castration with gonadotropin releasing hormone analogue (GnRHa) should be continued during treatment in patients not surgically castrated. If a dose is missed, it should be taken as soon as possible on the same day with a return to the normal schedule the following day. Extra tablets should not be taken to make up the missed dose. If a ≥ Grade 3 toxicity or an intolerable adverse reaction is experienced by the patient, dosing should be held rather than permanently discontinuing treatment until symptoms improve to ≤ Grade 1 or original grade, then should be resumed at the same dose or a reduced dose (180 mg or 120 mg), if warranted. **Children:** There is no relevant use of apalutamide in the paediatric population in the treatment of non-metastatic castration-resistant prostate cancer. **Elderly:** No dose adjustment is necessary for elderly patients. **Renal impairment:** No dose adjustment is necessary for patients with mild to moderate renal impairment. Caution is required in patients with severe renal impairment as apalutamide has not been studied in this patient population. If treatment is started, patients should be monitored for the adverse reactions and dose reduced as per section Dosage and administration. **Hepatic impairment:** No dose adjustment is necessary for patients with baseline mild or moderate hepatic impairment (Child-Pugh Class A and B, respectively). ERLEADA is not recommended in patients with severe hepatic impairment. **CONTRAINDICATIONS:** Pregnant women or women with potential to be pregnant. Hypersensitivity to the active substance or to any of the excipients. **SPECIAL WARNINGS & PRECAUTIONS:** ***Seizure***: ERLEADA is not recommended in patients with a history of seizures or other predisposing factors including, but not limited to underlying brain injury, recent stroke (within one year), primary brain tumours or brain metastases. 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 fractures and fall risk and should be monitored and managed continuously for fractures and use of bone-targeted agents should be considered. ***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 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. Patients on medication with ERLEADA should be advised about risk of seizures with regard to driving or operating machines. **SIDE EFFECTS:** ***Very Common***: skin rash (Skin rash associated with ERLEADA was most commonly described as macular or maculo-papular. Skin rash included rash, rash maculo-papular, rash generalised, urticaria, rash pruritic, rash macular, conjunctivitis, erythema multiforme, rash papular, skin exfoliation, genital rash, rash erythematous, stomatitis, drug eruption, mouth ulceration, rash pustular, blister, papule, pemphigoid, skin erosion, and rash vesicular), fracture (includes rib fracture, lumbar vertebral fracture, spinal compression fracture, spinal fracture, foot fracture, hip fracture, humerus fracture, thoracic vertebral fracture, upper limb fracture, fractured sacrum, hand fracture, pubis fracture, acetabulum fracture, ankle fracture, compression fracture, costal cartilage fracture, facial bones fracture, lower limb fracture, osteoporotic fracture, wrist fracture, avulsion fracture, fibula fracture, fractured coccyx, pelvic fracture, radius fracture, sternal fracture, stress fracture, traumatic fracture, cervical vertebral fracture, femoral neck fracture, tibia fracture), arthralgia, fatigue, weight decreased, fall. ***Common***: hypothyroidism (includes hypothyroidism, blood thyroid stimulating hormone increased, thyroxine decreased, autoimmune thyroiditis, thyroxine free decreased, tri-iodothyronine decreased), hypercholesterolaemia, hypertriglyceridaemia, pruritus. ***Uncommon***: seizure. ***Not known***: QT prolongation. **Refer to the SmPC for other side effects. FERTILITY/PREGNANCY/ LACTATION:** ERLEADA

ADT=androgen deprivation therapy; nmCRPC=non-metastatic castration resistant prostate cancer; PFS2=second progression-free survival; PSA=prostate-specific antigen.

*PFS2 is a novel endpoint recommended by the European Medicines Agency (EMA) for assessing outcomes of sequential therapies.[3] In the SPARTAN study, PFS2 was defined as the time from randomisation to investigator-assessed disease progression (PSA progression, detection of metastatic disease on imaging, symptomatic progression or any combination thereof) during the first subsequent treatment for mCRPC, or death from any cause.[1] **PSA doubling time ≤10 months despite ADT.[1] †PFS2: ERLEADA® prolonged PFS2 by 11.8 months (Median PFS2 for ERLEADA® + ADT: 55.6 months vs placebo: 43.8 months; HR=0.55; 95% CI=0.45–0.68; P<0.0001).[2]

Janssen Oncology

PHARMACEUTICAL COMPANIES OF Johnson & Johnson

PUSH BACK EARLY, PLAN FOR THEIR FUTURE

CHOOSE ERLEADA®, THE ONLY NOVEL ANTIANDROGEN IN nmCRPC THAT OFFERS SECOND PROGRESSION-FREE SURVIVAL (PFS2)* DATA[1–4]

With the power of ERLEADA® at hand, you can now give your patients with high-risk** nmCRPC proven sustained efficacy.[1,2,5]

ERLEADA® + ADT initiated early in nmCRPC:

- ☑ provides a sustained improvement in PFS2 vs placebo + ADT[†1,2,5]
- ☑ may delay further downstream progression[5]

is contraindicated in women who are or may become pregnant. ERLEADA may cause foetal harm when administered during pregnancy. There are no data available from the use of ERLEADA in pregnant 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. **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 classIII (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: April 2019. Based on 14 January 2019 EU Summary of Product Characteristics.

References: 1. Smith MR, *et al. N Engl J Med.* 2018;378:1408–18. 2. Small EJ, *et al. Ann Oncol.* 2019. pii: mdz397. doi: 10.1093/annonc/mdz397. [Epub ahead of print]. 3. Hussain M, *et al. N Engl J Med.* 2018;378:2465–7. 4. Fizazi K, *et al. N Engl J Med.* 2019;380(13):1235–46. 5. Oudard S and Juarex Soto A. *EMJ Urol.* 2019;7(suppl 2):2–11. 6. European Medicines Agency (EMA). Appendix 1 to the guideline on the evaluation of anticancer medicinal products in man. 13 December 2012. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/01/WC500137126.pdf. Accessed October 2019.

Date of preparation: November 2019 CP-108488

Erleada® (apalutamide) tablets ▽

▽ This medicinal product is subject to additional monitoring and it is therefore important to report any suspect adverse reactions related to this medicinal product.

Management of Moderate-to-Severe Atopic Dermatitis in the Era of Targeted Treatments

This symposium took place on 11th October 2019, as part of the 28th European Academy of Dermatology and Venereology (EADV) Congress in Madrid, Spain

Chairpeople: Matthias Augustin[1]

Speakers: Eric Simpson,[2] Marjolein de Bruin-Weller[3]

1. University of Hamburg, Hamburg, Germany
2. Oregon Health & Science University, Portland, Oregon, USA
3. University Medical Centre Utrecht, Utrecht, the Netherlands

Disclosure: Prof Augustin has received grants and/or honoraria as a consultant, speaker, and/or advisory board member from 3M, AbbVie, ALK-Abello, Almirall-Hermal, Amgen, AOK BV, AOK BW, Astellas, Barmenia, BarmerGEK, Basilea, Bayer Healthcare, Bauerfeind, Beiersdorf, Biogen, Birken, Boehringer Ingelheim, BMS, BSN, BVDD, Bvmed, Celgene, Centocor, Coloplast, DAK, DDG, Derma2go, Diabet concept, DPB, Geko, Gerromed, GlaxoSmithKline, Heigel.com, Hirschen Group, Janssen-Cilag, Johnson & Johnson, KCI, Leo, Lilly, Medac, Medi, Medovent, Menlo, Merck-Serono, Mölnlycke, MSD, Mundipharma, Novartis, NHS Trust, Pfizer, Pharmafacts, Pohl-Boskamp, Regeneron, Roche, Sandoz, Sanofi, Spirig, Schülke & Mayr, Söring, Stiefel, Swiss4Ward, Systagenix, Techniker, TFS, Tissuetherapies, Trevi, UCB, Uluru, Videoclinic, and Xenoport. Dr Simpson has received grants and/or personal fees from AbbVie, Eli Lilly, Galderma, Kyowa Hakko Kirin, LEO Pharma, Merck, Pfizer, Regeneron, Sanofi, Chugai, Genentech, MedImmune, Novartis, Tioga, Vanda, Amgen, Celgene, Boehringer-Ingelheim, Dermavant, Forte Bio, Incyte, Menlo Therapeutics, Pierre Fabre Dermo Cosmetique, Valeant, Roivant, and Anacor. Dr de Bruin-Weller has been a consultant/advisor for AbbVie, Regeneron, Sanofi Genzyme, Eli Lilly, UCB, and Pfizer; and has received grant/research support from Regeneron and Sanofi Genzyme.

Acknowledgements: Writing assistance was provided by Sam Coates, Sam Coates Medical Writing, Reading, UK.

Support: The publication of this article was funded by Sanofi Genzyme and Regeneron. The symposium on which this article is based was funded and organised by Sanofi Genzyme and Regeneron. The views and opinions expressed are those of the presenters. Content was reviewed by Sanofi Genzyme and Regeneron for medical accuracy.

Citation: EMJ. 2019;4[4]:14-23.

Meeting Summary

Prof Augustin opened the symposium by underlining how the management of patients with moderate-to-severe atopic dermatitis (AD) has become more dynamic over the past 2 years following the approval of the first biologic agent, dupilumab, in 2017. Prof Augustin explained that moderate-to-severe AD is a chronic Type II inflammatory disease that has significant effects on patients' and caregivers' lives. The multidimensional disease burden of AD, which includes signs and symptoms that impact physical, mental, social wellbeing, and quality of life (QoL), is proportional to disease severity

and lack of disease control. Sustained control of moderate-to-severe AD is essential to limit the burden caused by the disease. In the second presentation, Dr Simpson emphasised the importance of pointing out to each patient that AD is a chronic disease that requires long-term management. When discussing goals and treatment options with each patient, the importance of sustainable disease management should be emphasised. He presented outcomes from recent clinical trials investigating the long-term efficacy and safety of targeted agents in patients with AD. In the final presentation, Dr de Bruin-Weller discussed the importance of real-world evidence when considering treatment options for patients with AD. Real-world evidence for the effectiveness and tolerability of treatments can be gleaned from a number of sources, including registry-based clinical experience, survey data, centre-based clinical experience, and case studies. Consideration of real-world evidence, alongside outcomes from randomised controlled trials, enables selection of the most appropriate treatment option for each patient.

Burden of Moderate-to-Severe Atopic Dermatitis in Adult and Adolescent Patients

Professor Matthias Augustin

AD is a chronic Type II inflammatory disease characterised by dry and scaly eczematous lesions accompanied by intense itching.[1] Signs and symptoms of AD include xerosis (dryness), diffuse erythematous patches, oozing papulovesicles, lichenified and excoriated plaques, and frequent and intense pruritus (itch).[1]

Atopic Dermatitis is a Highly Prevalent Dermatological Condition Affecting Children and Adults Worldwide

AD most often starts in childhood but is highly prevalent in adults.[2,3] A recent international survey reported point prevalence (95% confidence intervals) of adult AD in the overall population of 4.9% (4.6–5.2%) in the USA, 3.5% (3.1–3.9%) in Canada, 4.4% (4.2–4.6%) in the European Union (EU), and 2.1% (1.8–2.3%) in Japan.[4] AD prevalence was generally lower for males versus females, and decreased with age.[4] In addition, 46–55% of adult patients had moderate-to-severe AD, as measured by the Patient-Oriented Eczema Measure (POEM).[4]

Prof Augustin explained that AD prevalence is higher in children than adults and has been increasing over recent decades. In fact, AD is the most common chronic inflammatory dermatosis in paediatric patients in industrialised countries, with prevalence rates in North America of 8.5–9.1%, the EU of 6.1–10.4%, and Japan of 10.5–16.9%.[5-8] Approximately, 10–40% of children with AD have moderate-to-severe disease.[7,9-15]

The Burden of Atopic Dermatitis is Substantial and Multidimensional

Patients with AD experience a multidimensional burden of disease (Figure 1). In addition to visible skin lesions[16] and symptoms that include severe pruritus, pain, and sleep disturbance,[17] AD impacts negatively on QoL,[17-19] social activities,[19] and work productivity.[20] AD is associated with mental health disorders, such as anxiety and depression,[17,21-24] and a number of comorbid conditions, including skin infections[25-29] and Type II inflammatory diseases.[17] Indeed, data from AD clinical trials show substantial prevalence of asthma (8.0–42.7%), allergic rhinitis (34.5–50.6%), chronic rhinosinusitis or nasal polyps (2.6%), and food allergy/intolerance (17.4–38.4%) in adult patients with moderate-to-severe AD.[30-32]

The Burden of Atopic Dermatitis Increases with Disease Severity

AWARE is an international, cross-sectional, observational study of adults with AD that aims to compare patient-reported burden across disease severity levels.[33] Significantly increased measures of pruritus, sleep disturbance, and negative impact on QoL were reported with greater disease severity based on Investigator's Global Assessment (IGA).[33] In addition, the presence of coexisting Type II inflammatory diseases, including asthma and food allergies, also increased with greater disease severity.[33] Analysis of work productivity in adult patients showed that even mild AD led to a mean number of 11.2 work/study days missed per year, with even more days missed in patients with moderate disease (24.7 days) and severe disease (45.4 days) ($p<0.001$ for mild and moderate versus severe AD).[34]

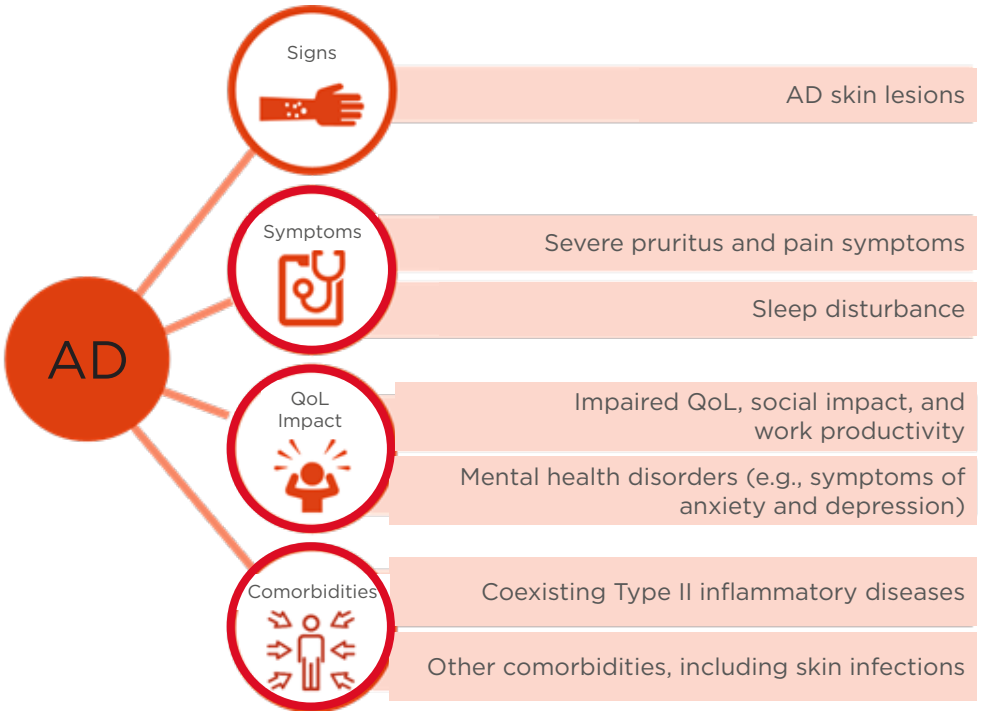


Figure 1: Patients with atopic dermatitis experience a multidimensional burden of disease.

In addition to visible skin lesions and symptoms that include severe pruritus, pain, and sleep disturbance, atopic dermatitis impacts negatively on quality of life, social activities, and work productivity. Atopic dermatitis is associated with mental health disorders such as anxiety and depression, as well as a number of comorbid conditions, including skin infections and Type II inflammatory diseases.

AD: atopic dermatitis; QoL: quality of life.

Additionally, significantly more patients with moderate or severe disease reported that AD influenced their career choice and had a negative effect on their pursuit of education compared with patients with mild AD.[34]

Children and adolescents with AD also experience a high burden of disease. Data from a German health insurance database showed increased prevalence of skin conditions (i.e., impetigo, vitiligo), Type II inflammatory diseases (i.e., bronchial asthma, allergic rhinitis), arthritis, and mental health disorders (i.e., depression) in patients aged <18 years compared with age-matched counterparts without AD.[8] In a Phase III study (N=251), 92% of patients aged 12–17 years with moderate-to-severe AD (n=230) had a self-reported history of ≥1 coexisting Type II inflammatory disease at baseline, including allergic rhinitis (65.6%), food allergy (60.8%), asthma (53.6%), hives (28.8%), and allergic conjunctivitis (22.8%).[35] In addition, QoL and social development were significantly impacted in this patient population, with a high proportion of patients reporting affected sleep, feelings of embarrassment and self-consciousness, and a negative impact on clothing choices, leisure activities, and social interactions, including friendships.[35] As with adults, the impact on QoL in adolescent patients with AD increases with greater disease severity, as does the impact on caregiver QoL and the number of missed school days.[36] This high burden experienced by children and adolescents with AD highlights a need for effective early intervention for this condition.

What are the Unmet Needs in Patients with Atopic Dermatitis?

Disease control is of great importance in patients with AD; inadequate control of AD is common and is associated with increased disease burden. The rate of inadequate disease control significantly increases with AD severity: 45.1% in patients with mild disease; 64.2% of patients with moderate disease; and 84.9% of those with severe disease.[37] Many patients report dissatisfaction with

commonly available AD treatments; adult patients in the USA reported low satisfaction with topical treatments, systemic immunosuppressants, and phototherapy, with 48.1% of survey respondents 'extremely' or 'very' dissatisfied.[38] In the EU, systemic immunosuppressants are commonly used to treat AD. These agents are associated with multiple risks that can contribute to patient burden, including lymphomas and other malignancies, osteoporosis, diabetes, glaucoma, infections such as eczema herpeticum, and hospitalisations.[39] In summary, there is an ongoing need for a safe and effective targeted treatment that provides sustained disease control for patients with moderate-to-severe AD.

Sustainable Management of Moderate-to-Severe Atopic Dermatitis Using Targeted Therapies: Emerging Data from Clinical Trials

Doctor Eric Simpson

Dr Simpson highlighted the importance of achieving sustained management of AD and the ongoing requirement for therapies that are safe and effective for both short and long-term use. He emphasised that the first step to sustainable management of AD is to talk to the patient about their treatment goals and preferences. Every patient is different, but the patient needs to realise that AD is a chronic disease that will require long-term treatment.

There is a paucity of data on the long-term use of systemic immunosuppressive drugs for the treatment of AD. In some countries, there are limits on the length of administration for some agents, for example in Japan the limit for cyclosporine (CsA) use is 8–12 weeks.[40] Guidelines published by the European Academy of Dermatology and Venereology (EADV) recommend cessation of CsA after 2 years, with careful monitoring for potential severe side effects.[41]

Recent advances in immunology research are driving the development of novel systemic therapies for AD, and a number of novel agents are now in Phase II and III clinical trials. Given the need for long-term AD treatment, Dr Simpson focussed on novel therapies with available evidence for long-term efficacy and safety. Two drugs were discussed: the approved therapy dupilumab, and the investigational therapy nemolizumab.

Dupilumab is a fully human IL-4 receptor α monoclonal antibody that potently inhibits signalling of IL-4 and IL-13, leading to decreased numbers of Type 2 cytokines implicated in numerous allergic diseases ranging from asthma to AD.[42,43] Dupilumab is the first approved biologic agent that targets Type II inflammation in patients with AD and is approved for the treatment of moderate-to-severe AD in adults and patients aged 12–17 years.[42] Efficacy and safety data for 52 weeks' treatment in a Phase III trial, as well as efficacy and safety data for 76 weeks' treatment in an open-label extension study, are available for dupilumab.[31,43]

Nemolizumab is an investigational drug that targets signalling pathways involved in pruritus via inhibition of IL-31.[30] Data from a 64-week open label Phase II trial are available in a limited number of patients.[44] A Phase III nemolizumab clinical trial is currently recruiting.[45]

Available Evidence for Sustainable Management of Atopic Dermatitis with Dupilumab

In the 1-year, randomised, double-blind, placebo-controlled CHRONOS study, treatment with dupilumab demonstrated significant and sustained improvements versus placebo in the proportion of patients achieving 75% improvement from baseline using the Eczema Area and Severity Index (EASI-75) at Week 16 that were sustained until Week 52.[43] In addition, dupilumab treatment resulted in rapid and sustained improvements in itch and sleep loss,[46] and a lower proportion of dupilumab-treated patients required rescue medication versus those who received placebo through Week 52.[43] Dupilumab was well tolerated during and up to 52 weeks of treatment, with nasopharyngitis, injection-site reactions (mainly pain and redness), and conjunctivitis comprising the main adverse events (AE) of interest.[43] Dr Simpson stated that the majority of conjunctivitis cases reported in dupilumab-treated patients were mild-to-moderate in severity and responded to treatment with anti-inflammatory agents. Furthermore, the incidence of conjunctivitis did not increase over time. The incidence of non-

herpetic skin infections in CHRONOS was lower in the groups receiving dupilumab (11% and 8%) than in the placebo group (18%).[43]

An open-label extension study is currently assessing additional long-term safety and efficacy of dupilumab in adults with mild-to-moderate AD. This is a heterogeneous patient population, enrolling patients who had participated or been screened in 13 prior dupilumab AD studies (3 Phase Ib studies, 5 Phase II studies, and 5 Phase III studies), and includes patients who originally received placebo.[31] By Week 76, from the total study population of 1,491 patients who received at least 1 dose of dupilumab, 1.8% of patients had discontinued dupilumab.[31] There were rapid and sustained improvements in mean EASI score through Week 76 in both dupilumab-naïve and dupilumab-retreated patients (Figure 2A).[31] There were also improvements in AD signs and symptoms and patient QoL at Weeks 52 and 76 with dupilumab treatment (Figure 2B).[31] Over 76 weeks, the dupilumab safety profile was consistent with previous studies, including CHRONOS.[31,43]

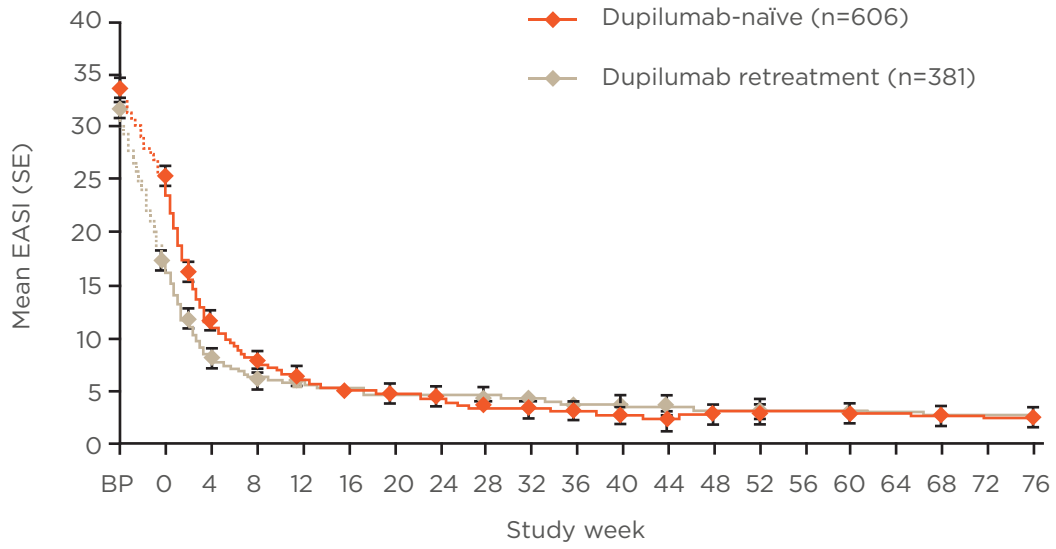
Dr Simpson presented a pooled analysis of safety data from seven Phase II and Phase III studies (n=1,841 for all dupilumab doses combined), and confirmed that dupilumab is not an immunosuppressant, with the overall number of treatment-emergent infections comparable to those observed in patients who received placebo (n=1,091).[47] Skin structure and soft tissue infections (risk ratio: 0.44; p<0.001) and clinically important herpes viral infections (eczema herpeticum and herpes zoster) (risk ratio: 0.31; p<0.01) were less common with dupilumab compared with placebo, respectively.[47]

In addition to clinical trials in adults, dupilumab has also been investigated in adolescent patients. The coprimary endpoints in a Phase III monotherapy study[48] were both met, with dupilumab (300 mg every 4 weeks [n=84] or 200/300 mg every 2 weeks [n=82]) treatment significantly improving IGA and EASI-75 response rates at Week 16 compared with placebo (n=85) in patients aged 12–17 years.[49] In addition, the dupilumab safety profile in the adolescent population was consistent with that observed in adult patients.[49] Consequently, in an open-label extension to the Phase III study that enrolled 36 adolescent patients with moderate-to-severe AD, sustained improvement in EASI-75 was observed in patients treated with dupilumab through Week 52.[50] In addition, there was sustained improvement in mean peak pruritus numeric rating scale (NRS) score over the same timeframe, with a 66% reduction from baseline.[50] The dupilumab safety profile in this open-label extension cohort was consistent with a previous Phase IIb study, and there were no treatment discontinuations resulting from AE.[50]

Available Evidence for Sustainable Management of Atopic Dermatitis with Investigational Therapies

Nemolizumab is a humanised monoclonal antibody against IL-31 receptor α that binds to IL-31 receptor α on a number of cells, including neurons, to inhibit IL-31 signalling, which may alleviate pruritus.[30] As it is the only investigational agent in AD currently with long-term (≥52 weeks) data available, Dr Simpson provided an overview of outcomes from a nemolizumab Phase II clinical trial. In the two-part study, three separate dosing regimens of nemolizumab resulted in improved signs and symptoms of AD versus placebo at Week 12.[30] A total of 191 nemolizumab-treated patients transitioned from the placebo-controlled phase of the study to the open-label phase.[44] During the open-label phase, 31.4% of nemolizumab-treated patients discontinued treatment by Week 64, leading to concerns that the remaining population may be those that respond best to this treatment. At Week 64, improvements in pruritus visual analogue scale score and mean EASI score were observed with all nemolizumab dosing schedules, although exacerbation of AD was reported in 21–28% of patients.[44] Nemolizumab was generally well tolerated and the safety profile in the 52-week long-term extension was comparable with that observed in the placebo-controlled period; the most common treatment-related AE were nasopharyngitis, AD exacerbation, increase in blood creatinine phosphokinase, upper respiratory tract infection, peripheral oedema, and injection-site reactions.[44]

A



B

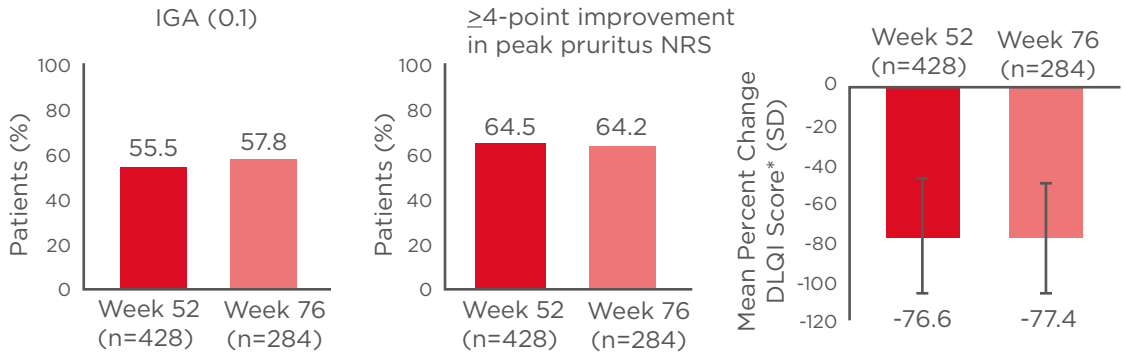


Figure 2: Sustained improvements in (A) mean EASI score over 76 weeks†, and (B) signs, symptoms, and quality of life at Week 52‡ and Week 76¶ with dupilumab treatment in an open-label extension study.

†Efficacy analyses were performed including all patients who reached the respective timepoint or would have reached that timepoint had they not discontinued earlier. Sensitivity analyses (e.g., multiple imputations) were consistent with efficacy in all observed patients, suggesting no bias on treatment outcomes due to patient withdrawal.

‡Efficacy at Week 52 observed for Week 52 cohort, which included all patients enrolled at least 53 weeks prior to data cutoff (accounting for ±1 week visit window).

¶Efficacy at Week 76 observed for Week 76 cohort, which included all patients enrolled at least 77 weeks prior to data cutoff (accounting for ±1 week visit window).

*DLQI was assessed every 12 weeks with the last timepoint for this analysis at Week 48.

DLQI: Dermatology Life Quality Index; EASI: Eczema Area and Severity Index; IGA: Investigator's Global Assessment; NRS: numeric rating scale; SD: standard deviation; SE: standard error.

Both figures adapted from Deleuran et al.[31]

Advances in the Management of Moderate-to-Severe Atopic Dermatitis in the Real World

Doctor Marjolein de Bruin-Weller

In the final symposium presentation, Dr de Bruin-Weller explained the importance of real-world evidence when considering treatment options for patients with AD. Patient populations in randomised, controlled trials differ from those seen in the clinic; for instance, they are generally more motivated and compliant with treatment than patients seen in real-world practice. In addition, physician time and resources are likely to be more limited in the 'real world' compared with the clinical trial environment. Real-world evidence for the effectiveness and tolerability of treatments can be gleaned from a number of sources, including registry-based clinical experience, survey data, centre-based clinical experience, and case studies.

Registry-Based Clinical Experience in Atopic Dermatitis

Data analyses from registries, especially prospective registries, are very useful for assessing the long-term efficacy and safety of treatments as they are used in daily clinical practice. There are a number of AD registries worldwide and Dr de Bruin-Weller provided an overview of three European-based registries: EUROSTAD,[51] TREATgermany,[52,53] and BioDay.[54]

EUROSTAD is a 5-year, observational, industry-sponsored study being performed in 10 European countries that is investigating patient characteristics and disease management in 308 adult patients with mostly moderate-to-severe AD.[51] At enrolment, 53.5% of patients were IGA 3 and 31.6% were IGA 4; the mean EASI score was 16.3. The most commonly prescribed systemic therapy at enrolment was CsA (40.5% of patients), with 23.3% of patients receiving methotrexate, 19.0% receiving corticosteroids, and 18.3% receiving dupilumab.[51] Outcomes from EUROSTAD are not yet available but are awaited with great interest.

TREATgermany is a long-term, prospective, industry-sponsored registry investigating the use, effectiveness, and safety of treatments for moderate-to-severe AD in >30 centres in Germany.[52,53] This registry is an initiative of the German Society of Dermatology (DDG) and is academia-led. Clinicians, and their patients with moderate-to-severe AD, complete assessments every 3–6 months for ≥2 years. In patients enrolled by 2014 (i.e., prior to the availability of dupilumab) (n=78), CsA was the most commonly prescribed systemic treatment and was received by 33.3% of patients.[53] In patients who were prescribed CsA (n=35), EASI-75 was achieved by 34% of patients at Weeks 12–24. Over the same time frame, there was no significant impact of CsA treatment on POEM or Dermatology Life Quality Index (DLQI) scores.[53] A second analysis, performed on data collected from June 2017 to June 2019, included real-world usage of dupilumab. Patients who received dupilumab showed mean reduction in EASI score at 12 weeks of 74.2% (n=105), which was stable through Week 24 (n=53). In addition, EASI-75 was achieved in 57.1% of patients by Week 12 and in 51.9% of patients at Week 24.[55] Improvements in AD symptoms (measured via POEM) and QoL (measured via DLQI) were achieved by Week 12 (both $p<0.001$ versus baseline) and maintained at Week 24.[55,56] The dupilumab safety profile in this real-world data analysis was consistent with clinical trial data: conjunctivitis was the most frequently reported AE (reported by 13.3% of patients at Week 12 and by 29.6% of patients at Week 24).[56]

The BioDay registry is a prospective, academia-led, industry-sponsored registry involving four university hospitals and seven regional hospitals in the Netherlands that commenced in January 2018. Initial results in 138 patients reported dupilumab efficacy consistent with that demonstrated in clinical trials, with mean reduction in EASI score from baseline of 73.1% and EASI-75 achieved by 61.7% of patients at Week 16.[54] Improvements in pruritus NRS and POEM were also consistent with those observed in the CHRONOS and CAFE clinical trials.[55] In a 'responder' algorithm developed by the investigators, 89.0% of patients achieved at least 1 of the following after 16 weeks treatment with dupilumab:[54]

- EASI-75
- pruritus NRS ≥4-point improvement
- DLQI ≥4-point improvement

In this patient population, in which 64.5% of patients reported a history of allergic conjunctivitis

at study baseline, 34.0% reported allergic conjunctivitis following dupilumab treatment. A total of 20.0% of patients experienced moderate-to-severe conjunctivitis that required treatment with anti-inflammatory agents.[54]

Centre-Based Clinical Experience in Atopic Dermatitis

Centre-based clinical studies provide outcomes from daily clinical practice for individual countries or regions. Dr de Bruin-Weller presented centre-based clinical experience from three separate studies, of which two were retrospective (performed in Italy [N=109][57] and France [N=241],[58] respectively) and one prospective (performed in the Netherlands [N=95]).[59] Differences in baseline characteristics and outcome measurements make cross-study comparisons difficult, but in each study dupilumab treatment resulted in rapid improvement in EASI and DLQI score.[57-59]

Survey Data in Atopic Dermatitis

Data from RELIEVE-AD, a prospective, longitudinal patient survey (N=674) conducted in the USA, have recently been made available. Patients complete a survey prior to receiving dupilumab and at Months 1, 2, 3, 6, 9, and 12 post-treatment commencement. In a 6-month interim analysis, the proportion of patients who were 'very' or 'extremely' satisfied with their AD treatment increased from 2.9% at baseline to 57.8% at Month 1, and 70.9% at Month 6 (both $p<0.001$ versus baseline).[38] The proportion of patients reporting use of concomitant systemic corticosteroids was 34.7% at baseline, 7.8% at Month 1, and 9.2% at Month 6.[38] The proportion of patients reporting use of concomitant AD therapies (excluding dupilumab) from ≥3 drug categories was 13.1% at baseline, 1.7% at Month 1, and 1.5% at Month 6 (both $p<0.001$ versus baseline). Furthermore, the proportion of patients without any concomitant AD therapy increased from 12.8% at baseline to 39.6% at Month 1 and 41.3% at Month 6 (both $p<0.001$ versus baseline).[38]

Case Studies in Special Populations

Dr de Bruin-Weller highlighted the importance of considering the efficacy and safety of novel treatments in special populations that are often excluded from clinical trials, for example patients with chronic hepatitis B virus (HBV), renal failure, or HIV. Case studies can be of value when considering the use of novel treatments in such populations. Dr de Bruin-Weller discussed a case report of two patients with AD and HBV who received dupilumab whilst receiving concomitant therapy for HBV.[60] Prior to treatment with dupilumab, AD signs and symptoms were uncontrolled in both patients despite topical or systemic AD treatment. Following treatment with dupilumab, both patients experienced good control of AD. Neither patient reported AE associated with dupilumab. In addition, both patients had normal liver function and an undetectable HBV load.[60]

Indirect Treatment Comparisons in Atopic Dermatitis

In the absence of head-to-head clinical trial data, indirect comparisons using logistic regression analysis can be used to predict the 'responder' rate for a comparator that was not originally tested within a study. The logistic regression model accounts for differences in study populations, including adjustments for baseline characteristics.

As there are no head-to-head comparisons for dupilumab versus 'standard of care' in the setting of moderate-to-severe AD, an indirect comparison analysis has been performed using data from patients treated with CsA in daily practice at University Medical Center Utrecht (N=57) and those treated with dupilumab (every 2 weeks) in the Phase III CHRONOS study (N=106). This analysis showed greater numbers of EASI-75 responders with dupilumab versus CsA in both data sets.[61]

Conclusion

In summary, there is a growing body of real-world evidence for the effectiveness of dupilumab treatment in patients with moderate-to-severe AD. Use of dupilumab in real-world clinical practice has been shown to improve AD signs, symptoms, and patient QoL, all in a manner consistent with clinical trial observations. The dupilumab safety profile in real-world studies is consistent with evidence from randomised controlled trials. There are reports in some real-world cohorts of increased incidence of conjunctivitis compared with clinical trials; conjunctivitis cases rarely led to treatment discontinuation and occurred more frequently in patients with a history of this condition. Reports from other registries, surveys,

centre-based studies, and case studies will further our understanding of real-world outcomes associated with the use of dupilumab and other novel systemic therapies. Going forward, there is a need for collaboration across registries because sharing data will further advance our understanding in this therapy area and ensure optimal outcomes for patients.

References

1. Weidinger S, Novak N. Atopic dermatitis. Lancet. 2016;387(10023):1109-22.
2. Guttman-Yassky E et al. Contrasting pathogenesis of atopic dermatitis and psoriasis - part 1: Clinical and pathologic concepts. J Allergy Clin Immunol. 2011;127(5):1110-8.
3. Schmitt J et al. Determinants of treatment goals and satisfaction of patients with atopic eczema. J Dtsch Dermatol Ges. 2008;6(6):458-65.
4. Barbarot S et al. Epidemiology of atopic dermatitis in adults: Results from an international survey. Allergy. 2018;73(6):1284-93.
5. Thestrup-Pedersen K. Clinical aspects of atopic dermatitis. Clin Exp Dermatol. 2000;25(7):535-43.
6. World Allergy Organization (WAO). Eczema, Atopic Eczema and Atopic Dermatitis. 2019. Available at: http://www.worldallergy.org/professional/allergic_diseases_center/atopiceczema/. Last accessed: 16 October 2019.
7. Williams H et al. Worldwide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. J Allergy Clin Immunol. 1999;103(1 Pt 1):125-38.
8. Augustin M et al. Epidemiology and comorbidity in children with psoriasis and atopic eczema. Dermatology. 2015;231(1):35-40.
9. Ballardini N et al. Eczema severity in preadolescent children and its relation to sex, filaggrin mutations, asthma, rhinitis, aggravating factors and topical treatment: A report from the BAMSE birth cohort. Br J Dermatol. 2013;168(3):588-94.
10. Brown SJ et al. Filaggrin null mutations and childhood atopic eczema: A population-based case-control study. J Allergy Clin Immunol. 2008;121(4):940-6.
11. Dhami S, Sheikh A. Estimating the prevalence of aero-allergy and/or food allergy in infants, children and young people with moderate-to-severe atopic eczema/dermatitis in primary care: Multi-centre, cross-sectional study. J R Soc Med. 2015;108(6):229-36.
12. Lebon A et al. Role of Staphylococcus aureus nasal colonization in atopic dermatitis in infants: The Generation R Study. Arch Pediatr Adolesc Med. 2009;163(8):745-9.
13. Olesen AB et al. Stable incidence of atopic dermatitis among children in Denmark during the 1990s. Acta Derm Venerol. 2005;85(3):244-7.
14. Saeki H et al. Prevalence of atopic dermatitis in Japanese elementary schoolchildren. Br J Dermatol. 2005;152(1):110-4.
15. Silverberg JI, Simpson EL. Association between severe eczema in children and multiple comorbid conditions and increased healthcare utilization. Pediatr Allergy Immunol. 2013;24(5):476-86.
16. Zuberbier T et al. Patient perspectives on the management of atopic dermatitis. J Allergy Clin Immunol. 2006;118(1):226-32.
17. Simpson EL et al. Patient burden of moderate to severe atopic dermatitis (AD): Insights from a Phase 2b clinical trial of dupilumab in adults. J Am Acad Dermatol. 2016;74(3):491-8.
18. Simpson EL et al. Association of inadequately controlled disease and disease severity with patient-reported disease burden in adults with atopic dermatitis. JAMA Dermatol. 2018;154(8):903-12.
19. Lange S et al. Predictors of the quality of life in patients with atopic dermatitis. Dermatol Psychsom. 2000;1:66-70.
20. Whiteley J et al. The burden of atopic dermatitis in US adults: Results from the 2013 National Health and Wellness Survey. Curr Med Res Opin. 2016;32(10):1645-51.
21. Strom MA et al. Association between atopic dermatitis and attention deficit hyperactivity disorder in U.S children and adults. Br J Dermatol. 2016;175(5):920-9.
22. Shrestha S et al. Burden of atopic dermatitis in the United States: Analysis of healthcare claims data in the commercial Medicare and Medi-Cal database. Adv Ther. 2017;34(8):1989-2006.
23. Patel KR et al. Association between atopic dermatitis, depression, and suicidal ideation: A systematic review and meta-analysis. J Am Acad Dermatol. 2019;80(2):402-10.
24. Eckert L et al. Impact of atopic dermatitis on health-related quality of life and productivity in adults in the United States: An analysis using the National Health and Wellness Survey. J Am Acad Dermatol. 2017;77(2):274-9.
25. Simpson AL. Comorbidity in atopic dermatitis. Curr Dermatol Rep. 2012;1(1):29-38.
26. Schmitt J et al. Atopic dermatitis is associated with increased risk for rheumatoid arthritis and inflammatory bowel disease, and a decreased risk for type 1 diabetes. J Allergy Clin Immunol. 2016;137(1):130-6.
27. Treudler R et al. Atopic dermatitis is associated with autoimmune but not with cardiovascular comorbidities in a random sample of the general population in Leipzig, Germany. J Eur Acad Dermatol Venerol. 2018;32(2):e44-6.
28. Zander N et al. Atopic dermatitis shows significant cutaneous comorbidity: Results from large-scale investigations in the working population. J Eur Acad Dermatol Venerol. 2019; doi:10.1111/jdv.15792. [Epub ahead of print].
29. Williams MR, Gallo RL. The role of the skin microbiome in atopic dermatitis. Curr Allergy Asthma Rep. 2015;15(11):65.
30. Ruzicka T et al. Anti-interleukin-31 receptor A antibody for atopic dermatitis. N Engl J Med. 2017;376(9):826-35.
31. Deleuran M et al. Dupilumab shows long-term safety and efficacy in moderate-to-sever atopic dermatitis patients enrolled in a Phase 3 open-label extension study. J Am Acad Dermatol. 2019; pii: S0190-9622(19)32465-X. [Epub ahead of print].
32. Guttman-Yassky E et al. Efficacy and safety of fezakinumab (an IL-22 monoclonal antibody) in adults with moderate-to-severe atopic dermatitis inadequately controlled by conventional treatments: A randomized, double-blind, Phase 2a trial. J Am Acad Dermatol. 2018;78(5):872-81.
33. De Bruin-Weller et al. The patient-reported disease burden in adults with atopic dermatitis: A cross-sectional study in Europe and Canada. J Eur Acad Dermatol Venereol. 2019; doi:10.1111/jdv.16003. [Epub ahead of print].
34. de Bruin-Weller M et al. Productivity losses in adults with atopic dermatitis (AD): A cross-sectional study from clinical practices in Europe and Canada. Abstract 714. Society for Investigative Dermatology (SID) Annual Meeting, 8-11 May, 2019.

35. de Bruin-Weller M et al. Disease burden in adolescents with moderate-to-severe atopic dermatitis: Screening data from a Phase 3 trial of dupilumab. Poster 267. European Academy of Dermatology and Venerology (EADV) Congress, 9-13 October, 2019.
36. Weidinger S et al. The patient-reported disease burden in adolescents with atopic dermatitis: A cross-sectional study in the United States and Europe. Poster 1459. European Academy of Dermatology and Venerology (EADV) Congress, 9-13 October, 2019.
37. Wei W et al. Extent and consequences of inadequate disease control among adults with a history of moderate to severe atopic dermatitis. J Dermatol. 2018;45(2):150-7.
38. Strober B et al. Dupilumab improves outcomes in patients with atopic dermatitis (AD) in clinical practice - results from the RELIEVE-AD study. Poster 261. European Academy of Dermatology and Venerology (EADV) Congress, 9-13 October, 2019.
39. Armstrong AW et al. Real-world utilization patterns of systemic immunosuppressants among US adult patients with atopic dermatitis. PLoS One. 2019;14(1):e0210517.
40. Saeki H et al. Clinical practice guidelines for the management of atopic dermatitis 2016. J Dermatol. 2016;43(10):1117-45.
41. Wollenberg A et al. Consensus-based European guidelines for the treatment of atopic eczema (atopic dermatitis) in adults and children: Part II. J Eur Acad Dermatol Venereol. 2018;32(6):850-78.
42. European Medicines Agency (EMA). Dupixent (dupilumab): Summary of product characteristics. Available at: https://www.ema.europa.eu/en/documents/product-information/dupixent-epar-product-information_en.pdf. Last accessed: 28 November 2019.
43. Blauvelt A et al. Long-term management of moderate-to-severe atopic dermatitis with dupilumab and concomitant topical corticosteroids (LIBERTY AD CHRONOS): A 1-year, randomised, double-blinded, placebo-controlled, Phase 3 trial. Lancet. 2017;389(10086): 2287-303.
44. Kabashima K et al. Nemolizumab in patients with moderate-to-severe atopic dermatitis: Randomized, Phase II, long-term extension study. J Allergy Clin Immunol. 2018;142(4):1121-30.
45. Galderma R&D. Efficacy and safety of nemolizumab in subjects with moderate-to-severe atopic dermatitis. NCT03985943. https://clinicaltrials.gov/ct2/show/NCT03985943.
46. De Abreu D et al. Presentation at CILAD 2018; November 14–17; Sao Paulo, Brazil.
47. Eichenfield LF et al. Infections in dupilumab clinical trials in atopic dermatitis: A comprehensive pooled analysis. Am J Clin Dermatol. 2019;20(3):443-56.
48. Regeneron Pharmaceuticals. Efficacy and safety of dupilumab in participants ≥12 to <18 years of age, with moderate-to-severe atopic dermatitis. NCT03054428. https://clinicaltrials.gov/ct2/show/NCT03054428.
49. Paller AS et al. Dupilumab for adolescents with moderate-to-severe atopic dermatitis: Results from a Phase 3, randomized, double-blinded trial. Abstract 10051. Annual Meeting of the American Academy of Dermatology (AAD), 1-5 March, 2019.
50. Cork MJ et al. Efficacy and safety of dupilumab in adolescent patients with moderate-to-severe atopic dermatitis. Abstract 605. Society for Investigative Dermatology (SID) Annual Meeting, 8-11 May, 2019.
51. de Bruin-Weller M et al. Poster 9761. Annual Meeting of the American Academy of Dermatology (AAD), 1-5 March, 2019.
52. Technische Universität Dresden. TREATgermany: German National Clinical Registry for patients with moderate-to-severe atopic dermatitis (TREATgermany). NCT03057860. https://clinicaltrials.gov/ct2/show/NCT03057860.
53. Schmitt J et al. Usage and effectiveness of systemic treatments in adults with severe atopic eczema: First results of the German Atopic Eczema Registry TREATgermany. J Dtsch Dermatol Ges. 2017;15(1):49-59.
54. Ariëns LFM et al. Poster presented at: ISDS 2018, Vienna, Austria, December 12–15, 2018 and EAACI 2019, Lisbon, Portugal. Accepted for publication, Allergy 2019.
55. Weidinger S. Presented at: European Society for Dermatological Research; September 18–21, 2019; Bordeaux, France, Poster 127.
56. Weidinger S. Presented at: Kuwait Dermatology Congress; April 25, 2019; Kuwait City, Kuwait. Oral presentation.
57. Fargnoli MC et al. A real-life italian experience on efficacy and safety of dupilumab in adult patients with atopic dermatitis. Abstract 409. World Congress of Dermatology, 10-15 June, 2019.
58. Faiz S et al. Effectiveness and safety of dupilumab for the treatment of atopic dermatitis in a real-life French multicentre adult cohort. J Am Acad Dermatol. 2019;81(1):143-51.
59. de Wijs L et al. Effectiveness of dupilumab treatment in 95 patients with atopic dermatitis: Daily practice data. Br J Dermatol. 2019; doi:10.1111/bjd.18179. [Epub ahead of print].
60. Ly K et al. Dupilumab in patients with chronic hepatitis B on concomitant entecavir. JAAD Case Rep. 2019;5(7):624-6.
61. Ariëns LFM et al. Dupilumab versus cyclosporine for the treatment of moderate-to-severe atopic dermatitis in adults: Indirect comparison using the eczema area and severity index. Acta Derm Venereol. 2019;99(10):851-7.

SAGLB.DUP.19.11.1710 12/19

Improving Intravenous Immunoglobulin Dosing in Autoimmune Neuropathies

This symposium took place on Monday 24th June 2019, as part of the 2019 Peripheral Nerve Society (PNS) Annual Meeting in Genoa, Italy

Chairpeople: John England[1]

Speakers: Bart Jacobs,[2] John England,[1] Jean-Marc Léger[3]

1. LSUHSC School of Medicine, New Orleans, Louisiana, USA
2. Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
3. National Referral Center for Rare Neuromuscular Diseases, University Hospital Pitié-Salpêtrière, Paris, France

Disclosure: The authors received support from Grifols for their presentation at this symposium and have reviewed the article. No author received any financial remuneration for the development of the article. Prof England has received Spreakers' Bureau from Grifols and CSL Behring; has been a consultant for Grifols; and has received a stipend from Elsevier for being the Editor-in-Chief of the Journal of the Neurological Sciences. Prof Jacobs has received unrestricted financial support for research from the Prinses Beatrix Spierfonds, GBS-CIDP Foundations International, CSL-Behring, Grifols, Annexon, and Hansa Biopharma. Prof Léger has acted as investigator and/or member of Steering Committee/Advisory Board for clinical trials, and/or invited lecturer for Baxalta, CSL-Behring, Kedrion, LFB, Novartis, Pfizer, and UCB.

Acknowledgements: Medical writing assistance was provided by Content Ed Net, Madrid, Spain.

Support: This symposium was funded by Grifols, who also funded medical writing support which was performed in line with Good Publishing Practice (GPP-3) guidelines.

Citation: EMJ. 2019;4[4]:24-32.

Meeting Summary

This symposium took place on Monday 24th June 2019, as part of the 2019 Peripheral Nerve Society (PNS) Annual Meeting in Genoa, Italy. Immune-mediated neuropathies such as Guillain–Barré Syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP), and multifocal motor neuropathy (MMN) are diverse inflammatory peripheral nerve disorders. International consensus guidelines recommend intravenous Ig (IVIG) as Level A for the treatment of GBS, CIDP, and MMN. Suggested induction doses of IVIG are 2 g/kg divided over 2–5 days, but maintenance doses are purposely less clearly defined and left up to the judgement of the clinician, depending upon the specific needs of the individual patient. Community-based neurologists treating patients with these rare inflammatory neuropathies may be unaware of optimal dosing regimens and patient response to treatment may therefore be inadequate. In this symposium, world-renowned experts in GBS, CIDP, and MMN shared their expertise and review of the literature to provide reasonable dosing regimens for neurologists who may rarely encounter these conditions.

Intravenous Immunoglobulin Dosing in Guillain–Barré Syndrome

Professor Bart Jacobs

The Dutch Guillain–Barré Study Group first published their experience with IVIG in GBS in 1992.[1] In this study, the dosage employed, 0.4 g/kg/day for 5 consecutive days, was based upon previous experience in autoimmune diseases and it was uncertain whether it would be effective in acute GBS. The study showed that IVIG was at least as effective as plasma exchange in this clinical setting and was associated with less frequent complications. An important question arising from the study was whether 0.4 g/kg/day for 5 days is the optimal IVIG dose for all patients, or whether greater improvements could be achieved with a higher dose.

To investigate this question, a pharmacokinetic (PK) study was performed in which 174 patients with GBS received IVIG 0.4 g/kg/day for 5 days.[2] After 2 weeks, there was a significant increase in serum IgG levels which then slowly declined (Figure 1A).[2] Notably, there was a marked variation in PK profile. Patients were stratified into four groups according to the change in serum IgG levels and correlated with outcome; the proportion of patients who recovered the ability to walk unaided was largest in patients with the highest increase in IgG levels (Figure 1B).[2]

There are a number of questions concerning IVIG treatment of GBS regarding treatment failure, treatment-related fluctuations (TRF), treatment of mild GBS, treatment of Miller Fisher syndrome, and other variants.[3]

Treatment Failure

Treatment failure after IVIG 0.4 g/kg/day for 5 days is an important issue, especially if a patient has reached a stage requiring intensive care and respiratory support and shows no sign of recovery. When asked how to manage patients with apparent treatment failure after IVIG, neurologists at the PNS meeting most frequently opted to watch and wait. Other management choices, including an additional course of IVIG, methylprednisolone, or plasma-exchange, were frequently endorsed but there is a lack of evidence to support these choices. To help address this lack of evidence, a prospective observational cohort study (The International GBS Outcome Study [IGOS]) was designed to address clinical and biological determinants and predictors of GBS.[4] The initial aim was to include at least 1,000 patients with a follow-up of 1–3 years using a web-based data entry system.[5]

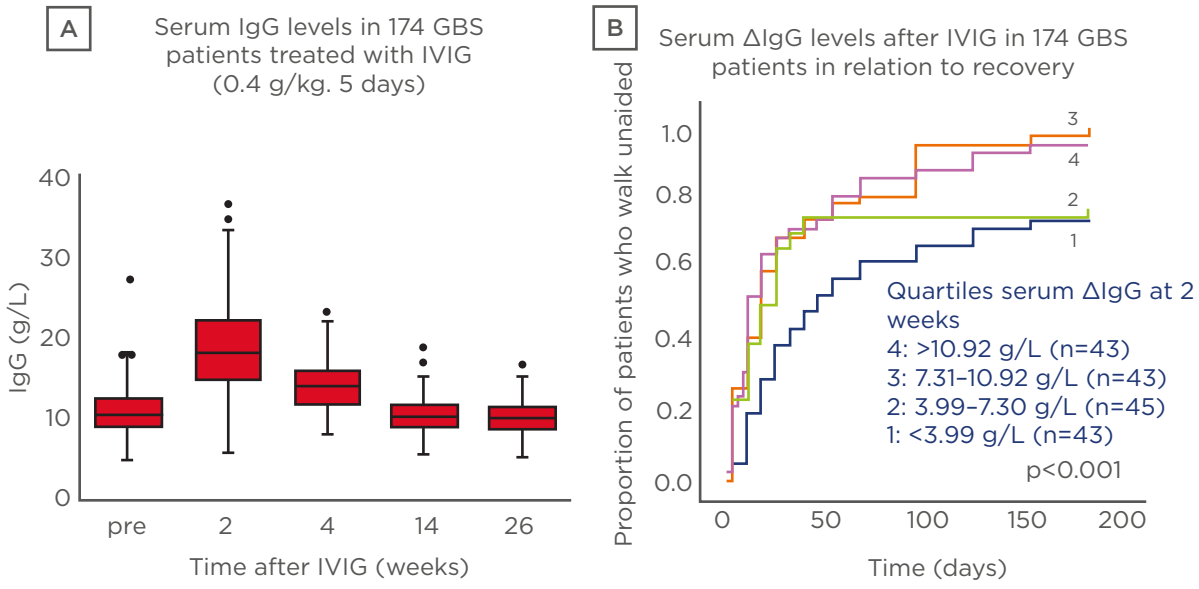


Figure 1: Intravenous Ig pharmacokinetics (A) and outcome (B) in patients with Guillain–Barré syndrome treated with intravenous Ig 0.4 g/kg/day for 5 days.[2]

5 Box: interquartile range; line in box: median; whiskers: range without outliers; dots: outliers.

GBS: Guillain–Barré syndrome; IVIG: intravenous Ig.

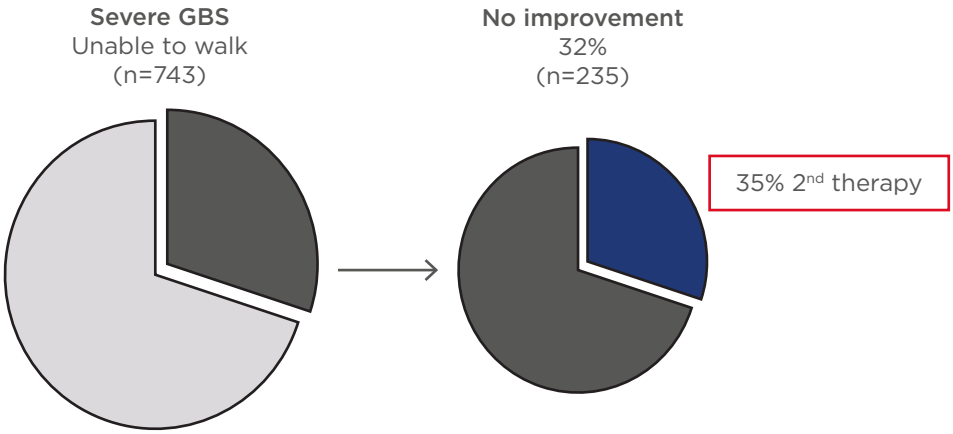


Figure 2: Response to first course of immunotherapy in patients with severe Guillain–Barré syndrome and percent of these patients receiving a second course of treatment.[5]

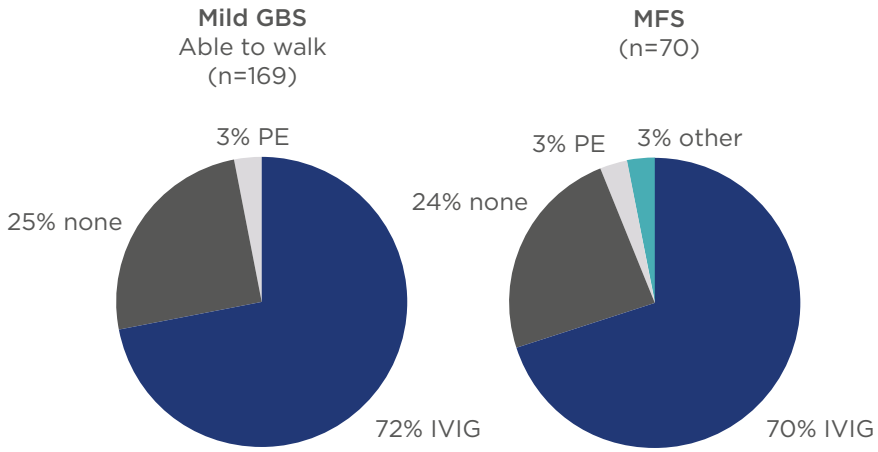
GBS: Guillain–Barré syndrome.

In a recent update on the current practice of treatment, 1,023 patients were assessed including 743 with severe disease (unable to walk unaided), 168 with mild disease (able to walk independently), 70 with Miller Fisher syndrome, and 40 with other variants. Within these groups, 724, 126, 53, and 33 patients, respectively, were treated with IVIG, plasma exchange, or other immunotherapy. In the severe GBS group, 32% had no improvement with initial treatment and, of these, 35% received a second course of immunotherapy (Figure 2).[5] In a nonrandomised, observational study in the IGOS cohort, the clinical course was compared in patients with a second course of IVIG and a predicted poor outcome. It was reported that 38/237 patients treated with a second IVIG course versus 199/237 given only 1 course showed no between-group differences in ability to walk unaided at 4 weeks.[5] However, the groups were unbalanced because patients receiving a second course of immunotherapy were more severely affected.

To resolve this issue, the Erasmus MC team in Rotterdam, the Netherlands, have initiated a placebo-controlled, randomised controlled trial (RCT) investigating the therapeutic effect of a second IVIG dose (SID-GBS trial),[6] and the results are awaited with interest.

Treatment-Related Fluctuations

TRF are characterised by secondary deterioration after initial clinical improvement or stabilisation, and occur in up to 10% of patients with GBS.[7] It has been hypothesised that relapses after treatment may occur when there is ongoing immune (re)activation (inflammation), resulting in a more protracted clinical course that is longer than the effective duration of action of immunotherapy.[8] If the patient experiences >2 TRF, or if a TRF occurs >8 weeks after the onset of the illness, then a diagnosis of acute-onset, chronic inflammatory polyneuropathy, a variant of CIDP, needs to be considered.[9,10] It has been reported that 3% of patients presenting with GBS have acute-onset CIDP.[9] In these patients, maintenance treatment therapeutic options include IVIG or switching to an alternative therapy such as corticosteroids. It has been reported that relapsing patients respond well to a second course of immunotherapy.[7] In the IGOS study, 53/1,023 GBS patients experienced TRF; of 50 patients initially treated with IVIG, 60% were retreated with IVIG, 8% switched to plasma exchange, and 32% received no treatment for their TRF. In patients who were re-treated for their TRF, the TRF occurred at an earlier time point than in untreated patients (median: 21 days versus 32 days; p=0.008).[5]



Majority of mild and variant forms are already treated.

Figure 3: Treatment of mild Guillain–Barré syndrome and variant forms such as Miller Fisher syndrome.[5]

GBS: Guillain–Barré syndrome; IVIG: intravenous Ig; MFS: Miller Fisher syndrome; PE: plasma exchange.

Treatment of Mild Guillain–Barré Syndrome and Variant Forms of the Disease

There does not appear to be any RCT with IVIG published on patients with mild or variant forms of GBS; however, based on the findings of the IGOS analysis it is clear that in current clinical practice the majority of patients with mild GBS, as well as those with variants such as Miller Fisher syndrome, receive immunotherapy (Figure 3).[5]

Conclusion

Based on evidence to date, the optimal IVIG dose in patients with GBS is not well characterised, although guidelines recommend a dosage of 2 g/kg given over 5 days as the first-line treatment. This may be explained, at least in part, by a variability in IVIG PK properties in the patient population, resulting in inconsistent therapeutic responses. Consequently, neurologists are faced with several potential treatment dilemmas with GBS patients. This has led to variability in clinical practice and there is a need for additional clinical data from RCT to support the development of evidence-based treatment guidelines.

Intravenous Immunoglobulin Dosing in Chronic Inflammatory Demyelinating Polyneuropathy

Professor John England

CIDP is an acquired immune-mediated disorder of the peripheral nervous system. It is a heterogeneous, chronic, progressive disease that usually causes weakness, sensory loss, and neuropathic pain in the limbs.[10] The efficacy of IVIG has been established in five randomised, placebo-controlled clinical trials, including the ICE study.[11] This study, the largest reported trial of any CIDP treatment at the time, demonstrated the short-term and long-term efficacy and safety of IVIG and 10% caprylate-chromatography purified immune globulin intravenous (IGIV-C), and supports the use of IGIV-C as the standard for use in CIDP patients. This is despite there being very little evidence to support the relative benefits of different dosages and no true dose-response curves being established.

The PK parameters of IVIG are characterised by rapid attainment of peak levels, followed by a slow decrease in serum IgG levels with a mean/median half-life of 20–40 days as the Ig equilibrates between plasma and extravascular fluid. Equilibrium was achieved in 3–5 days.[12] This extended half-life has been used to calculate a

dose infusion frequency for IVIG of 'every 3 weeks'. In terms of therapeutic effect, it is not known which IgG PK parameter helps define the best possible outcome (peak versus trough level, change in IgG level, steady-state level, etc.). The picture is further clouded by the fact that IgG can present in different forms (monomeric, dimeric, etc.) with various sialylation/glycosylation patterns that can impact terminal galactose and sialic acid residues.[13] Better understanding of these factors may enable personalisation of dosing schedules.

European Federation of Neurological Societies (EFNS)/PNS guidelines recommendations on the management of CIDP with IVIG are based upon the findings of the ICE study: 2.0 g/kg as a baseline loading dose divided over 2–4 days, followed by maintenance infusions of 1.0 g/kg over 1–2 days every 3 weeks.[14] However, this does not take into consideration patient weight status, or whether actual or adjusted bodyweight should be used. Consequently, IVIG dosing can vary widely and a recent USA survey reported that most practitioners used 0.4–1.2 g/kg every 2–6 weeks.[15]

Some key responses from qualitative interviews with external medical experts in the field of neurology in the USA regarding the treatment of CIDP are summarised as follows:

1. IVIG was considered to be first-line treatment (approximately 70–90% of the time), because it was effective and had a better side-effect profile than steroids.

2. However, if IVIG fails or is contraindicated, then steroids were the second-line choice.

3. IVIG dosing: all experts gave a loading dose of 2 g/kg over 2–5 days which was typically followed by maintenance with 1 g/kg every 3–4 weeks. However, three respondents stated that for some patients this may be undertreatment and that they would freely give more IVIG, implying some disagreement about the correct maintenance dosage.

4. No consensus on third-line therapy could be reached by the experts.

5. All experts stated the need to establish expectations prior to treatment; any decisions to adjust dose will be based on strength and function only, and not on perceived sensory symptoms, such as tingling, pain, or fatigue. Patient agreement at the outset is considered important.

6. All experts sought an objective response and relied heavily on clinical examination to determine treatment success or failure. In some cases, lack of improvement constituted failure, for others it was worsening of symptoms. However, one expert mentioned that if a patient had advanced CIDP, slowing disease progression may be an acceptable outcome.

7. If there was a good response, experts tended to continue the maintenance dose until the patient achieved a maximal response and plateaued; then they started tapering the dosage. Experts typically felt that eventually they could wean up to 50% of patients. The preferred method for weaning patients off IVIG was by increasing the interval between doses.

8. Two respondents incorporated a dose-reduction strategy to help maintain IgG levels at a steadier state (avoiding peaks and troughs).

9. Diagnosing a patient as IVIG-dependent was rare, with most noting that they would resume a trial of weaning (i.e., cycle through the maintenance, weaning, and discontinuation process).

10. When asked about the management of CIDP, expert neurologists indicated that community neurologists generally under or overtreated CIDP, with respect to dosing.

11. Both over and underdiagnosis of CIDP are relatively frequent, and this can lead to problems with treatment.

12. Undertreating CIDP is a concern because patients may be considered treatment failures, whereas giving a higher dose may have produced clinical improvement.

13. Literature 'wish list' items included: dose weaning to a minimum tolerated dose, dose weaning to discontinue treatment (when/how to discontinue), and best treatment options for patients' refractory to both IVIG and steroids.

14. The idea of a validated patient outcome tool is appealing if it contains measures of strength, function, and disability.

15. All experts mentioned using an 'activities of

daily living' outcome tool, either Inflammatory Neuropathy Cause and Treatment (INCAT) Disability Score, Rasch-built Overall Disability Scale (RODS), Neuropsychological Impairment Scale (NIS), or other.

To evaluate how neurologists in community practice make decisions concerning the diagnosis and treatment of CIDP, Prof England's group conducted an anonymous cross-sectional quantitative survey involving 100 practitioners in the USA.[15] There was a wide variation in the use of specific guidelines to diagnose CIDP, with only 13% of respondents indicating that they used the globally accepted EFNS/PNS guidelines and almost 40% stating they did not routinely use a specific guideline. Many respondents had difficulty recognising the electrodiagnostic criteria used to diagnose CIDP or identifying atypical variants of the disease.

Regarding treatment, 44% of community practice neurologists chose IVIG and 20% indicated a preference for steroids; however, 24% of the respondents reported using steroids plus IVIG, despite guidelines stating that there is no evidence of clinical benefit for this combination. With respect to dosing, 67% of respondents indicated that they would use actual bodyweight, whereas 25% said they would use adjusted or ideal bodyweight, and 7% said they would start with a fixed dose regardless of the patient's weight. For initial CIDP treatment, 55% of respondents used the recommended dose of 2 g/kg over 3–5 days. However, the remaining 45% of respondents quoted a wide range of doses from very low (0.01–0.04 g/kg) to unreasonably high (10.00–40.00 g/kg). Regarding maintenance treatment, the range was not as wide, but >40% neurologists used low (immune replacement) dosages of 0.01–0.50 g/kg. Most neurologists gave IVIG at least 3 months before deciding the response to treatment; however, 25% of survey respondents indicated that they treat their patients for 6 months. Finally, in terms of communication with the patient regarding weaning off IVIG therapy, responses were varied with no consensus. Most patients were not well-informed regarding this process and the timing of it. Others were simply told that they would be monitored and that treatment would be discontinued at some point in the future. Additionally, 21% of neurologists indicated that they made IVIG dosing decisions concerning the patient over the phone without a physical examination. To assess the patient's overall condition, as well as strength, function, and disability, a consultation to ascertain how the IVIG is working is essential. There was no consensus on the duration of maintenance therapy prior to attempted weaning, and this is another area where more information is required to optimise overall patient management.

Conclusion

Based on the findings from a survey of 100 neurologists in community practice, diagnosis of CIDP remains problematic. Treatment of CIDP patients with IVIG is extremely variable, and under and overtreatment commonly occurs. Undoubtedly, CIDP is a difficult disease to manage because it is heterogeneous and there is a lack of good evidence in relation to various aspects of diagnosis and treatment. This should encourage the use of available evidence, perhaps in the form of an evidence-based clinical practice guideline that is brief, clear, and actionable. Ideally, IVIG dosage regimens should be derived empirically from high-quality studies that employed objective measures to ascertain the overall response to therapy.

Intravenous Immunoglobulin Dosing in Multifocal Motor Neuropathy

Professor Jean-Marc Léger

As way of background, a case of MMN involving a 46-year-old woman who presented with left foot drop was discussed. Over the next 5 years, she developed right hand weakness. At this stage, clinical examination revealed distal motor deficits of the left tibialis anterior and peroneus muscles, and in the distribution of the right median nerve. Tendon reflexes were normal except for the left ankle. There was no sensory or cranial nerve involvement and the Overall Neuropathy Limitations Scale (ONLS) score was 3. Electrophysiological studies confirmed conduction block in the right median nerve and denervation in the area of the fibula. Laboratory investigations showed that tests for antiganglioside antibodies (GM-1 and GM-2) were negative, the cerebrospinal fluid protein

count (0.35 g/L) was normal, and MRI of roots and plexuses revealed 2 foci of hyperintensity on the right median nerve (proximal and distal), and hypertrophy and a hyperintensity in the left L2–S1 roots. At this stage, treatment was started with IVIG 100 g over 3 days, repeated every 4 weeks from June 2015 to January 2017. There was marked improvement of the motor deficits in the right hand, but lesser improvement in the left lower limb, and the patient relapsed 3 weeks after each infusion. In 2017, the patient relapsed 2 weeks post-infusion and electrophysiological studies identified a new conduction block in the left upper limb and ONLS was rated at 4.

Discussions with colleagues resulted in two suggested treatment approaches: the first was to maintain IVIG treatment, but to increase the dosage or reduce the duration between doses; the second was to add an immunomodulator to the current treatment regimen. Prof Léger and his team decided to continue with IVIG, but to reduce the dosing interval to every 3 weeks. The patient responded and has done well since this change in treatment with stable conduction blocks.

The challenges to Prof Léger's understanding of MMN treatment are numerous and some questions that his group are wrestling with include:

Q1. Can we learn anything from the pathophysiology and natural history of MMN?

Q2. What are the best outcome measures?

Q3. What have we learned regarding short-term treatment with IVIG from prospective clinical trials?

Q4. What have we learned about long-term use of IVIG from retrospective and cohort studies?

Q5. How do we manage patients whose MMN is deteriorating despite maintenance therapy?

MMN is characterised by slowly progressive, asymmetrical, predominantly distal limb weakness, usually starting and predominating in the upper limbs, with no sensory loss and persistent conduction block. While the pathophysiology is still unknown, some elegant research is being performed in relation to peripheral nerve nodopathies that may be relevant.[16] In recent years, there has been growing support for the concept that nerve dysfunction in MMN is probably related to autoantibody attack of proteins in the nodal, paranodal, and juxtaparanodal regions of the nerve, and this results in sodium ion channel dysfunction and conduction block. This may also explain why there is conduction block in MMN without true demyelination and a rapid initial response to IVIG.[17]

It is therefore important to identify the best outcome measures to use in MMN to guide repeat IVIG administration. Currently, there is no consensus on best outcome measures. A European Neuromuscular Centre (ENMC) workshop meeting proposed that the primary outcome measure in clinical trials should be the RODS for MMN, which has 25 selected items and is the only disease-specific outcome measure. This could be expanded in the future using the AMC Linear Disability Score (ALDS) or the ABILHAND questionnaire.[1] However, at this stage it remains an exploratory outcome measure.

A Cochrane review evaluated 4 RCT[19-22] that assessed the effects of IVIG on motor scales in 34 MMN patients.[23] This analysis showed that strength improved in 78% of patients treated with IVIG versus 4% of placebo-treated patients. Disability improved in 39% of patients after IVIG treatment versus 11% after placebo (not statistically significant). Following the Cochrane review, a placebo-controlled RCT was published involving 44 adults with MMN randomised 1:1 to either double-blind treatment of IVIG followed by placebo for 12 weeks each, or the reverse. This study had the advantages of a longer follow-up duration (3 months) and disability assessment.[24] Mean maximal grip strength of the more affected hand decreased 31.0% on placebo and increased 3.8% on IVIG (p=0.005). In 36% of participants, Guy's Neurological Disability Scale (GNDS) scores for upper limbs worsened only during the placebo period, while 12% deteriorated only during IVIG treatment (p=0.021). A total of 69% of blinded patients switched prematurely from placebo to open-label IVIG, whereas <3% switched from IVIG to open-label IVIG (p<0.001). This RCT confirms the beneficial effects of IVIG in MMN, but further research is needed to investigate what level of improvement in disability can be achieved and its relative cost-effectiveness. EFNS/PNS recommendations for MMN treatment are that IVIG (2 g/kg given over 2–5 days) should be first-line treatment (Level A) when disability is sufficiently severe to warrant

treatment, corticosteroids are not recommended, toxicity makes cyclophosphamide a less desirable option, if initial IVIG treatment is effective then repeat treatment should be considered in selected patients (Level C), the frequency of IVIG maintenance therapy should be guided by the response (good practice point), and typical treatment regimens for maintenance are 1 g/kg every 2–4 weeks or 2 g/kg every 1–2 months (good practice point).[25] Globally, IVIG is considered a first-line treatment option for MMN.[25-28]

Since 2002, six observational studies have been published to provide information regarding the benefit of long-term IVIG treatment in MMN.[29-34] The first of these studies reported that long-term maintenance (4–8 years) with IVIG had a beneficial effect on muscle strength and upper limb disability, but may not prevent a slight decrease in muscle strength. Electrophysiological results suggested a favourable influence of IVIG on remyelination and reinnervation in MMN patients, but axon loss could not be prevented.[29] In Prof Léger's study, 40 MMN patients were treated with IVIG and confirmed a significantly high short-term response.[32] At the end of follow-up (mean 2.2 years), only 8 patients had significant remission and 25 patients (68%) were dependent on periodic (4–8 weekly) IVIG infusions. A similar conclusion was reached in a Dutch study involving 88 patients followed for a median of 6 years.[33] Initially, 94% of patients responded to IVIG and nonresponders had longer disease duration before the first treatment. At the time of the analysis, 76% of patients were receiving maintenance treatment and the median dose increased from 12 to 17 g/week during follow-up. In contrast, a more recent Austrian study reported a reduction in IVIG dosage in MMN patients during long-term follow-up (mean: 7.5 years) from a mean of 1.8 g/kg at baseline to 1.1 g/kg at the last visit.[34]

Regarding the management of patients whose MMN is deteriorating despite maintenance therapy, EFNS guidelines recommend that if IVIG is not sufficiently effective then immunosuppressive therapy may be considered. However, no agent to date has been shown to be beneficial in a clinical trial and data from case series are conflicting. Preliminary data from uncontrolled studies show that a small number of patients improve on immunosuppressive therapy;[35] however, RCT are needed to confirm overall efficacy and optimal treatment schedules. More recently, a Cochrane review investigated the use of immunosuppressants in MMN and concluded that mycophenolate did not significantly improve strength or function or reduce the need for IVIG. There were some reports of clinical benefit, but also serious adverse effects with cyclophosphamide, and there is very little evidence for drugs such as azathioprine, IFN beta, rituximab, or cyclosporine.[36]

Conclusion

Until definitive evidence is available for alternative treatments, IVIG remains the mainstay of treatment in MMN and there is a need to obtain the best outcomes with this agent.[35] In the future, a better understanding of the pathogenesis and natural history of MMN may help optimise treatment approaches and possibly identify new therapeutic targets. Identification of the best outcome measures to predict clinical benefit with IVIG will also be important. Learning from the results of RCT, real-world retrospective studies, and extrapolation into clinical practice will be important for patients. There is a need to better understand factors associated with the stabilisation of MMN when patients are not being treated or their disease is worsening. Finally, the best candidates for long-term disease-modifying therapy need to be identified.

References

1. van der Meché FG, Schmitz PI. A randomized trial comparing intravenous immune globulin and plasma exchange in Guillain-Barré syndrome. Dutch Guillain-Barré Study Group. N Engl J Med. 1992;326(17):1123-9.
2. Kuitwaard K et al. Pharmacokinetics of intravenous immunoglobulin and outcome in Guillain-Barré syndrome. Ann Neurol. 2009;66(5):597-603.
3. Verboon C et al. Treatment dilemmas in Guillain-Barré syndrome. J Neurol Neurosurg Psychiatry. 2017;88(4):346-52.
4. Jacobs BC et al. International Guillain-Barré Syndrome Outcome Study: Protocol of a prospective observational cohort study on clinical and biological predictors of disease course and outcome in Guillain-Barré syndrome. J Peripher Nerv Syst. 2017;22(2):68-76.
5. Verboon C et al. Second IVIg course

in Guillain-Barré syndrome with poor prognosis: The non-randomised ISID study. J Neurol Neurosurg Psychiatry. 2019. doi: 10.1136/jnnp-2019-321496. [Epub ahead of print].

6. Walgaard C et al. Second IVIg course in Guillain-Barré syndrome patients with poor prognosis (SID-GBS trial): Protocol for a double-blind randomized, placebo-controlled clinical trial. J Peripher Nerv Syst. 2018;23(4):210-5.
7. Donofrio PD. Guillain-Barré Syndrome. Continuum (Minneap Minn). 2017;23(5):1295-309.
8. Visser LH et al. Risk factors for treatment related clinical fluctuations in Guillain-Barré syndrome. J Neurol Neurosurg Psychiatry. 1998;64(2):242-4.
9. Ruts L et al. Distinguishing acute-onset CIDP from fluctuating Guillain-Barré syndrome: A prospective study. Neurology. 2010 May 25;74(21):1680-6.
10. Vallat JM et al. Chronic inflammatory demyelinating polyradiculoneuropathy: Diagnostic and therapeutic challenges for a treatable condition. Lancet Neurology. 2010;9(4):402-12.
11. Hughes RA et al. Intravenous immune globulin (10% caprylate-chromatography purified) for the treatment of chronic inflammatory demyelinating polyradiculoneuropathy (ICE study): A randomised placebo-controlled trial. Lancet Neurol. 2008;7(2):136-44.
12. Berger M et al. Rapid and reversible responses to IVIG in autoimmune neuromuscular diseases suggest mechanisms of action involving competition with functionally important autoantibodies. J Peripher Nerv Syst. 2013;18(4):275-96.
13. Schwab I, Nimmerjahn F. Role of sialylation in the anti-inflammatory activity of intravenous immunoglobulin - $F(ab')_2$ versus Fc sialylation. Clin Exp Immunol. 2014;178(Suppl 1):97-9.
14. Van Den Bergh PY et al. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy: Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society – First Revision. Eur J neurol. 2010;17(3):356-63.
15. Gelinas D et al. Current practice patterns in CIDP: A cross-sectional survey of neurologists in the United States. J Neurol Sci. 2019;397:84-91.
16. Uncini A, Kuwabara S. Nodopathies of the peripheral nerve: An emerging concept. J Neurol Neurosurg Psychiatry. 2015;86(11):1186-95.
17. Beadon K et al. Multifocal motor neuropathy. Curr Opin Neurol. 2018;31(5):559-64.
18. Vanhoutte EK et al. 196th ENMC international workshop: Outcome measures in inflammatory peripheral neuropathies 8-10 February 2013, Naarden, the Netherlands. Neuromuscul Disord. 2013;23(11):924-33.
19. Azulay JP et al. Intravenous immunoglobulin treatment in patients with motor neuron syndromes associated with anti-GM1 antibodies: A double-blind, placebo-controlled study. Neurology. 1994;44(3 Pt 1):429-32.
20. Van den Berg LH et al. Treatment of multifocal motor neuropathy with high dose intravenous immunoglobulins: A double blind, placebo-controlled study. J Neurol Neurosurg Psychiatry. 1995;59(3):248-52.
21. Federico P et al. Multifocal motor neuropathy improved by IVIg: Randomized, double-blind, placebo-controlled study. Neurology. 2000;55(9):1256-62.
22. Léger JM et al. Intravenous immunoglobulin therapy in multifocal motor neuropathy: A double-blind, placebo-controlled study. Brain. 2001;124(Pt 1):145-53.
23. van Schaik IN et al. Intravenous immunoglobulin for multifocal motor neuropathy. Cochrane Database Syst Rev. 2005;(2):CD004429.
24. Hahn AF et al. A controlled trial of intravenous immunoglobulin in multifocal motor neuropathy. J Periph Nerv Syst. 2013;18(4):321-30.
25. Joint Task Force of the EFNS and the PNS. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on management of multifocal motor neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society – first revision. J Periph Nerv Syst. 2010;15(4):295-301.
26. Patwa HS et al. Evidence-based guideline: Intravenous immunoglobulin in the treatment of neuromuscular disorders. Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology. 2012;78(13):1009-15
27. Elovaara I et al. EFNS guidelines for the use of intravenous immunoglobulin in treatment of neurological diseases: EFNS task force on the use of intravenous immunoglobulin in treatment of neurological diseases. Eur J Neurol. 2008;15(9):893-908.
28. Tegeline 50 mg/mL, powder and solvent for solution for infusion: A report from the National Agency for the Safety of Medicines and Health Products. Available at: http://base-donnees-publique.medicaments.gouv.fr/extrait.php?specid=69174658. Last accessed: 21 November 2019.
29. van den Berg-Vos RM et al. Multifocal motor neuropathy: Long-term clinical and electrophysiological assessment of intravenous immunoglobulin maintenance treatment. Brain. 2002;125(Pt 8):1875-86.
30. Terenghi F et al. How long is IVIg effective in multifocal motor neuropathy? Neurology. 2004;62(4):666-8.
31. Vucic S et al. Multifocal motor neuropathy: Decrease in conduction blocks and reinnervation with long-term IVIg. Neurology. 2004;63(7):1264-9.
32. Léger JM et al. Intravenous immunoglobulin as short- and long-term therapy of multifocal motor neuropathy: A retrospective study of response to IVIg and of its predictive criteria in 40 patients. J Neurol Neurosurg Psychiatry. 2008;79(1):93-6.
33. Cats EA et al. Correlates of outcome and response to IVIg in 88 patients with multifocal motor neuropathy. Neurology. 2010;75(9):818-25.
34. Löscher WN et al. Multifocal motor neuropathy in Austria: A nationwide survey of clinical features and response to treatment. J Neurol. 2018;265(12):2834-40.
35. Nobile-Orazio E, Gallia F. Multifocal motor neuropathy: Current therapies and novel strategies. Drugs. 2013;73(5):397-406.
36. Umapathi T et al. Immunosuppressant and immunomodulatory treatments for multifocal motor neuropathy. Cochrane Database Syst Rev. 2015;(3):CD003217.

Anticipate Your Next Move in Chronic Myeloid Leukaemia Patient Management

This symposium took place on the 13th June 2019, as part of the 24th European Hematology Association (EHA) Congress in Amsterdam, the Netherlands

Chairpeople: Jeroen Janssen[1]

Speakers: Jeroen Janssen,[1] Jane Apperley,[2] Simona Soverini,[3] Hugues de Lavallade[4]

1. Amsterdam UMC, Amsterdam, the Netherlands
2. Imperial College London, London, UK
3. University of Bologna, Bologna, Italy
4. King's College London, London, UK

Disclosure: Dr Janssen has received research funding from BMS and Novartis; received honoraria from Incyte, Novartis, and Pfizer; and is the founder of HematologyApp, supported by BMS, Daiichi-Sankyo, Incyte, Janssen, MSD, Pfizer, Roche, and Takeda. Prof Apperley has received honoraria and research funding from Ariad, Incyte, Novartis, and Pfizer; and honoraria from BMS. Prof Soverini has provided consultancy for BMS, Incyte, and Novartis. Dr de Lavallade has received honoraria from BMS, Incyte, Novartis, and Pfizer; and has received research grants from ARIAD/Incyte and BMS.

Acknowledgements: Medical writing assistance was provided by Tom Feys, Ariez International, Bruges, Belgium.

Support: The symposium and the writing and publication of the article was funded by Incyte. The views and opinions expressed are those of the speakers and not necessarily of Incyte.

Citation: EMJ. 2019;4[4]:33-36.

Meeting Summary

Treatment decisions in chronic myeloid leukaemia (CML) are complex and require the evaluation of many factors at each stage of therapy. Many patients will become resistant or intolerant to the first and subsequent lines of tyrosine kinase inhibitors (TKI) they receive, requiring them to switch to a different TKI. Clinicians are faced with many considerations when choosing subsequent treatments and an important issue is how best to manage failure on a second-generation TKI. During an interactive and case-based, Incyte-sponsored, satellite symposium at the 2019 European Hematology Association (EHA) congress, Dr Janssen and Prof Apperley discussed the current best practices for managing patients failing imatinib or second-generation TKI, considering whether second-generation TKI should be used sequentially and the timing of the introduction of a third-generation TKI (ponatinib). Dr Soverini and Dr de Lavallade discussed how regular *BCR-ABL* response monitoring and mutational analysis are integral to CML patient management. They highlighted the clinical relevance of low-level mutations and the necessity to prevent clonal expansion of these TKI-resistant mutants, and the accumulation of additional mutations, by switching to an effective TKI in a timely manner.

Introduction

The significant advances in the treatment of patients with CML over the last two decades have resulted in an improved prognosis for most patients, contributing to their life expectancy approaching that of the general population.[1] Improvements in the prognosis of CML patients was also accompanied by a shift in the management of the disease and the treatment objectives. While in 2001 the treatment objective was to prolong the survival of patients, the main treatment goal today is for patients to achieve a deep molecular response, which gives them the best chance to successfully stop treatment. The introduction of imatinib revolutionised the treatment landscape of CML, and with this agent the majority of patients will eventually attain a deep molecular response;[2,3] however, for a proportion of patients the treatment outcomes with imatinib are unsatisfactory. Imatinib and second-generation TKI can become inactive once point mutations in the *BCR-ABL* TKI binding domain appear.[4] When this occurs, there is a need for an alternative TKI that is active in the presence of such resistant mutations. During an interactive and case-based satellite symposium, hosted by Incyte during the 2019 EHA meeting, Dr Janssen and Prof Apperley discussed the current best practices in CML patients failing on imatinib or a second-generation TKI, after which Dr Soverini and Dr de Lavallade discussed the technical aspects related to mutation testing in CML.

Dealing with Imatinib or Second-Generation Tyrosine Kinase Inhibitor Failure in Chronic Myeloid Leukaemia

Doctor Jeroen Janssen

Over the last decade, regular *BCR-ABL* response monitoring has become an important part of managing CML patients who are treated with a TKI. This approach allows physicians to quickly identify patients with a suboptimal response ('warning' or 'failure'), as defined by the European Leukemia Network (ELN) criteria, and switch them to an alternative TKI. In the case of a 'warning', the outcome might improve, but close follow-up is warranted. In the case of 'failure', immediate action is required (i.e., a TKI switch).[5] In the case of a 'warning' or 'failure', the ELN and National Comprehensive Cancer Network (NCCN) guidelines on CML recommend a mutational analysis of the *BCR-ABL* kinase domain.[5-7] If a mutation is discovered, it is important to choose the appropriate next TKI, e.g., by using the traffic-light coded heat map that lists the sensitivity of most common mutations for all currently available TKI.[8]

What can we Expect from Switching Between Second-Generation Tyrosine Kinase Inhibitors?

Professor Jane Apperley

There is a lack of clinical data on the effect of switching from one second-generation TKI to another (i.e., switching between nilotinib, dasatinib, or bosutinib) after initial failure on imatinib. The scarce data available indicate that the rate of complete cytogenetic responses to a second-generation TKI in the third-line setting was low (ranging from 11% to 32%).[9-11] Furthermore, the durability of these responses was limited;[10-12] for example, in a Phase I/II study 71% of the patients who received bosutinib after imatinib and dasatinib/nilotinib failure discontinued therapy within 2 years.[12] Notably, almost half of the patients in these trials switched between second-generation TKI for reasons of intolerance, and the proportion of truly resistant patients was low. As such, these studies do not provide firm support for a switch between second-generation TKI in TKI-resistant patients.[9-12]

When switching from a second to a third-generation TKI (i.e., ponatinib), deep and durable responses can be achieved. In the PACE trial, chronic-phase CML patients treated with ponatinib after resistance or intolerance to dasatinib or nilotinib resulted in a 49% complete cytogenetic response and a 35% major molecular response. An MR4.5 was achieved by 20% of patients (Figure 1).[13] Importantly, the response to ponatinib proved to be durable with 59% of the responders remaining in major molecular response after 5 years. The latter translated into an estimated overall survival of 73% at 5

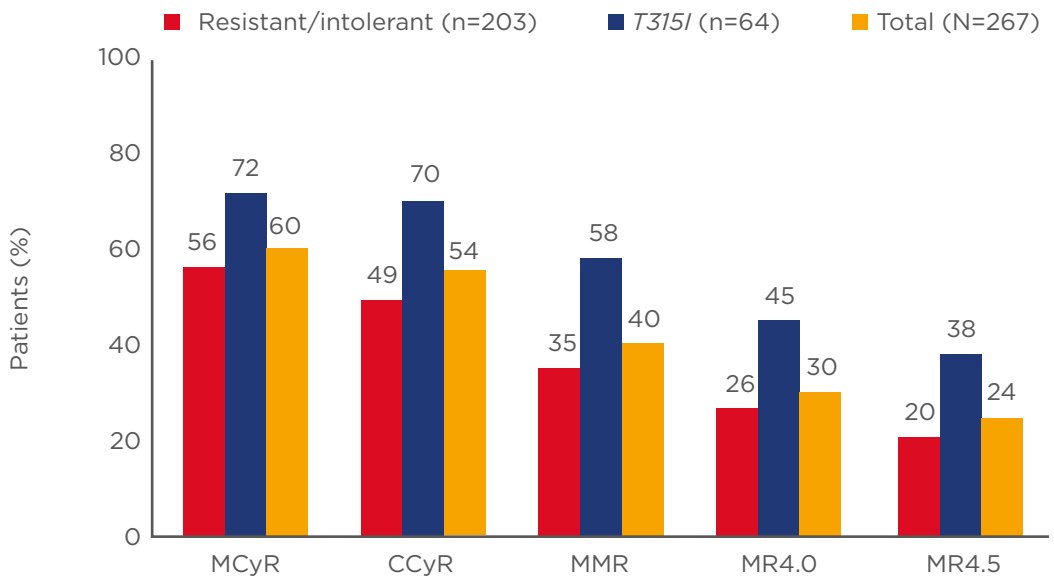


Figure 1: Rates of responses to ponatinib in the PACE trial in patients with chronic-phase chronic myeloid leukaemia.

CCyR: complete cytogenetic response; MCyR: minor cytogenetic response; MMR: major molecular response; MR4.0/4.5: deep molecular response 4.0/4.5.

Adapted from Cortes et al.[13]

years.[13] For patients treated with ponatinib, close monitoring and the use of preventive measures are warranted to decrease the risk of toxicity.[14] Finally, for the small proportion of patients who are not responding to multiple lines of therapy, including ponatinib, a donor search for an allogeneic stem cell transplantation can be started.[14]

Optimising Mutation Testing in Chronic Myeloid Leukaemia

Doctor Simona Soverini and Doctor Hugues de Lavallade

As indicated before, patients with a suboptimal response or a TKI treatment failure should undergo mutational analysis, as recommended by the ELN and NCCN guidelines.[5-7] Sanger sequencing has long been the gold standard to perform this mutational analysis, but evidence has accumulated showing that next-generation sequencing (NGS) is markedly more sensitive. NGS can detect mutations with a sensitivity of approximately 3%, while Sanger sequencing has a sensitivity of 15–20%.[15,16] As such, NGS allows the detection of TKI-resistant mutations much earlier and at lower frequency levels. Data generated by Dr Soverini indicate that the detection of low-level mutations is of clinical relevance given the fact that all these low-level mutations expand if there is no switch to an appropriate TKI.[17,18] In addition, recent data reported by Schmitt et al.[19] indicate that advanced CML and Philadelphia chromosome-positive acute lymphoblastic leukaemia patients with *BCR-ABL* mutations have a greater likelihood of acquiring additional mutations. With this in mind, Dr Soverini and Dr de Lavallade concluded that it is essential to prevent the clonal expansion of these TKI-resistant mutants and the accumulation of additional mutations by switching to an appropriate TKI in a timely manner.

References

1. Bower H et al. Life expectancy of patients with chronic myeloid leukemia approaches the life expectancy of the general population. J Clin Oncol. 2016;34(24):2851-7.
2. Hehlmann R et al. Deep molecular response is reached by the majority of patients treated with imatinib, predicts survival, and is achieved more quickly by optimized high-dose imatinib: Results from the randomized CML-study IV. J Clin Oncol. 2014;32(5):415-23.
3. Hochhaus A et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. N Engl J Med. 2017;376(10):917-27.
4. Soverini S et al. Implications of *BCR-ABL1* kinase domain-mediated resistance in chronic myeloid leukemia. Leuk Res. 2014;38(1):10-20.
5. Baccarani M et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872-84.
6. Soverini S et al. *BCR-ABL* kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: Recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118(5):1208-15.
7. Radich JP et al. Chronic myeloid leukemia, Version 1.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2018;16(9):1108-35.
8. Redaelli S et al. Three novel patient-derived *BCR/ABL* mutants show different sensitivity to second and third generation tyrosine kinase inhibitors. Am J Hematol. 2012;87(11):E125-8.
9. Ibrahim AR et al. Efficacy of tyrosine kinase inhibitors (TKIs) as third-line therapy in patients with chronic myeloid leukemia in chronic phase who have failed 2 prior lines of TKI therapy. Blood. 2010;116(25):5497-500.
10. Garg RJ et al. The use of nilotinib or dasatinib after failure to 2 prior tyrosine kinase inhibitors: Long-term follow-up. Blood. 2009;114(20):4361-8.
11. Giles FJ et al. Nilotinib is active in chronic and accelerated phase chronic myeloid leukemia following failure of imatinib and dasatinib therapy. Leukemia. 2010;24(7):1299-301.
12. Khoury H et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. Blood. 2012;119(15):3403-12.
13. Cortes JE et al. Ponatinib efficacy and safety in Philadelphia chromosome–positive leukemia: Final 5-year results of the Phase 2 PACE trial. Blood. 2018;132(4):393-404.
14. Müller MC et al. Ponatinib in chronic myeloid leukemia (CML): Consensus on patient treatment and management from a European expert panel. Crit Rev Oncol Hematol. 2017;120:52-9.
15. Baer CR et al. Prospective next-generation sequencing of mutations in the *ABL1* kinase domain in CML patients lacking optimal response according to ELN Guidelines. Blood. 2016;128(22):3100.
16. Rohlin A et al. Parallel sequencing used in detection of mosaic mutations: Comparison with four diagnostic DNA screening techniques. Hum Mutat. 2009;30(6):1012-20.
17. Soverini S et al. Clinical relevance of low burden *BCR-ABL1* mutations detectable by amplicon deep sequencing (DS) in Philadelphia-positive (Ph+) acute lymphoblastic leukemia (ALL) patients (pts): The type of mutation matters. Blood. 2015;126(23):2489.
18. Soverini S et al. Next generation sequencing for sensitive detection of *BCR-ABL1* mutations relevant to tyrosine kinase inhibitor choice in imatinib-resistant patients. Oncotarget. 2016;7(16):21982-90.
19. Schmitt MW et al. Single-molecule sequencing reveals patterns of preexisting drug resistance that suggest treatment strategies in Philadelphia-positive leukemias. Clin Cancer Res. 2018;24(21):5321-34.

Integrating Selective Targeted Monoclonal Antibody Therapies for Improved Outcomes in Uncontrolled Asthma

These posters were presented Between September 28th and October 2nd 2019, as part of the European Respiratory Society (ERS) International Congress, Madrid, Spain

Speakers: Mario Castro,[1] Warner W. Carr,[2] Ian Pavord,[3] Alberto Papi[4]

1. Washington University School of Medicine, Saint Louis, Missouri, USA
2. Allergy & Asthma Associates of Southern California, Mission Viejo, California, USA
3. NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, UK
4. St. Anna University Hospital, Ferrara, Italy

Disclosures: Prof Castro has received consultation fees from Aviragen, Boston Scientific, Genentech, Nuvaire, Sanofi-Aventis, and Teva; speaker fees from AstraZeneca, Boehringer Ingelheim, Boston Scientific, Genentech, and Teva; has conducted contracted research for Amgen, Boehringer Ingelheim, Gilead, GlaxoSmithKline, Invion, MedImmune, Sanofi-Aventis, Teva, and Vectura; and is on the data safety and monitoring board for GlaxoSmithKline. Dr Carr has received honoraria or consultation fees from AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Novartis, Sanofi, and Teva. Prof Pavord has received consultation fees from Aerocrine, Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Novartis, Sanofi/Regeneron, and Teva; grants and research support from Chiesi; and other support from AstraZeneca, Bayer, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Insmed, Merck, Sanofi/Regeneron, and Teva. Prof Papi has received consultant or advisory board fees from Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi Farmaceutici, GlaxoSmithKline, Mundipharma, Novartis IT, TEVA, and Zambon; speaker fees from Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi Farmaceutici, Gentili, GlaxoSmithKline, Merck Sharp & Dohme, Mundipharma, Novartis, Pfizer, and TEVA; and sponsored grants from AstraZeneca, Boehringer Ingelheim, Chiesi Farmaceutici, Fondazione Maugeri, Fondazione, GlaxoSmithKline, Menarini, and Merck Sharp & Dohme.

Acknowledgement: Medical writing was provided by Bronwyn Boyes, London, UK.

Support: The publication of this article was funded by Sanofi Genzyme and Regeneron Pharmaceuticals, Inc. The views and opinions expressed are those of the presenters and not necessarily of Sanofi Genzyme and Regeneron Pharmaceuticals, Inc.

Citation: EMJ. 2019;4[4]:37-44.

Summary

Asthma is one of the most common chronic diseases, with ≤25% of patients experiencing uncontrolled disease.[1] Patients with uncontrolled, moderate-to-severe asthma are at increased risk of recurrent exacerbations, accelerated decline in lung function, fixed airway obstruction, and have increased utilisation of health care resources.[2,3] Furthermore, reduced lung function, as assessed by measures such as forced expiratory volume in 1 second (FEV_1), is a strong independent predictor of exacerbations,

progressive decline in lung function, and all-cause pulmonary and cardiovascular mortality in patients with asthma.[2] Achieving asthma control in these patients is therefore critical. The recognition of distinct inflammatory phenotypes within this population has been instrumental in addressing this need. In these patients, there is robust evidence of the pathogenic role of Th2 cytokines, such as IL-4 and IL-13, in the eosinophilic and allergic inflammatory processes.[4] This in turn has driven the development of targeted biological therapies, particularly selective targeted monoclonal antibodies such as dupilumab which inhibit the biological effects of both IL-4 and IL-13.[5]

This article reviews four posters displayed at the European Respiratory Society (ERS) International Congress 2019 that presented results demonstrating the efficacy and safety of dupilumab, an anti-IL-4 receptor human monoclonal antibody, compared to placebo for the treatment of uncontrolled, moderate-to-severe asthma, as measured by a range of outcomes.

Background

Atypical production of several Th2 cytokines, including IL-4, IL-5, and IL-13, play a central pathogenic role in multiple atopic conditions.[6-9] Specifically, IL-4 and IL-13 are associated with the pathogenesis of certain types of asthma, including allergic and nonallergic forms.[5,6,9-12] IL-4 and IL-13 were historically thought to mediate identical signalling pathways because they share receptor complexes; however, IL-4 and IL-13 elicit distinct allergic hallmarks. IL-4 is the central mediator of Th2 cell differentiation, isotype class switching (especially to IgE), B cell growth, and eosinophil (EoS) recruitment.[12-14] IL-13 has roles in goblet cell hyperplasia induction and smooth muscle contractility.[9,15] Therefore, IL-4 and IL-13 activate multiple cell types and induce various mediators involved in inflammation, contributing to airflow limitation and increasing the risk of severe exacerbations.[11-15] Little is currently known regarding the roles of IL-4/IL-13 in IgE and non-IgE-mediated inflammatory pathways, or the effect of inhibiting IL-4/IL-13 in these pathways in asthma.

Dupilumab, a fully human anti-IL-4Rα monoclonal antibody, inhibits signalling of both IL-4 and IL-13 by specifically binding to the IL-4Rα subunit shared by both receptor complexes.[5,10-12] This effect is associated with the marked suppression of biomarkers of Type 2 inflammation including total serum IgE, thymus and activation regulated chemokine, eotaxin-3, and fractional exhaled nitric oxide (FeNO).[13]

Liberty Asthma QUEST Trial

The Liberty Asthma QUEST[16] was a Phase III, randomised, placebo-controlled, parallel-group trial in 1,902 patients with persistent asthma, receiving continuous inhaled corticosteroids (ICS), plus up to two additional controller medications.[17] Patients with uncontrolled, moderate-to-severe asthma (based on the Global Initiative for Asthma [GINA] 2015 guidelines),[18] with a history of one or more exacerbations in the previous year and without a minimum requirement for baseline blood EoS count or any other Type 2 biomarkers (FeNO or serum total IgE),[19] were randomised in a 2:2:1:1 ratio to receive 52 weeks of add-on therapy with subcutaneously administered dupilumab 200 mg or 300 mg every 2 weeks, or matched placebo.[5]

The co-primary efficacy endpoints included an annualised rate of severe exacerbation events during the 52-week treatment period and absolute change from baseline in pre-bronchodilator (BD) FEV_1 at Week 12. A secondary endpoint was the percentage change from baseline to Week 12 in pre-BD FEV_1.[19]

This study showed that add-on dupilumab significantly reduced severe asthma exacerbations; improved lung function, asthma control, and quality-of-life measures; and was generally well-tolerated.[13] Moreover, treatment effects were greater in patients with elevated Type 2 biomarkers at baseline (blood EoS and FeNO).[17,19]

Dupilumab Effect on Lung Function in Patients with Uncontrolled, Moderate-to-Severe Asthma with an Allergic Phenotype

Professor Mario Castro

This post hoc subset analysis of the Liberty Asthma QUEST trial assessed the effect of dupilumab on lung function parameters in patients with uncontrolled, moderate-to-severe asthma with and without evidence of allergic asthma. In this study, allergic asthma was defined as total serum IgE ≥30.00 IU/mL and ≥1.00 perennial aeroallergen-specific IgE ≥0.35 kU/L. The study assessments included the change from baseline in pre-BD FEV_1 (L), post-BD FEV_1 (L), pre-BD forced expiratory flow at 25–75% of pulmonary volume ($FEF_{25-75\%}$, L/s), and pre-BD FEV_1/forced vital capacity (FVC) ratio (%) during the 52-week treatment period in patients receiving dupilumab 200 mg every 2 weeks, 300 mg every 2 weeks, or matched placebos stratified by evidence of allergic asthma.[20]

Of the patients, 57% had allergic asthma (n=1,083) with a mean age of 44.40 years, 58.40% were female, and the mean number of severe exacerbations was 1.96. In the nonallergic asthma group, the mean age was 52.70 years, 70.40% were female, and the mean number of severe exacerbations was 2.32.[20]

This post hoc analysis showed that dupilumab improved pre and post-BD FEV_1, pre-BD $FEF_{25-75\%}$ (L/s), and FEV_1/FVC ratio (%) at Weeks 12 and 52 in patients with uncontrolled, moderate-to-severe asthma with and without evidence of allergic asthma. Dupilumab 200 mg and 300 mg every 2 weeks versus placebo also improved lung function parameters at Week 12 (change from baseline least squares [LS] mean difference pre-BD FEV_1: 0.13/0.16 L; post-BD FEV_1: 0.13/0.11 L; $FEF_{25-75\%}$: 0.14/0.22 L/s; FVC: 0.15/0.11 L; FEV_1/FVC ratio: 0.56/2.78%; all $p<0.05$ except dupilumab 200mg, FEV_1/FVC ratio [p=0.35]). Sustained or better improvements were observed at Week 52 (all $p<0.05$).[20]

The incidence of treatment-emergent adverse events (TEAE) was similar across treatment groups and the most common TEAE reported were viral upper respiratory tract infections (18.2% versus 19.6%), injection-site erythema (13.8% versus 5.5%), upper respiratory tract infection (11.6% versus 13.6%), and bronchitis (11.4% versus 14.0%) in dupilumab versus placebo, respectively.[20]

Prof Castro concluded that in addition to reducing severe asthma exacerbations and biomarkers of Type 2 inflammation, including total serum IgE,[21] dupilumab therapy demonstrated rapid and sustained improvement in lung function in uncontrolled, moderate-to-severe asthma patients, with or without evidence of allergic asthma, during the 52-week treatment period. Dupilumab improved both large (pre and post-BD FEV_1) and small (pre-BD $FEF_{25-75\%}$) airway function, as well as airway obstruction (pre-BD FEV_1 /FVC). The magnitude of improvement was consistent between patients with and without evidence of allergic inflammation and the maximum effect was achieved by Week 12 and sustained to Week 52.[20] These results are supported by a previous post hoc analysis, in which similar results were observed in QUEST patients with and without evidence of allergic asthma.[21]

Dupilumab Efficacy in Patients with Uncontrolled, Moderate-to-Severe Asthma by Immunoglobulin E Levels at Baseline

Doctor Warner W. Carr

This post hoc analysis assessed the effect of dupilumab on severe exacerbations and FEV_1, as well as the impact on overall asthma control in patients with uncontrolled, moderate-to-severe asthma as defined by baseline IgE levels. The aim was to investigate whether there was a differential effect on these efficacy measures defined by baseline IgE levels. The study assessments included the annualised rate of severe exacerbations, LS mean change from baseline in pre-BD FEV_1 (L), and LS mean change from baseline in the 5-item Asthma Control Questionnaire (ACQ-5) score during the 52-week treatment period.[22]

Patients with uncontrolled, moderate-to-severe asthma were characterised at baseline by IgE level (381 patients had an IgE level <100; 782 patients had ≥100 to <500; 419 patients had ≥500; 313 patients had ≥700; and 212 patients had ≥1,000 IU/mL [Figure 1]). Baseline demographics and disease characteristics were generally similar across IgE groups.[22]

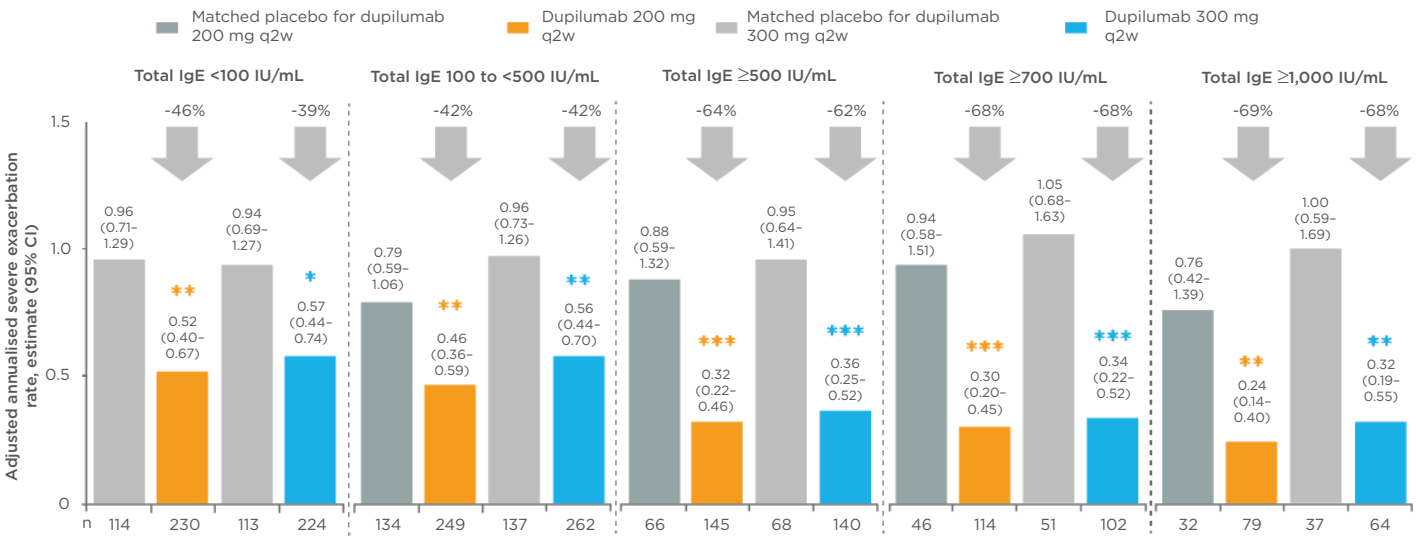


Figure 1: Dupilumab significantly reduced severe exacerbations in all baseline IgE groups.[22]

*p<0.05; **p<0.01; ***p<0.001 versus matched placebo.

CI: confidence interval; IgE: immunoglobulin-E.

Dupilumab 200 mg and 300 mg every 2 weeks versus placebo significantly reduced severe exacerbations in all baseline IgE groups (-38.9 to -67.9%; all p<0.05) and significantly improved pre-BD FEV_1 at Weeks 24 and 52 in all baseline IgE groups (LS mean difference: 0.11–0.31 L; all p<0.05), except for 300 mg in IgE <100 IU/mL and ≥1,000 IU/mL groups (Figure 2).[22]

In the overall safety population, the incidence of TEAE was similar across treatment groups. Conjunctivitis was observed in 2.3% versus 3.3% of patients receiving dupilumab versus placebo, respectively.[22]

Dr Carr concluded that in general, baseline demographics and disease characteristics were balanced between treatment groups across the patient subgroups by total serum IgE levels at baseline. Dupilumab reduced severe asthma exacerbation rates and improved FEV_1 and asthma control in patients with moderate-to-severe asthma in all IgE subgroups. For exacerbations, these effects reached statistical significance for both dupilumab 200 mg and 300 mg every 2 weeks groups in all IgE subgroups. For FEV_1 and asthma control, these effects reached statistical significance for patients receiving dupilumab 200 mg every 2 weeks in all IgE subgroups; but for where dupilumab was prescribed 300 mg every 2 weeks dose, not all IgE subgroups reached statistical significance. In conclusion, regardless of atopic status as categorised by baseline IgE levels, dupilumab can reduce severe exacerbations and improve FEV_1.[22]

Dupilumab Efficacy in Type 2 Inflammatory Asthma: Liberty Asthma QUEST Study (Poster OA3807)

Professor Ian D. Pavord

The new GINA report for difficult-to-treat and severe asthma proposes baseline blood Eos ≥150 cells/µL and/or baseline FeNO ≥20 parts per billion (ppb) as cut-offs to define Type 2 inflammatory asthma.[23]

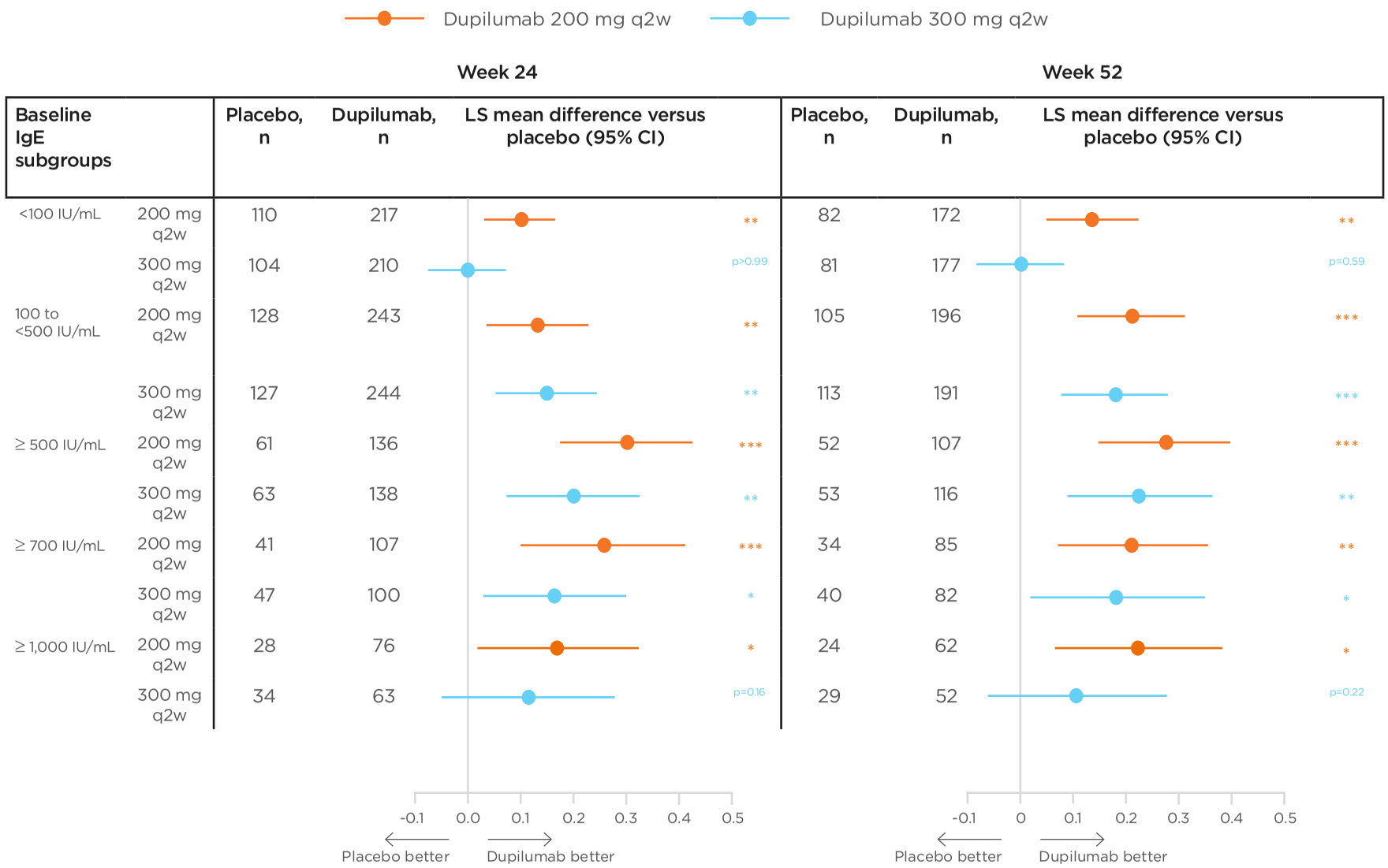


Figure 2: Effect of dupilumab on the change from baseline in least squares mean pre-bronchodilator forced expiratory volume in 1 second by IgE levels at Weeks 24 and 52.[22]

*p<0.05; **p<0.01; ***p<0.001 versus matched placebo.

CI: confidence interval; IgE: immunoglobulin-E; LS: least squares; q2w: every 2 weeks.

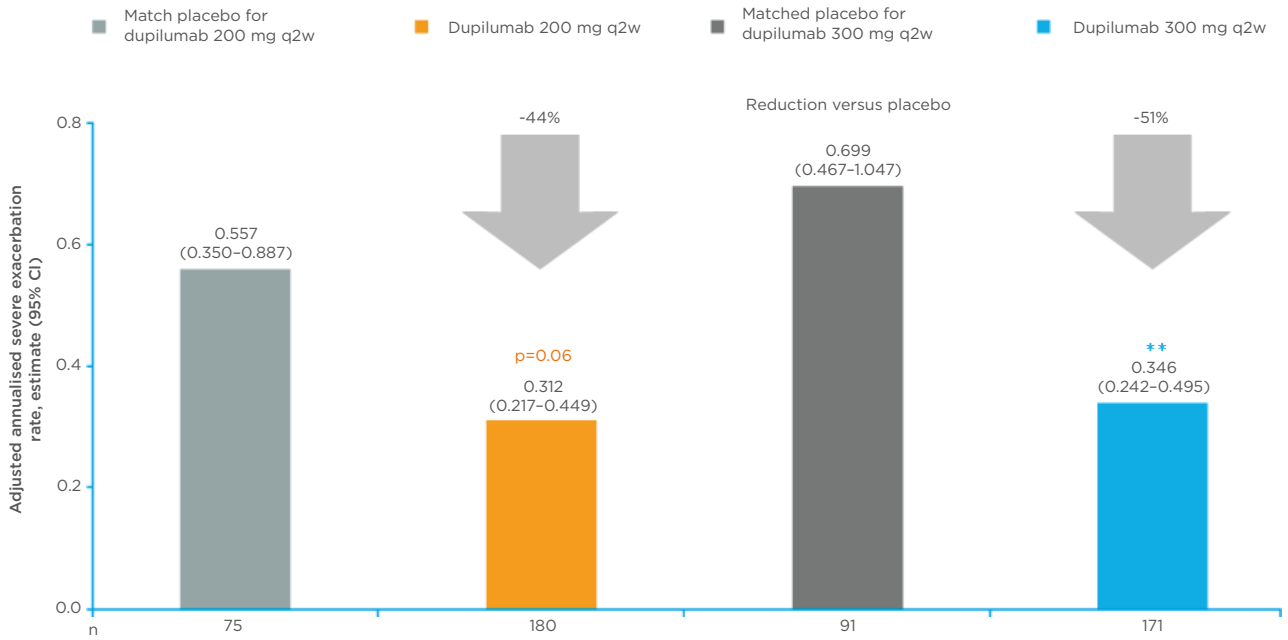


Figure 3: Dupilumab reduced severe exacerbations in patients with baseline pre-bronchodilator forced expiratory volume in 1 second 60–80% predicted (or 60–90% in adolescents <18 years) on medium-dose inhaled corticosteroids .[25]

**p<0.01 versus matched placebo.

Dupilumab 200 mg and 300 mg q2w reduced annualised severe exacerbation rates versus placebo. The annualised rates of severe exacerbations during the 52-week treatment period were analysed using negative binomial regression models.

CI: confidence interval; q2w: every 2 weeks.

This post hoc analysis assessed dupilumab efficacy in patient subgroups defined by the GINA proposed markers for Type 2 asthma, namely in patients with baseline Eos ≥150 cells/µL, FeNO ≥20 ppb, and in other quadrant subgroups. The endpoints assessed included annualised rate of severe exacerbations during the 52-week treatment period and change from baseline in pre-BD FEV_1 (L) at Week 12.[24]

Baseline disease characteristics were generally comparable across the subgroups. The mean age was 47.20 years, 59.00% were female, the mean percent predicted pre-BD FEV_1 was 58.55, the mean exacerbations in the past year was 2.22, and the mean ACQ-5 score was 2.76.[24]

In patients with baseline Eos ≥150 cells/µL and FeNO ≥20 ppb (n=922), dupilumab 200 mg and 300 mg every 2 weeks versus placebo significantly reduced severe exacerbations by 66% and 63% respectively, and improved FEV_1 by 0.26 L and 0.22 L, respectively (all p<0.0001). Similar results were observed at Week 52 and dupilumab efficacy was not significant in the other patient subgroups.[24]

Overall, the most frequently reported AE in the dupilumab versus placebo group was injection-site reactions.[24]

Prof Pavord concluded that dupilumab significantly reduced severe exacerbations and improved FEV_1 in patients with Type 2 inflammatory asthma. Moreover, the effect of dupilumab treatment in reducing exacerbations and improving FEV_1 was greatest in patients with elevation of both baseline blood EoS count (≥150 cells/µL) and FeNO (≥20 ppb).[24]

Dupilumab Efficacy in Asthma Patients with FEV_1 60–80% Predicted on Medium-Dose Inhaled Corticosteroids : LIBERTY ASTHMA QUEST Study

Professor Alberto Papi

This post hoc analysis aimed to assess dupilumab efficacy in patients with moderate asthma defined as asthma with baseline pre-BD FEV_1 60–80% predicted (60–90% in adolescents <18 years), on medium-dose ICS (implying milder asthma than other QUEST patients), and one or more additional controller therapy, without a minimum requirement for baseline blood Eos count or FeNO. The co-primary endpoints were the annualised severe asthma exacerbation rates during the 52-week treatment period and the change from baseline in pre-BD FEV_1 at Week 12, analysed using negative binomial models and mixed-effects models with repeated measures, respectively. Study assessments included the annualised severe exacerbation rates, LS mean change from baseline in pre-BD FEV_1 (L), and LS mean change from baseline in the ACQ-5 score during the 52-week treatment period. The medium ICS dose was fluticasone propionate at a total daily dose of 250–500 µg or an equipotent equivalent.[25]

Twenty-seven percent (517/1,902) of patients had pre-BD FEV_1 60–80% predicted and were on medium-dose ICS at baseline. The mean age was 43.50 years, 61.90% were female, the mean percent predicted pre-BD FEV_1 was 69.49, the mean exacerbations in the past year was 1.82, and the mean ACQ-5 score was 2.56.[25]

In these patients, dupilumab 200 mg and 300 mg every 2 weeks versus placebo reduced annualised severe exacerbation rates by 44% and 51%, respectively (p=0.06; p=0.01; [Figure 3]).

Dupilumab 200 mg and 300 mg every 2 weeks versus placebo also improved FEV_1 at Week 12 with a LS mean difference of 0.11 L/ 0.09 L, respectively (p=0.01/p=0.05).[25]

Overall, the most frequent adverse event reported in the dupilumab 200 mg and 300 mg groups versus placebo groups were injection-site reactions (15%/18% versus 5%/10%).[25]

Conclusion

Prof Papi concluded that dupilumab demonstrated meaningful reductions in severe exacerbations and significantly improved pre-BD FEV_1 in the studied patient population. The magnitude of these effects was comparable to those previously seen in the LIBERTY ASTHMA QUEST patients with severe asthma. Numerical improvements in ACQ-5 were observed at all time points, with the trend comparable to results observed in the overall QUEST population and, furthermore, dupilumab was generally well-tolerated.[25]

Dupilumab is approved in the European Union (EU) for patients >12 years as an add-on maintenance treatment for severe asthma with Type 2 inflammation. This is characterised by raised blood EoS and/or raised FeNO; inadequately controlled with high dose ICS and another medicinal product for maintenance treatment; and in certain patients with asthma, chronic rhinosinusitis with nasal polyps, or atopic dermatitis in a number of countries.[17,26-33] These four posters presented at the ERS International Congress 2019 demonstrate that dupilumab treatment is relatively well-tolerated and could significantly improve FEV_1, symptoms, asthma control, quality of life, and reduce severe exacerbation risk in patients with uncontrolled asthma. This therapy offers an important new option for respiratory clinicians to manage their patients with uncontrolled asthma.

References

1. Peters SP et al. Uncontrolled asthma: A review of the prevalence, disease burden and options for treatment. Respir Med. 2006;100(7):1139-51.
2. Ratageri VH et al. Lung function tests in asthma: Which indices are better for assessment of severity? J Trop Pediatr. 2001;47(1):57-9.
3. Kerkhof M et al. Healthcare resource use and costs of severe, uncontrolled eosinophilic asthma in the UK general population. Thorax. 2018;73(2):116-24.
4. Chung KF. Targeting the interleukin pathway in the treatment of asthma. Lancet. 2015;386(9998):1086-96.
5. Busse WW et al. Liberty Asthma QUEST: Phase 3 randomized, double-blind, placebo-controlled, parallel-group study to evaluate dupilumab efficacy/safety in patients with uncontrolled, moderate-to-severe asthma. Adv Ther. 2018;35(5):737-48.
6. Hall S, Agrawal DK. Key mediators in the immunopathogenesis of allergic asthma. Int Immunopharmacol. 2014;23(1):316-29.
7. Chen L et al. IL-4 induces differentiation and expansion of Th2 cytokine-producing eosinophils. J Immunol. 2004 Feb 15;172(4):2059-66.
8. Rael EL, Lockey RF. Interleukin-13 signaling and its role in asthma. World Allergy Organ J. 2011;4(3):54-64.
9. Gour N, Wills-Karp M. IL-4 and IL-13 signaling in allergic airway disease. Cytokine. 2015;75(1):68-78.
10. Macdonald LE et al. Precise and in situ genetic humanization of 6 Mb of mouse immunoglobulin genes. Proc Natl Acad Sci U S A. 2014;111(14):5147-52.
11. Murphy AJ et al. Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normal mice. Proc Natl Acad Sci U S A. 2014;111(14):5153-8.
12. Gandhi NA et al. Commonality of the IL-4/IL-13 pathway in atopic diseases. Expert Rev Clin Immunol. 2017;13:425-37.
13. Wynn TA. Type 2 cytokines: Mechanisms and therapeutic strategies. Nat Rev Immunol. 2015;15(5):271-82.
14. Chatila TA. Interleukin-4 receptor signaling pathways in asthma pathogenesis. Trends Mol Med. 2004;10(10):493-9.
15. Chiba Y et al. Interleukin-13 augments bronchial smooth muscle contractility with an up-regulation of RhoA protein. Am J Respir Cell Mol Biol. 2009;40(2):159-67.
16. Sanofi. Evaluation of Dupilumab in Patients With Persistent Asthma (Liberty Asthma Quest). NCT02414854. https://clinicaltrials.gov/ct2/show/NCT02414854.
17. Castro M et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med. 2018;378(26):2486-96.
18. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. 2015. Available at: https://ginasthma.org/wp-content/uploads/2016/01/GINA_Report_2015_Aug11-1.pdf. Last accessed: 8 November 2019.
19. Maspero JF et al. Dupilumab efficacy in uncontrolled, moderate-to-severe asthma with self-reported chronic rhinosinusitis. J Allergy Clin Immunol Pract. 2019;pii:S2213-2198(19)30633-6. [Epub ahead of print].
20. Castro M et al. Dupilumab effect on lung function in patients with uncontrolled, moderate-to-severe asthma with an allergic phenotype. Eur Respir J. 2019;54(Suppl 63):PA540.
21. Corren J et al. Dupilumab efficacy in patients with uncontrolled, moderate-to-severe allergic asthma. J Allergy Clin Immunol Pract. 2019;pii:S2213-2198(19)30775-5. [Epub ahead of print].
22. Carr WW et al. Dupilumab efficacy in patients with uncontrolled, moderate-to-severe asthma by immunoglobulin e levels at baseline. Eur Respir J. 2019;54(Suppl 63):PA536.
23. Global Initiative for Asthma (GINA). Difficult-to-treat and severe asthma in adolescents and adults. 2019. Available at: https://ginasthma.org/wp-content/uploads/2019/06/GINA-2019-main-report-June-2019-wms.pdf. Last accessed: 8 November 2019.
24. Pavord IS et al. Dupilumab efficacy in Type 2 inflammatory asthma: Liberty asthma quest study. Eur Respir J. 2019;54(Suppl 63):OA3807.
25. Papi A et al. Dupilumab efficacy in asthma patients with FEV1 60–80% predicted on medium-dose ICS: Liberty asthma quest study. Eur Respir J. 2019;54(Suppl 63):OA3807. PA538.
26. European Medicines Agency (EMA). DUPIXENT® (dupilumab). 2017. Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/dupixent. Last accessed: 10 November 2019.
27. U.S. Food and Drug Administration (FDA). DUPIXENT® (dupilumab). Highlights of prescribing information. 2019. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/761055s014lbl.pdf. Last accessed: 10 November 2019.
28. Pharmaceuticals and Medical Devices Agency (PMDA). DUPIXENT® (dupilumab). 2019. Available at: http://www.pmda.go.jp/PmdaSearch/iyakuDetail/ResultDataSetPDF/780069_4490405G1024_1_04. Last accessed: 10 November 2019.
29. Wenzel S et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β2 agonist: A randomised double-blind placebo-controlled pivotal Phase 2b dose-ranging trial. Lancet. 2016;388(10039):31-44.
30. Rabe KF et al. Efficacy and safety of dupilumab in glucocorticoid-dependent severe asthma. N Engl J Med. 2018;378(26):2475-85.
31. Blauvelt A et al. Long-term management of moderate-to-severe atopic dermatitis with dupilumab and concomitant topical corticosteroids (LIBERTY AD CHRONOS): A 1-year, randomised, double-blinded, placebo-controlled, Phase 3 trial. Lancet. 2017;389(10086):2287-303.
32. Simpson EL et al. Two Phase 3 trials of dupilumab versus placebo in atopic dermatitis. N Engl J Med. 2016;375(24):2335-48.
33. Thaçi D et al. Efficacy and safety of dupilumab in adults with moderate-to-severe atopic dermatitis inadequately controlled by topical treatments: A randomised, placebo-controlled, dose-ranging Phase 2b trial. Lancet. 2016;387(10013):40-52.

Identifying Shared Features and Addressing Common Challenges in Clinical Trials for Chronic Inflammatory Diseases: An Overview

EDITOR'S PICK

In this edition's Editor's Pick, La Noce and Ernst explore the important topic of executing clinical trials for chronic inflammatory diseases that share common features, especially those that assess biologic drugs that could treat multiple diseases. By examining the common challenges that arise in this endeavour, the authors offer their ideas on how to best implement the most successful clinical trials for these diseases.

Authors: *Anna La Noce,[1] Marcin Ernst[2]

1. Executive Medical Director, General Medicine, Syneos Health, Italy
2. Vice President, General Medicine, Syneos Health, Poland
*Correspondence to anna.lanoce@syneoshealth.com

Disclosure: The authors have declared no conflicts of interest; the research for this manuscript was conducted independently of their positions within Syneos Health.

Received: 13.03.19

Accepted: 07.06.19

Citation: EMJ. 2019;4[4]:45–52.

Abstract

Chronic inflammatory diseases (CID) share many common features, such as debilitating illness, increased mortality, impaired quality of life and productivity, and high economic burden. The approach to treating CID has shifted over the last 20 years from symptom to mechanism of action-targeted therapy following the development of primarily biologic drugs, in which the same therapy can potentially treat multiple diseases. Developing these drugs requires novel strategies and a multidisciplinary approach for implementation. This article provides an overview of shared features for CID clinical trials and addressing common challenges in their planning and execution. Since CID studies often test the same drug for treating different pathologies, knowledge of the drug from previously investigated therapeutic indications can be leveraged when planning clinical trials. Given the variety of CID signs and symptoms, eligibility criteria need to clearly define the target patient population by minimising ambiguity and risk of misunderstanding. Other common challenges include an elevated response in the placebo arm, the subjectivity of investigator assessments, and the use of appropriate patient-reported outcomes. Several measures can help minimise the impact of the aforementioned issues on study outcome, including centralised eligibility review and endpoint adjudication, tight control of background therapy and concomitant medications, and intensive training of assessors. The above common features support an approach to CID as a largely interconnected therapeutic area in which a multidisciplinary approach, application of common strategies, and lessons learnt across different indications represent crucial factors for effectively planning and executing clinical trials.

INTRODUCTION

Chronic inflammatory diseases (CID) encompass a wide range of pathologies affecting different organs or bodily functions. Besides signs and symptoms specific to the organ or the function, they all present common features, such as a long-lasting and debilitating illness, increased mortality, impaired quality of life and productivity, and high social and economic burden.

Most CID are characterised by a dysregulation of the innate or adaptive immune system, leading to and perpetuating a chronic inflammatory condition. Their estimated prevalence varies from 5% to nearly 10%, and is increasing.[1,2] A systematic review to identify the incidence and prevalence of immune-mediated inflammatory diseases over the period 1985–2015 found mean net percentage increases per year (standard deviations) of 19.1±43.1 and 12.5±7.9, respectively.[3] Among the most prevalent CID, psoriasis accounts for >120 million cases worldwide, while rheumatoid arthritis and psoriatic arthritis amount to >70 million cases each.[4,5,6]

In the following sections, an overview of the main features shared by clinical trials of CID is provided, highlighting challenges and potential mitigation.

Since, as stated initially, a huge number of pathologies can be classified as CID spanning across almost all therapeutic areas, it is beyond the scope of this review to cover all of them. While the following discussion will focus primarily on select CID within rheumatology, dermatology, and gastroenterology, the majority of the identified features and challenges can also apply to other disease areas.

CHALLENGES AND COMMON FEATURES OF CHRONIC INFLAMMATORY DISEASES CLINICAL TRIALS

One Drug for Multiple Indications

Since the introduction of the first TNFα inhibitor for the treatment of rheumatoid arthritis, the strategy of CID management has focussed on the identification of altered inflammatory or immune pathway(s) and the search for related targets for drug development. The approach to treating CID has substantially shifted from symptom to mechanism of action-targeted therapy.[7] As a result, the same drug can potentially treat multiple diseases, as the same immune or inflammatory pathway can be shared by different pathologies.

A crucial role in the pathogenesis of CID is played by several proinflammatory cytokines such as TNF, IL-6, or interferon γ. These are considered pleiotropic cytokines, meaning that they show multiple biological actions and, therefore, they can represent an optimal target for drugs directed to treat multiple CID. [8,9] TNF-inhibitors are currently approved for treatment of several rheumatologic, skin, and gastrointestinal autoimmune CID. Similarly, IL-6 inhibitors are presently approved to treat various rheumatologic diseases, giant cell arteritis, and Castleman's disease, and are being tested for other types of CID. New cytokines have more recently emerged as targets for multiple CID, for example IL-17 or IL-23.[10] Other targets that have proved adequate to treat multiple CID are intracellular signal transducers such as those of the JAK or signal transducers and activators of the transcription family.[11]

Planning the development of these new classes of drugs that are either monoclonal antibodies or small molecules has required novel strategies. These include upfront selection of the potential CID to treat with a choice of therapeutic indications to pursue for first marketing authorisation, or a choice of the most adequate clinical model for a preliminary demonstration of the drug's biological efficacy. Implementation of such development strategies in the early clinical phases with possible subsequent adjustments require a multidisciplinary approach, including feasibility assessments, market analyses, and clinical development expertise in multiple therapeutic areas. A multidisciplinary approach with sharing experience across different therapeutic indications also proves helpful at the time of planning and executing clinical trials because of the common features they present, as this review discusses in greater detail.

Treatment-Associated Features

Because the same drug is tested to treat different pathologies, knowledge of the drug from previously investigated therapeutic indications can be leveraged when planning clinical trials. For example, even if differences exist among

different patient populations receiving the same product, knowledge of drug-related side effects, pharmacokinetic characteristics, immunogenicity in the case of biological drugs, administration modalities, and pharmacodynamic markers all contribute to increase a multidisciplinary team's familiarity with the study drug and facilitate planning and execution of clinical trials.

In addition, patients enrolled in CID trials are often receiving background standard of care with immunosuppressive drugs such as methotrexate, azathioprine, mycophenolate mofetil, or with corticosteroids. Knowledge of side effects and precautions associated with the use of these treatments can be also shared across different therapeutic indications to help design adequate study protocols.

Disease and Patient Characteristics

Different CID, even if affecting primarily a specific organ or bodily function such as the joints or skin, often present with a variety of common signs and symptoms that involve the entire body, such as fatigue, fever, anaemia, myalgia, arthralgia, and ocular or renal symptoms. A patient can also suffer from a combination of different CID or develop different CID over time. Moreover, each of these diseases can present a wide spectrum of severity. Medical management can also greatly vary across geographies, depending especially on access to novel therapies and local reimbursement policies.[12,13]

Therefore, it appears evident that enrolling a relatively homogeneous patient population with a definite diagnosis may be challenging. Special attention is to be paid to designing a study protocol with eligibility criteria that clearly define the target patient population by minimising ambiguity and risk of misunderstanding. Eligibility criteria should include severity of disease assessed by standardised methods, a list of concomitant pathologies that are exclusionary, and allowed or prohibited concomitant medications with pre-defined minimal washout duration. On the other hand, when defining such criteria, evolving standard of care is to be taken into account to be able to achieve recruitment goals. For example, while enrolling biologic-naïve patients is becoming more difficult, even in regions with poor access to expensive therapies, it may be more feasible to establish a limit to prior exposure to biologics (e.g., not more than one previous biologic agent) to facilitate recruitment and at the same time avoid recruiting subjects with refractory disease.

Conducting a survey assessment across potential investigational sites on the main features of the protocol and consultation with key opinion leaders is crucial to get a clear picture of the feasibility of the study protocol and recruitment capability.

Patients with CID are commonly known by the clinics taking care of them, given the chronic nature of their condition. This means that study sites have a database of patients, from which they can pre-select subjects who could be eligible for trial participation and can therefore plan for enrollment of patients in a timely fashion and in co-ordination with their clinical activity. In the authors' experience, patients who have a long-standing relationship with a study site also tend to be more compliant with protocol requirements and to remain in the study until completion.

In addition, there are well-organised advocacy groups for most CID, which can be leveraged for disseminating information on the clinical trial and facilitate recruitment.

Placebo Response

One of the major challenges and reasons for study failure in CID is an elevated response rate in the placebo arm. Many factors can contribute to increased placebo response in clinical trials, some of which are especially important for CID. They include subjectivity of disease assessments at screening and in the course of the study, and the need for background therapy in all study participants[14-17]. This last point is of particular interest for CID that often require keeping all patients enrolled in a clinical trial on a stable background therapy, given the chronicity and the severity of the condition. It is well known that patients with such chronic diseases tend to show poor compliance with therapy. But their compliance usually increases when enrolled in a clinical trial and therefore they are subject to closer medical control. As a result, the effectiveness of their background therapy improves together with the response to placebo given on top of it.

In rheumatology, dermatology, or gastroenterology indications of CID, composite indices of disease activity are often used that

heavily rely on assessments performed by the investigator or the patient. In rheumatoid arthritis, count of swollen and tender joints, physician assessment of disease, patient assessment of disease and pain, and health assessment questionnaire are all evaluations that are heavily dependent on individual experience or the subject's perception of their own condition. Two recent studies have shown how these assessments can impact placebo response. In a meta-analysis of 165 randomised controlled trials in rheumatoid arthritis, a placebo effect size for pain relief of 0.28 (95% confidence interval: 0.19, 0.37) was found.[14] Significant placebo effect size for other outcomes was also found, such as physician and patient disease assessment, tender joint count, swollen joint count, and function. Surprisingly, a meta-analysis of 10 Phase II or III randomised controlled trials in rheumatoid arthritis, which enrolled and treated with placebo on top of methotrexate nearly 1,000 subjects, found that assessments performed by investigator or site staff were more sensitive to placebo response compared to patient-reported outcomes (PRO).[15]

Several measures can help limit or at least keep under control placebo response: adequate training of site staff to standardise and make their assessments as objective as possible; training of patients on correct completion of PRO; introduction of a run-in period on background therapy prior to randomisation; preference for objective measures of disease, like blood tests analysed at a central laboratory, or central review of key data; consideration of the cultural or geographical differences (e.g., higher placebo response commonly reported from Latin America[15]); and realistic statistical assumptions for sample size estimates.

Selection of appropriate patients as per protocol is also crucial to reduce the risk of elevated placebo response. Enrolling subjects with a lower than required disease severity is a common issue for CID trials, despite well-defined eligibility criteria.[18] Close control of screening procedures and possibly implementation of a central eligibility review can help. Traditionally, a central eligibility review is implemented in systemic lupus erythematosus (SLE) trials[18] wherein the risk for misclassifying disease severity at study entry is high. For example, the majority of SLE trials require a certain level of disease severity based on a composite index called Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), which is also an important component of the study primary endpoint. The SLEDAI is based on the assessment of various symptoms or signs attributable to lupus, each of which is assigned an individual score, with the overall score being the sum of the individual scores. Petri et al.[19] have shown that mistakenly reporting the presence of headache or vasculitis due to lupus, which both have a very high SLEDAI score, can erroneously inflate the screening and baseline total SLEDAI scores. In a post-hoc analysis of a Phase III study in SLE, the authors observed that the high placebo response could be partly explained by the 'disappearance' of headache or vasculitis in the course of the trial, which was having a strong impact on the patient's response.[19]

Subjectivity of Assessments

As mentioned previously, the majority of CID trials are negatively impacted by subjectivity of assessments used to define the study efficacy endpoints. A few examples of such assessments for different pathologies are listed in Table 1, which is not intended to be exhaustive in terms of either diseases or assessments. Some of the assessments included in the table are a mix of objective and subjective (either from investigator or patient) evaluations, such as the Disease Activity Score-28, Crohn's Disease Activity Index, or Mayo/Ulcerative Colitis Disease Activity Index score.

Even assessments that appear to be objective because they are based on observation and physical examination, such as count of swollen and tender joints, have been shown to strictly depend on the assessor's experience and employed methodology. Several studies have reported considerable variability among individual assessors or clinical sites for both tender and swollen joint count.[20,21]

Interobserver variability seems to be higher for smaller joints and for swollen joints compared to tender joints.[19,22] For the Crohn's Severity Activity Index, which is essentially based on a physician's interpretation of patient symptoms, significant interobserver differences were noted in various studies.[23] Other studies reported assessment reliability within a moderate-to-good range.[24]

Table 1: Assessments of chronic inflammatory diseases.

Disease	Assessment
Rheumatoid arthritis	Swollen and tender joint count Patient Global Assessment (PGA) Physician's Global Assessment Disease Activity Score -28 (DAS28)
Psoriasis	Psoriasis Area Severity Index (PASI) Investigator's Global Assessment (IGA)
Lupus	Systemic Lupus Erythematous Disease Area Index (SLEDAI) British Isle Lupus Assessment Group (BILAG) Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI)
Systemic sclerosis	modified Rodnan Skin Score (mRSS)
Ulcerative colitis	Ulcerative Colitis Disease Activity Index (UCDAI)
Crohn's Disease	Crohn's Disease Activity Index (CDAI)

The modified Rodnan Skin Score, an assessment of skin thickness, is commonly used in clinical trials of systemic sclerosis as a surrogate endpoint for disease activity and severity. It has been shown to exhibit a high interobserver variability.[25]

Because in almost all assessments discussed above the intraobserver variability tends to be significantly lower than the interobserver one, the first recommendation for CID studies is that key disease activity assessments are performed by the same assessor throughout the trial or at least within the same subject. All assessors need to be properly trained and qualified to standardise the method of assessment across study sites. Differences among assessors may be due not only to different levels of experience but also to differences in local practice and the adopted methodology. Training can be conducted by employing a combination of different tools, for example the use of video demonstration, Webex training, implementation of dedicated educational web portals, and/or live demonstration by qualified trainers at investigator meetings. It is recommended that trainees are required to pass a test at the end of a training session to receive certification of training. Training should also be tailored to each individual study.

When feasible and appropriate, central adjudication can be used so that the assessment for all patients enrolled in the study is performed by an adjudication committee. This is the case for the British Isles Lupus Assessment Group's (BILAG) scoring index where usually centralised review and adjudication on an ongoing basis is implemented in clinical trials for lupus. The adjudication process is commonly supported by onsite review conducted by experienced study monitors and preprogrammed automatic edit checks to clean local assessments and correct inconsistencies.[18]

Central reading is also implemented for instrumental evaluations like endoscopy in inflammatory bowel diseases, spirometry in respiratory diseases, or joint radiography in rheumatology to guarantee standardisation and unbiased evaluation.

Biomarkers

In recent years, attention has focussed on the identification of biomarkers specific for individual or groups of CID.[26,27] Besides being used to expedite initial proof of mechanism studies and screening of drug candidates, biomarkers can allow for the prediction of response to treatment

or stratification of patients. As such, they can support the attempt to develop personalised medicine for CID, similarly to what is being done in oncology and other therapeutic areas. There are several examples of such biomarkers. Systemic lupus is a CID in which the search for biomarkers is particularly active due to the heterogeneity of disease and efficacy of drugs that apparently is limited to subgroups of patients.[28,29] Among others, *Type I interferon* gene signature has emerged as a marker of disease severity and as a potential tool to stratify patients for tailored treatment.[30]

Therefore, incorporating biomarker evaluation into CID clinical trials from early to late phase has become a common practice.[31]

Patient Reported Outcomes

As recommended by regulatory agencies,[32,33] use of PRO in clinical trials is increasing and also extended to early phase studies. In CID, PRO usually include evaluation of patient pain, global disease, functional limitation, and quality of life, among others. The same or very similar PRO are used across multiple pathologies. For example, the health assessment questionnaire that allows for assessment of functional limitations in daily activities due to the disease is commonly employed in all rheumatologic conditions.[34] Fatigue is a typical symptom of CID that can become more pronounced during flares and can impact a patient's functioning and quality of life. It is often evaluated by means of the Functional Assessment of Chronic Illness-Fatigue questionnaire. Several tools are available for quality of life evaluations. Some of these, such as the 36-Item Short Form Health Survey or the EuroQoL Research Foundation's EQ-5D instruments, are generic and are employed across many different pathologies, while other tools are disease-specific. Depression scales, such as the Hospital Anxiety and Depression Scale, are also often administered because depression tends to be more frequent in subjects with CID.[35,36] Pain scales can include the Visual Analog Scale, Numeric Rating Scale, or more specific tools. Some of the aforementioned scales or questionnaires can be administered during clinic visits, while others are to be completed daily in a patient's diary.

When selecting PRO for a clinical trial, some practical aspects are to be considered upfront. First of all, the most appropriate device used for recording the assessment is to be chosen. Nowadays, electronic devices are preferred over the traditional paper diary or questionnaire; however, attention should be paid to the age of the target patient population and their functional capability that can pose limitations to the use of some electronic devices or require special adaptations. Electronic devices present the big advantage of real-time data capture, which allows for monitoring on an ongoing basis via a dedicated web portal. This can increase patient compliance and help prevent or prompt identification of any issues that may impact data quality or patient safety.[37] Even if use of PRO in clinical trials is encouraged, this should not represent an excessive burden for the patient with consequent impact on compliance and quality. Therefore, the number of PRO, their relevance for the specific trial, time needed for completion, and frequency of assessment should all be considered to find the right balance between study needs and patient comfort and acceptance.

Lastly, patients and site staff both need to be trained on PRO completion and delivery.

CONCLUSION

CID present several common features in terms of patient and treatment characteristics, and with respect to operational aspects of clinical trials. They also share important challenges that could be overcome or mitigated by adopting proper strategies, as summarised in Table 2. This supports an approach to CID as a largely interconnected therapeutic area where multidisciplinarity and application of common strategies and lessons learnt across different indications represent crucial factors for planning and executing clinical trials in an effective and timely fashion.

Table 2: Addressing challenges of chronic inflammatory disease trials.

Challenges	Mitigating strategies
Treatment-associated features	• Leverage knowledge from previously investigated therapeutic indications for same therapy • Take note of side effects and precautions from background standard of care
Target patient population	• Establish clear study eligibility criteria • Assess feasibility of study protocol and recruitment capability
Placebo response	• Train site staff on objective assessment and patients on correct completion of PRO • Introduce run-in period on background therapy prior to randomisation, as feasible • Establish central review of disease measures and other key data, as feasible • Consider cultural and geographic differences • Set realistic statistical assumptions for sample size estimate • Verify that the right patients are enrolled according to eligibility review
Subjectivity of assessments	• Ensure all assessors are qualified and receive proper training tailored to study • Have key disease activity assessments performed by same assessor throughout trial or at least within same study subject • Use central adjudication and reading, when feasible and appropriate
PRO	• Select most appropriate device to record PRO • Consider number, frequency, and completion time for PRO to avoid excessive patient burden • Train patients and site staff on PRO completion and delivery

PRO: patient-reported outcomes.

References

1. El-Gabalawy H et al. Epidemiology of immune-mediated inflammatory diseases: Incidence, prevalence, natural history, and comorbidities. J Rheumatol Suppl. 2010;85:2-10.
2. Cooper GS et al. Recent insights in the epidemiology of autoimmune diseases: Improved prevalence estimates and understanding of clustering of diseases. J Autoimmun. 2009;33(3-4):197-207.
3. Lerner A et al. The world incidence and prevalence of autoimmune diseases is increasing. Int J Celiac Dis.

2015;3(4):151-5.

4. Griffiths C et al. The global state of psoriasis disease epidemiology: A workshop report. Br J Dermatol. 2017;177(1):e4-7.
5. Gibofsky A. Epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis: A Synopsis. Am J Manag Care. 2014;20(7 Suppl):S128-35.
6. Ogdie A, Weiss P. The epidemiology of psoriatic arthritis. Rheum Dis Clin North Am. 2015;41(4):545-68.
7. Kuek A et al. Immune-mediated inflammatory diseases (IMIDs) and biologic therapy: a medical revolution. Postgrad Med J. 2007;83:251-60.
8. Kalliolias G, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nature Rev Rheumatol 2016;12:49–62.
9. Tanaka T et al. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6: a016295.
10. Lai Y, Dong C. Therapeutic antibodies that target inflammatory cytokines in autoimmune diseases. Int Immunol. 2016;28:181-8.
11. O'Shea JJ, Plenge R. JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. Immunity. 2012;36:542-50.
12. Orlewska E et al. Access to biologic treatment for rheumatoid arthritis in Central and Eastern European (CEE) countries. Med Sci Monit. 2011;17:SR1-13.
13. Baumgart DC et al. Biological Therapies in Immune-Mediated Inflammatory Diseases: Can Biosimilars Reduce Access Inequities? Front Pharmacol. 2019;10:279.
14. Abdullah N. THU0189 placebo effect in the treatment of rheumatoid arthritis: A systematic review and meta-analysis of randomized controlled trials. Ann Rheum Dis. 2015;74(2):263.
15. Xu X et al. Physician/site staff assessments contribute to high placebo response in rheumatoid arthritis clinical trials. Abstract 2594. ACR/AHRP Annual Meeting, Washington, DC, USA, 11-16 November, 2016.
16. Kalunian KC et al. Impact of standard of care treatments and disease variables on outcomes in systemic lupus erythematosus trials: analysis from the Lupus Foundation of America Collective Data Analysis Initiative. Eur J Rheumatol. 2016 Mar;3(1):13-19.
17. Merrill JT. Lupus community panel proposals for optimising clinical trials: 2018. Lupus Sci Med. 2018;5:e000258.
18. Pike MC, Kelley L. Data quality challenges in systemic lupus erythematosus trials: How can this be optimized? Curr Rheumatol Rep. 2012;14(4):324-33.
19. Petri M et al. Systemic lupus erythematosus responder index assessment of responders in EMBLEM, a Phase IIb study in patients with moderate to severe systemic lupus erythematosus. Presentation 1378. ACR/AHRP Annual Meeting, Chicago, Illinois, USA, 5-9 November, 2011.
20. Salaffi F et al. Inter-observer agreement of standard joint counts in early rheumatoid arthritis: A comparison with grey scale ultrasonography—A preliminary study. Rheumatology (Oxford). 2008;47(1):54-8.
21. El-Hadidi K et al. Inter-observer agreement of standard joint count examination and disease global assessment in a cohort of Egyptian Rheumatoid Arthritis patients. Reumatol Clin. 2017; pii: S1699-258X(17)30263-2. [Epub ahead of print].
22. Cheung PP et al. Reliability of joint count assessment in rheumatoid arthritis: A systematic literature review. Semin Arthritis Rheum. 2014;43(6):721-9.
23. de Dombal FT, Softley A. IOIBD report no 1: Observer variation in calculating indices of severity and activity in Crohn's disease. International Organisation for the Study of Inflammatory Bowel Disease. Gut. 1987;28(4):474-81.
24. Freeman HJ. Use of the Crohn's disease activity index in clinical trials of biological agents. World J Gastroenterol. 2008;14(26):4127-30.
25. Khanna D et al. Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. J Scleroderma Relat Disord. 2017;2(1):11-8.
26. Giacomelli R et al. Guideline for biomarkers in autoimmune rheumatic diseases – Evidence based analysis. Autoimmune Rev. 2019;18(1):93-106.
27. Neelakshi RJ, James JA. Biomarkers in connective tissue diseases. J Allergy Clin Immunol. 2017;140(6):1473-83.
28. La Paglia GMC et al. One year in review 2017: systemic lupus erythematosus. Clin Exp Rheumatol. 2017;35:551-561.
29. Ahearn JM et al. Biomarkers for systemic lupus erythematosus. Transl Res. 2012;159:326-42.
30. Crow MK, Rönnblom L. Report of the Inaugural Interferon Research Summit: Interferon in inflammatory diseases. Available at: https://www.semanticscholar.org/paper/Report-of-the-inaugural-Interferon-Research-Summit%3A-Crow-R%C3%B6nnblom/8bd17396a912874dae56e805835276d279246ecd. Last accessed: 7 June 2019.
31. Pisetsky DS et al. New Perspectives in Rheumatology: Biomarkers as Entry Criteria for Clinical Trials of New Therapies for Systemic Lupus Erythematosus: The Example of Antinuclear Antibodies and Anti-DNA. Arthritis Rheumatol. 2017;69:487-493.
32. Campbell M. Measuring how patients feel and function. Available at: https://www.fda.gov/media/104969/download. Last accessed 27 Aug 2019.
33. Center for drug evaluation and research. Clinical outcome assessment (COA) compendium. Aug 2019. Available at: www.fda.gov/media/130138/download. Last accessed 27 Aug 2019.
34. Bruce B, Fries JF. The Health Assessment Questionnaire (HAQ). Clin Exp Rheumatol. 2005;23(Suppl 39):S14-8.
35. Lamb RC et al. Screening for anxiety and depression in people with psoriasis: a cross-sectional study in a tertiary referral setting. Br J Dermatol. 2017;176:1028-1034.
36. Dregan A et al. Common mental disorders within chronic inflammatory disorders: a primary care database prospective investigation. Ann Rheum Dis. 2019;78:688-695.
37. Bingham CO et al. Use of daily electronic patient-reported outcome (PRO) diaries in randomized controlled trials for rheumatoid arthritis: rationale and implementation. Trials. 2019;20:182.

Clustering by Health Professionals in Individually Randomised Controlled Trials

Authors: *Mohammad Waleed,[1] Isma Kazmi,[2] Mishah Farooq,[3] Abdul Hamid,[4] Fazal Karam,[5] Victoria Allgar,[6] Kenneth Y.K. Wong[7]

1. Department of Cardiology, Leeds General Infirmary, Leeds, UK
2. Department of Renal medicine, St James' University Hospital, Leeds, UK
3. Bradford Royal Infirmary, Bradford, UK
4. Department of Anaesthesia, Northern General Hospital, Sheffield, UK
5. Department of Orthopedics, Saidu Group of Teaching Hospital, Saidu Sharif, Pakistan
6. York Medical School, University of York, York, UK
7. Department of Cardiology, Blackpool Teaching Hospitals NHS Foundation Trust (Blackpool Victoria Hospital), Blackpool, UK

*Correspondence to waleed1280@yahoo.com

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: This research was started as a part of dissertation project while the authors were completing a master's degree in public health from the University of Sheffield. With the author's contributions, the dissertation was reviewed and amended for publication purposes as a review article. This work has not been presented previously in any scientific meeting. The authors confirm that no financial support or grants were received for this work.

Recieved: 16.07.18

Accepted: 16.01.19

Keywords: Adjustment for clustering, clustering, health professional, individual randomised controlled trials (iRCT), natural clustering.

Citation: EMJ. 2019;4[4]:53-61.

Abstract

Purpose: The aim of this study was to investigate the prevalence of clustering by health professionals in individually randomised controlled trials (iRCT), and its adjustment in both the sample size calculation estimates and the analysis of the data collected in iRCT (that is, trials that randomise individuals only). As a result, cluster randomised controlled trials will not be the part of this review study. Additionally, the authors aimed to discover the prevalence of the various forms of clustering in iRCT.

Methods: iRCT, in which the intervention was delivered by a health professional, were electronically searched in three medical journals. The dates searched were from 1st January 2000–31st August 2009. The retrieved trials were then screened to exclude those with complex designs and trials with more than two parallel arms. The selected trials were then fully reviewed for the presence of clustering effects and any corresponding adjustment. Data about the sample size calculation in the selected trials were also included. A basic form was generated for the purpose of data extraction from each of the selected trials.

Results: Of the 130 iRCT reviewed, clustering of outcomes was present in 127 (98%) trials. Only 61 trials (47%) had adjusted for the clustering effects in their design and analysis, while 53% of the trials had ignored the clustering effect, and hence no adjustment had been made in the trial design or analysis.

Regarding the various forms of clustering, clustering by centre in multicentre trials was found in 79 trials (60%), followed by natural clustering in 26 trials (20%), and clustering imposed by the design of the study in 23 trials (18%).

Conclusion: Potential clustering of outcomes exists in almost all iRCT; however, this review found that <50% of iRCT took clustering into account and adjusted the sample size calculation and statistical analysis of this data for clustering. Almost half of the reviewed iRCT ignored the clustering effect. As a result, inaccurate and nongeneralisable results could have been generated.

BACKGROUND

Researchers that individually randomised controlled trials (iRCT) assume that the observed outcomes of participants are independent. In practice, there are a number of situations in which there is some doubt about the validity of this assumption. One example is the correlation, or clustering, of the observed outcomes in participants treated by the same health professional.[1] The importance of this issue was emphasised by Lee and Thompson,[1] who assessed clustering in 42 iRCT and concluded that clustering of outcomes exists in almost all iRCT, but is usually ignored in the analysis, which leads to underestimates of uncertainty and overly extreme p values.[1] In another article, Lee and Thompson proposed random effect models to allow for such clustering and investigated their effect on estimation and interpretation of the treatment effect.[2]

INTRODUCTION

What Does the Term Clustering Mean?

The term clustering usually diverts the mind of the reader towards the cluster randomised controlled trials, wherein the groups, or clusters, of patients (rather than individuals) are randomised to a treatment either because of the nature of the treatment or to prevent contamination between treatment groups.[3] However, in this review, clustering in iRCT will be analysed.

In iRCT, clustering means that the observation(s) about patients and observed outcomes in iRCT may be correlated due to differences in the behaviours of the health professionals actively delivering the intervention, sociodemographic differences between the patients, or the design of the study.[1] Observed outcomes clustering can also occur by single centres when participating in larger multicentre randomised controlled trials.[2]

Why is it Important to Consider the Clustering Effect?

Clustering of outcomes in randomised trials reduces the effective sample size, reducing the power of a trial to detect an intervention effect.[4-6]

Additionally, clustering also affects the generalisability of the results and conclusions.[1] The results obtained and conclusions drawn from a trial cannot be generalised to the whole population if the potential of clustering for outcomes exists in a trial. For example, in therapy trials the sample of therapists in the trial should be representative of those who are going to deliver the intervention in practice,[7] otherwise, the results obtained cannot be generalised.

In What Forms Does Clustering Exist in Individually Randomised Controlled Trials?

Clustering may be imposed by the design of the trial; this inherent clustering as a result of trial design has been noted in a trial comparing a new one-stop clinic with a dedicated breast clinic for breast cancer screening.[8] In another form, clustering can be natural rather than imposed either because of the sociodemographic differences between patients or because of the general practitioner's or the practice's influence on delivering the intervention, as observed in a trial comparing fusidic acid cream with placebo for the treatment of impetigo.[9]

Clustering can also appear by centre in a multicentre trial, which was seen in a study comparing the cytological surveillance with immediate referral for colposcopy in management of women with low-grade cervical abnormalities.[10] Observations from the same centre were similar and therefore more correlated and clustered than those from different centres: this phenomenon defines the centre effect.[11]

How Can Clustering Effects Be Adjusted?

In iRCT the clustering effects can be accounted for by anticipating them at the time of trial design and increasing the sample size accordingly.[12]

Adjustment for the clustering effects in iRCT can also be conducted during the statistical analysis of a trial by using various statistical models.[4] When analysing data from a multicentre trial, the estimation of the main treatment effect must take into account the differences seen between each centre.[13-15] This statistical method to limit the effect of clustering is widely accepted, but there is no real consensus on the statistical model to use.[16-20] However, the selected method depends on the application of the trial's conclusion.[11] If conclusions apply across the participating centres or if the centres cannot be considered as a random sample from a population, the analysis of data will involve a fixed effects regression model. On the contrary, if one wants to extend the results to all the centres that could be concerned by the experimental treatment, the analysis of data will involve a mixed effects model.[11]

Time Trend of Clustering in Individually Randomised Controlled Trials

At present, there is no obvious trend of clustering prevalence and its accommodation in iRCT. One of the objectives of this review is to identify the time trend of clustering prevalence in iRCT and the according adjustments in such trials. The aim of this study is to conduct a systematic analysis of the iRCT for the potential effects of clustering by health professionals.

Objectives of this study

- To identify the prevalence of clustering in iRCT.
- To identify whether the researchers have allowed for clustering effects or ignored clustering effects in the selected iRCT.
- To explore the different ways used to accommodate for clustering effects in the selected iRCT.
- Analyse the time trend analysis of the presence of clustering in iRCT and any according adjustment.

METHODOLOGY

Trials to be Included in This Study

Only iRCT conducted by healthcare professionals have been included in this study. The term healthcare professional encompasses doctors, nurses, physiotherapists, and acupuncturists. iRCT involving a pharmaceutical and/or health technology have not been included in the final selection of the studies that were reviewed. Likewise, cluster randomised controlled trials were excluded during the final selection of the articles.

Study Design

This study is a systematic review of iRCT published in three selected journals from 1st January 2000–31st August 2009. The journals were selected for this study due to their diverse impact factors (at the time of the study) and the ease of access to the fully published articles from the university portal of the University of Sheffield. The retrieved articles were fully reviewed for the presence of various forms of clustering, relevance of the assumptions made while calculating sample size, and adjustment made for the effect of clustering during statistical analyses.

Strategic Plan for the Search

Basic literature search

The electronic databases searched for relevant literature were Medline® via OVIDSP online, the university portal/OVID online, and Google scholar. The database was searched from 1950 to date. The keywords used for electronic searching were "clustering", "clustering effects", "statistical models", "randomised controlled trials", "randomized controlled trials", "natural clustering", "multicentre trial", "adjustment for clustering", "health professional", "individual randomized trials", "individual randomized trials", "sample size", "sample size calculation", and "random effect model".

The keyword "clustering" yielded 27,084 papers, while the terms "randomized controlled trials" and "randomized controlled trials" yielded 286,121 and 6,006 papers, respectively. The search was narrowed on the basis the of forms of clustering, models to allow for clustering, adjustment

methods, sample size, and sample size calculation. The search was further narrowed by only selecting iRCT and excluding cluster randomised trials. Finally, iRCT conducted by pharmacists and those with technological subjects, such as *Helicobacter pylori*, cardiac markers, and other tests were also excluded as these studies did not meet the inclusion criteria.

Individual journal search

A separate search strategy was used for accessing and scrutinising each of the three journals. The portal and search strategy used for each of the journals is briefly discussed below.

BMJ

The yearly archives of the online issue of BMJ were accessed via the HighWire Press Free line using the university library electronic journal database. Within each year, the full reports of the articles regarding the primary care/general practice were accessed, and the retrieved articles were categorically arranged on the basis of their study design. The articles in the education and debate portion of the archives were not accessed.

The Lancet

The electronic search was made using ScienceDirect via the university library electronic journals database. The terms "randomized controlled trials" and "randomised controlled trials" were searched via ScienceDirect.

Articles were then selected or excluded by applying the limitations of the iRCT in the specified time period (1st January 2000–31st August 2009), which reduced the number of the articles to 82.

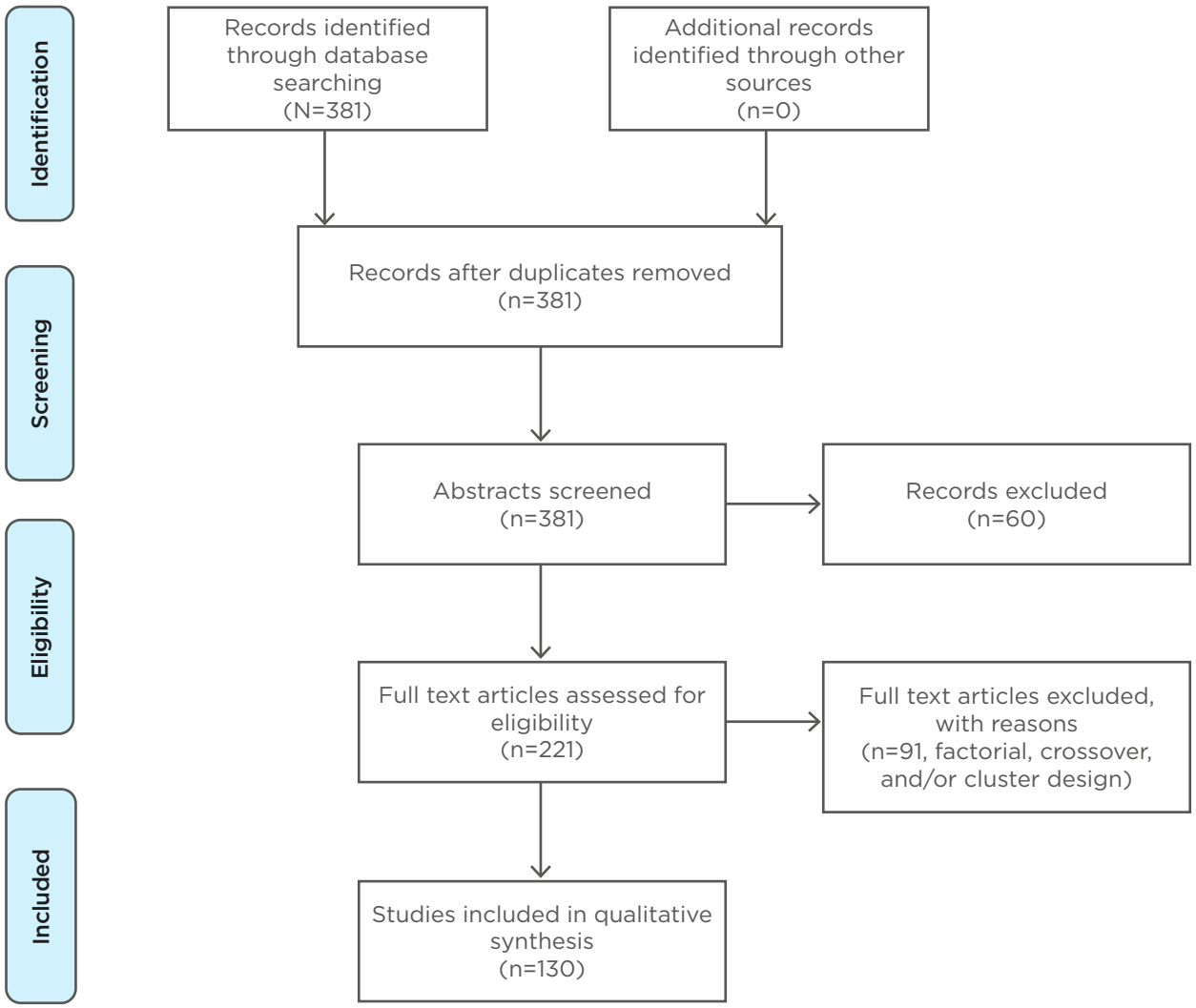


Figure 1: The identification, screening, and selection of the studies detailing individually randomised controlled trials included in this investigation.

Journal of Psychiatry and Neurosciences

PubMed Central, accessed via the electronic journal database of the university library portal, was used to search for JPN articles. The volumes of the JPN were scrutinised in detail from January 2000–July 2009. This search yielded 30 randomised controlled trials.

Screening of the titles and abstracts of the retrieved articles

The full literature search yielded 381 relevant articles. Two independent researchers screened the titles and abstracts of the retrieved articles, and, as a result, 160 articles were excluded.

Full text review of the retrieved articles

A review of the full text of the selected articles was conducted, and, as a result, 91 articles were excluded on the basis of full text review because they did not meet the inclusion criteria. Among these, trials with the factorial, crossover, and/or cluster design were excluded. Additionally, the follow-up studies were excluded from the retrieved articles (Figure 1).

Data Extraction

The iRCT selected for the final analysis were thoroughly reviewed for data extraction. A form was generated and used for the data extraction during the analysis of the selected iRCT. Data was extracted both from full text published iRCT and online extra material and registration websites.

Data extraction from the full text of the articles

The full text articles were studied and data regarding the general characteristics of the iRCT, sample size calculation, and statistical adjustments made for clustering were recorded.

General characteristics of the selected studies

The journal of publication, the year of publication, the type of intervention, number of multicentre trials, the health professionals conducting the trial, and the presence of clustering were recorded.

Sample size calculation

The methodology sections of the selected articles were studied in full detail, with particular focus placed on sample size calculation. All of the parameters used for the calculation of the sample size were collected. Any assumptions made and justification for the assumptions were also recorded. These parameters included Type I error, Type II error or power, one or two tailed tests, type of test, assumptions made in the control group, and predicted treatment effect.

Adjustment during statistical analysis

The results of the trials were studied and analysed for cluster effect adjustments, especially during statistical analysis of the results. Various models used for the statistical adjustment were also recorded.

Data extraction from the online extra material and trial registration websites

The trial registration websites were accessed and the target sample size and all the parameters used for the sample size calculation for the retrieved articles were recorded. Additionally, the extra materials related to the selected articles which were available online were also accessed and searched for the target sample size calculation and the parameters used for the sample size calculation.

RESULTS

Description of the 130 Included Articles

Table 1 describes the characteristics of the 130 selected articles. In 60 trials (46%), the intervention was delivered by the doctors, including general practitioners, physicians, psychiatrists, and surgeons. In the remaining included studies, the intervention was delivered by nurses in 43 trials (33%), physiotherapists in 16 trials (12%), and acupuncturists in the remaining 11 trials (9%). In half of the selected trials, the intervention was pharmacological, with a nonpharmacological intervention used in 45 (35%) trials. In the remaining 20 trials (15%), mixed method interventions were evaluated.

Table 1: Baseline characteristics of the articles and details of sample size calculation.

General characteristics of the 130 included individually randomised controlled trials			
Journal	**Number (%)**	**Health professional**	**Number (%)**
BMJ	75(58)	Doctors	60(46)
The Lancet	40(31)	Nurses	43(33)
Journal of Psychiatry and Neurosciences	15(11)	Physiotherapist	16(12)
		Acupuncturist	11(9)
Year of publication			
2000	13(10)	**Intervention**	
2001	10(8)	Pharmacological	65(50)
2002	7(5)	Nonpharmacological	45(35)
2003	10(8)	Both	20(15)
2004	8(6)		
2005	11(8)	**Clustering**	
2006	21(16)	Natural	26(20)
2007	18(14)	Imposed	23(18)
2008	19(15)	By centre in multicentre trials	79(60)
2009	13(10)	Not evident	2(2)
Details of sample size calculations for the 130 selected articles			
Parameter		**Number (%)**	
Articles not reporting a sample size calculation		16(12)	
Articles reporting sample size calculations		114(88)	
1) Reporting all required parameters		61(47)	
2) Reporting the power of the study		110(85)	
› 80%		66(60)	
› 85%		7(6)	
› 90%		27(26)	
› 95%		5(4)	
› Other values		5(4)	
3) Reporting the α risk		90(82)	
› 0.05		81(90)	
› 0.025		4(4)	
› Interim analysis		5(6)	
Articles not reporting the target sample size		9(7)	

Types and Prevalence of Clustering

Clustering by centre in multicentre trials was found in 79 trials (60%), followed by natural clustering, which was found in 26 trials (20%). Clustering imposed by the design of the study was noticed in 23 trials (18%), while the form of clustering was not clear in 2 trials (2%).

Allowance for Clustering in the 130 Selected Articles

Table 2 shows the frequency of the trials that corrected for the clustering effect in their study either by making adjustments while calculating the sample sizes or during the statistical analysis. Out of the 130 selected articles, 13 were published during the year 2000. Among these 13 trials, only 2 trials (15%) had adjusted for the clustering effect.

Table 2: Frequency of article allowing and/or ignoring for clustering effect.

Year of study	Number of articles studied	Total Number of iRCT	Number of articles with clustering	Articles with adjustment for clustering, n (%)	iRCT not allowing for clustering, n (%)
2000	39	13	12	2 (15)	11 (85)
2001	32	10	10	2 (20)	8 (80)
2002	20	7	7	2 (29)	5 (71)
2003	28	10	10	3 (33)	7 (67)
2004	24	8	8	3 (38)	5 (62)
2005	32	11	11	5 (45)	6 (55)
2006	63	21	20	12 (57)	9 (43)
2007	56	18	18	12 (67)	6 (33)
2008	48	19	19	12 (63)	7 (37)
2009	39	13	13	8 (62)	5 (38)
Total	381	130	128	61 (47)	69 (53)

iRCT: individually randomised controlled trials.

Likewise, among the 10 trials published during 2001, only 2 trials (20%) had made allowances for the clustering effect. In 2002, 7 articles out of the 130 selected articles were published, and among these 7, only 2 articles (29%) had taken the clustering effect into account. The percentage of trials that took various forms of clustering into account and had made adjustments in their study increased to 45% in 2005 and reached the maximum percentage recorded of 67% during the year 2007. During the year 2008, 12 trials out of 19 (63%) had taken the clustering effect into account. In 2009, of all the trials published up to the search end date, 31st August 2009, 8 trials (62%) showed proper adjustments for various forms of the clustering effect.

Ignoring the Clustering Effects in the 130 Selected Articles

Table 2 summarises the frequency of articles that ignored the clustering effect and made no allowance in their design and analysis. Overall, 69 articles (53%) made no adjustment for the clustering effect in their design and analysis.

Reporting sample size calculations

Table 1 summarises the data about sample size calculation; 16 articles (12%) did not report the sample size calculation, while 9 trials (7%) did not mention the target sample size, neither in the full text report nor on the trial registration database. Even though a sample size calculation was reported by the majority of articles (88%), some of the required parameters for sample size calculation were frequently absent in reports.

In total, 61 articles (47%) included the required parameters for sample size calculations, including the assumptions made for the treatment effect and the control group. Moreover, 110 trials (85%) reported the power of the study. Finally, the α risk was mentioned by 90 trials (82%), which mostly mentioned the two-tailed test.

DISCUSSION

Principal findings

In this study of 130 iRCT published in three medical journals during the 10-year period from 1st January 2000–31st August 2009, the potential for clustering was found to be very common (98%) and only 47% (61/130) of trials studied in this time period have made allowances and adjustments for the clustering effect in their study design and analysis. The time trend analysis showed that the trend of taking clustering into account in iRCT has

increased over recent years, from 15% of studies implementing anticlustering measures in 2000 to 67% in 2007, and 63% in 2008. This trend still remains high (62%) for the selected trials published up to the 31st August 2009 in the three selected medical journals.

A plateau in the time trend analysis graph during the years 2008 and 2009 warrants further investigation into the importance of clustering adjustment to further increase the percentage of iRCT that adjust for clustering.

Sample size calculations were reported in 88% (114/130) of articles. Reporting of the sample size calculation has greatly increased in the past decades, from 4% of reports describing a calculation in 1980 to 83% of reports in 2002.[21,22] However, some of the required parameters for replication of the sample size calculation are frequently absent in reports.

STRENGTHS OF THE STUDY

Familiarity with the data

Unlike secondary data analysis, in which the data is collected by others and a period of familiarisation is necessary, in this study, the data has been extracted and collected by the researcher, and the data set is self-generated so there is marked familiarity with the structure and contours of the data.

Presence of key variables

Secondary analysis entails the analysis of data collected by others for their own purposes, so one or more key variables may not be present.[23] In this study the data has been collected by the researcher, so the data about the key variables (potential clustering effect, sample size, accommodation for clustering) was collected with special attention.

Long study span and multiple medical journals reviewed

In this study, all the papers published in the three medical journals within the 10 years has been reviewed. Two of them are general medical journals with high impact factors: BMJ and The Lancet, while JPN is a specialist medical journal with a low impact factor.

Time trend analysis

It was difficult to have a long enough study span to analyse the change in trend with time on the potential clustering and its accommodation in the trials.

LIMITATIONS OF THE STUDY

It is difficult to assess whether the assumptions made in the iRCT during the sample size calculation to adjust for the clustering effect had been manipulated or not, as only the published data about the sample size has been used for the study. To obtain feasible sample sizes, the assumptions could be manipulated during the study.[24] Additionally, the sample size calculations can be manipulated after the completion of the study, as recently shown by Chan et al.[25] by comparing protocols to final articles.

The trials with complex designs, such as factorial designs, crossover trials, and trials with >2 parallel arms, were excluded during the screening process to obtain a homogeneous sample of articles. Therefore, the clustering effect and its adjustment in trials with more complex trials have not been assessed. This may limit the generalisability of the results.

Another limitation is that the treatment-by-centre interaction is not considered. However, as the main objective of a trial is often to assess the overall treatment effect, it is recommended to investigate the treatment effect using a model that only contains the centre effect.[14,26]

CONCLUSION

The issue of clustering by health professionals in iRCT has gained attention in the last few years, and there is need for further research in this field to elicit some more facts about this matter and to provide further guidelines about the anticipation of, and accommodation for, potential clustering. A simulation study will be helpful to demonstrate the clustering affect on the results of an iRCT. Although each and every form of the clustering may not need to be accounted for at the analysis stage,[27] this paper highlights the existence of this issue to the readers and reviewers and the need for analysis adjustment in certain cases.

References

1. Lee K, Thompson SG. Clustering by health professional in individually randomized trials. BMJ. 2005;330:142-4.
2. Lee K, Thompson SG. The use of random effect models to allow for clustering in individually randomized trials. Clinical Trial. 2005;2(2):12.
3. Donner A, Klar N, Design and analysis of cluster randomization trials in health research (2000), London: Arnold.
4. Roberts C. The implications of variation in outcome between health professionals for the design and analysis of randomized control trials. Stat Med. 1999;18(19):2605-15.
5. 5. Martindale C. The therapist as fixed effect fallacy in psychotherapy research. J Consult Clin Psychol. 1978;46:1526-30.
6. Greenfield S et al. Profiling care provided by different groups of physicians: Effects of patient case-mix (bias) and physician-level clustering on quality assessment results. Ann Int Med. 2002;136:1(2)11-21.
7. Crits-Christoph P, Mintz J. Implications of therapists effect for the design and analysis of comparative studies of psychotherapies. J Consult Clin Psychol. 1991;59(1):20-6.
8. Dey P et al. Costs and benefits of a one stop clinic compared with a dedicated breast clinic: randomized controlled trial. BMJ. 2002;324:507-11.
9. Koning S et al. Fusidic acid cream in the treatment of impetigo in general practice: Double blind randomized placebo controlled trial. BMJ. 2002;324:203-6.
10. TAMBOLA Group. Cytological surveillance compared with immediate referral for colposcopy in management of women with low grade cervical abnormalities: Multicentre randomised controlled trial. BMJ. 2009;339:b2546.
11. Vierron E, Giraudeau B. Sample size calculation for multicenter randomized trial: Taking the center effect into account. Contemp Clin Trials. 2007;28(4):451-8.
12. Hoover DR. Clinical trials of behavioral interventions with heterogeneous teaching subgroup effects. Stat Med. 2002;21:1351-64.
13. Anello C et al. Multicentre trials: A US regulatory perspective. Stat Methods Med Res. 2005;14(3):303-18.
14. European Medicines Agency. ICH Topic E 9. Note for guidance on statistical principles for clinical trials (CPMP/ICH/363/96) (1998). Available at: https://www.ema.europa.eu/documents/scientific-guideline/ich-e-9-statistical-principles-clinical-trials-step-5_en.pdf. Last accessed: 23 January 2019.
15. Localio AR et al. Adjustments for center in multicenter studies: An overview. Ann Intern Med. 2001;135(2):112-23.
16. Fedorov V et al. Estimation of the treatment difference in multicenter trials. J Biopharm Stat. 2004;14(4):1037-63.
17. Jones B et al. A comparison of various estimators of a treatment difference for a multi-centre clinical trial. Stat Med. 1998;17:1767-77 [discussion 1799-800].
18. Moerbeek M et al. A comparison between traditional methods and multilevel regression for the analysis of multicenter intervention studies. J Clin Epidemiol. 2003;56(4):341-50.
19. Permutt T. Probability models and computational models for ANOVA in multicenter clinical trials. J Biopharm Stat. 2003;13(3):495-505.
20. Senn S. Some controversies in planning and analysing multi-centre trials. Stat Med. 1998;17(15-16):1753-65 [discussion 1799-800].
21. Mills EJ et al. The quality of randomized trial reporting in leading medical journals since the revised CONSORT statement. Contemp Clin Trials. 2005;26(4):480-7.
22. Meinert CL et al. Content of reports on clinical trials: A critical review. Control Clin Trials. 1984;5(4 Suppl 1):328-47.
23. Bryman A, Bell A, Social Research Methods (Fourth Canadian Edition, 2008), Oxford: Oxford University Press.
24. Charles P et al. Reporting of sample size calculations in randomized controlled trials: Review. BMJ. 2009;338:b1732.
25. Chan AW et al. Discrepancies in sample size calculations and data analyses reported in randomised trials: Comparison of publications with protocols. BMJ. 2008;337:a2299.
26. Brown H, Prescott R, Applied mixed models in medicine, 3rd edition (1999), Chichester: John Wiley and Sons.
27. Kahan BC, Morris TP. Improper analysis of trials randomised using stratified blocks or minimisation. Stat Med. 2011;31(4):328-40.

A new day for genomic profiling

A new world of NGS

Introducing the Oncomine™ Precision Assay on the Ion Torrent™ Genexus™ System
Now your own lab can go from specimen to comprehensive genomic profile in one day.

Find out more at **oncomine.com**

ThermoFisher SCIENTIFIC

Immunotherapy and Oral Immunotherapy with Omalizumab for Food Allergies

Authors: *Shoichiro Taniuchi, Masahiro Enomoto, Hirotaka Minami

Department of Pediatrics, Takatsuki General Hospital, Osaka, Japan
*Correspondence to taniuchishoichiro@gmail.com

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors thank Ellen Knapp from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript. Dr Taniuchi is guarantor for this work and drafted the manuscript. Critical revision was performed by Dr Enomoto and supervision was under Dr Enomoto and Dr Minami.

Received: 25.04.2019

Accepted: 09.07.2019

Keywords: Desensitisation, food allergy, omalizumab (OMB), oral immunotherapy (OIT).

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

Abstract

Food allergy is potentially life-threatening and has a major impact on quality of life. Avoidance is currently the only approved therapy, and, although effective, avoidance diets can be difficult and may also put children at risk of nutritional deficiencies and impaired growth. At least 80% of milk and egg-allergic children are expected to achieve natural tolerance to these foods by adulthood, and 15–20% of peanut or tree nut-allergic individuals 'outgrow' their allergies. Effective therapies for food allergies are therefore highly desirable. There have been several immunotherapies for food allergy such as oral immunotherapy (OIT), sublingual immunotherapy (SLIT), epicutaneous immunotherapy (EPIT), and OIT combined with anti-IgE monoclonal antibodies (omalizumab [OMB]). However, efficacy and safety have only been demonstrated in one large Phase III trial for peanut allergies. Additionally, there have only been three randomised, controlled studies of OMB–OIT combination and these were low-powered, single-centre trials; therefore, evidence levels were low in these trials. Studies that included long-term follow-up observations and clinical tolerance are rare. Additionally, clinical tolerance is not well-defined and remains unknown. Therefore, several problems remain to be resolved, but hopefully OIT in combination with OMB will resolve these problems in the future. Although there are only three randomised, controlled trials of OMB–OIT, the combination therapy enabled high dose desensitisation for a short duration without any adverse events, resulting in the sustained unresponsiveness in IgE-related food allergy. It is speculated that this combination therapy will be the most effective immunotherapy in the future.

INTRODUCTION

The prevalence of IgE-mediated food allergy is increasing in Western and developed countries,[1] and the most common food allergies are milk, egg, wheat, peanut, tree nuts, soy, fish, shellfish, and sesame.[2] Treatment of food allergies has been carried out on strict avoidance of the offending food(s) and is performed when accidental ingestions occur. Food allergies are potentially

life-threatening and have a major effect on the quality of life.[3,4] Avoidance is currently the only approved therapy for food allergies, and although avoidance diets are effective, they can be difficult and may also put children at risk of nutritional deficiencies and impaired growth.[5-7] At least 80% of milk and egg-allergic children are expected to achieve natural tolerance to these foods by adulthood, and 15–20% of peanut or tree nut-allergic individuals outgrow their allergies.[7] Therefore, effective therapies for food allergies are highly desirable. There have been several immunotherapies for food allergies, such as oral immunotherapy (OIT), sublingual immunotherapy (SLIT), epicutaneous immunotherapy (EPIT), and OIT combined with anti-IgE monoclonal antibodies (omalizumab [OMB]; OMB–OIT). This review focusses on the advantages, disadvantages, and differences in these immunotherapies, especially OMB–OIT therapy.

ORAL IMMUNOTHERAPY

OIT as an active intervention for food allergies has been mainly performed in Europe, USA, and Japan.[8] OIT for eggs, milk, wheat, and peanuts has been reported in these countries;[8,9] however, OIT is not recommended as a general practice because of problems with safety.[10] Considering that there have been many recent outstanding reviews on individual OIT studies,[10-15] in this review, the authors will briefly focus on a general overview of OIT, current challenges, and clinical trials that are in progress.

With OIT, patients ingest food daily between dose escalations. Notably, there is a wide range of dosing protocols in terms of the build-up and maintenance phases. In peanut trials, studies have used maintenance doses ranging from 1 peanut (approximately 200 mg) to 17 peanuts. Furthermore, there have been significant differences in the reporting of outcomes, which are likely related to varied definitions used in differing studies. These differences could also be attributable to the effects of the time and the dose of therapy.

Patients who are able to proceed through these desensitisation protocols can often tolerate considerable quantities of the food while on therapy, which would provide significant protection against accidental ingestions. The majority of patients experience some side effects of therapy, especially oropharyngeal symptoms and abdominal pain, but more severe reactions, including anaphylaxis, can occur. Side effects, especially gastrointestinal effects, often limit a patient's ability to achieve the target maintenance dose. Studies have reported that ≤30% of patients fail to complete desensitisation,[16] and rates of sustained unresponsiveness have been low.[17,18] The optimal duration of immunotherapy is unknown, but it is likely that treatment of a long duration promotes sustained unresponsiveness.[17] A Phase III trial investigating an experimental OIT for peanut allergy (AR101) was recently published at the end of 2018.[19] Participants aged 4–55 years old who completed the regimen (i.e., received 300 mg per day of the maintenance regimen for approximately 24 weeks) underwent a double-blind, placebo-controlled food challenge (DBPCFC) at trial exit. In the study, 551 participants received AR101 or placebo, of whom 496 were 4–17 years old. Of the 372 participants who received active treatment, 250 (67.2%) were able to ingest a dose of ≥600 mg peanut protein without dose-limiting symptoms at the exit food challenge, compared with 5/124 participants (4.0%) who received placebo (difference= +63.0%; 95% confidence interval [CI]: 53.0–73.3; p<0.001). Efficacy was not shown in participants aged ≥18 years. Treatment with AR101 resulted in higher doses of peanut protein that could be ingested without dose-limiting symptoms and lower symptom severity during peanut exposure at the exit food challenge compared with placebo.

OMALIZUMAB

The U.S. Food and Drug Administration (FDA) approved OMB (Xolair®), a humanised anti-IgE mouse monoclonal antibody, for treating mild-to-severe allergic asthma and chronic spontaneous urticaria.[20-23] OMB acts by binding to circulating free IgE; therefore, OMB reduces the amount that would normally be available to bind FcεRI on mast cells and basophils. In an early Phase I study of 15 allergic and asthmatic patients with serum levels of IgE between 187 and 1,210 ng/mL, intravenous injection of OMB resulted in a reduction of IgE to 1% of the pretreatment levels.[24] Binding of IgE to FcεRI on mast cells and basophils enhances FcεRI expression;[25-29] consequently, a reduction in free IgE by OMB

leads to diminished FcεRI expression on the surface of mast cells, basophils, and dendritic cells.[24,29-31] In a previous study, treatment of atopic individuals with OMB for 3 months reduced FcεRI expression in basophils by 97% from 220,000 to 8,300 receptors per basophil.[24] An *in vitro* study with *in situ*-matured mast cells from human skin showed that IgE-dependent enhancement of FcεRI on human skin mast cells was prevented and reversed by OMB.[29] In this study, OMB prevented upregulation of FcεRI by 90% when added simultaneously with polyclonal IgE at a molar ratio of 2.9 (OMB to IgE). Additionally, OMB dose-dependently decreased FcεRI expression in human skin mast cells when added to cultures after FcεRI had already been upregulated with IgE, which suggested that OMB could disassemble preformed IgE–FcεRI complexes; this was later confirmed with a cell-free system and human basophils.[32,33] The efficacy and safety of OMB as a treatment against allergic asthma and urticaria have clearly been demonstrated, including as an add-on therapy with traditional treatments, such as glucocorticoids.[20,21] The therapeutic potential of OMB in other IgE-mediated disorders in which FcεRI plays a role, including food allergies,[34-36] allergic rhinitis,[37,38] and atopic dermatitis,[39,40] has also been shown. However, OMB is not available for children with severe bronchial asthma with >1,500 IU/mL of total IgE, those <6 years old, and those with severe food allergies. The effect of OMB in bronchial asthma was reported to be related to free IgE levels.[41] In patients with high IgE levels (>1,500 IU/mL), high-volume OMB administration is required to maintain low free IgE concentrations (≤10 ng/mL). The maximum doses administered are limited by the product of IgE levels, body weight, and age. This explains why OMB is not available for patients with high IgE levels.

OMALIZUMAB COMBINED WITH ORAL IMMUNOTHERAPY FOR FOOD ALLERGIES

Milk Allergies

In 2011, OMB–OIT was administered to 13 patients with severe cow's milk (CM) allergy in the USA.[42] The OMB–OIT combination was efficacious in 11/13 patients; this finding suggests that OIT can be escalated more rapidly when combined with OMB, although adverse reactions are still relatively common.

Wood et al.[43] studied the addition of OMB or placebo to open-label milk OIT. Open-label milk OIT was initiated after 4 months of OMB/placebo with escalation to maintenance over 22–40 weeks, followed by daily maintenance dosing to 28 months. At Month 28, OMB was discontinued, and subjects who passed an oral food challenge (OFC) continued OIT for 8 weeks. After this time, OIT was discontinued with a rechallenge at Month 32 to assess sustained unresponsiveness (SU), which was defined as the ability, after several months of OIT and subsequent avoidance of consuming the offending food for 4–8 weeks, to consume 2–4 g of the offending food allergen without developing clinically significant symptoms.[44] At Month 28, 24 (88.9%) OMB-treated subjects and 20 (71.4%) placebo-treated subjects passed the 10 g 'desensitisation' OFC (p=0.18). At Month 32, SU was achieved by 48.1% of subjects in the OMB group and 35.7% of subjects in the placebo group (p=0.42). Adverse reactions were markedly reduced during OIT escalation in OMB-treated subjects for percentages of doses per subject provoking symptoms (2.1% versus 16.1%; p=0.0005), dose-related reactions requiring treatment (0.0% versus 3.8%; p=0.0008), and doses required to achieve maintenance (198 versus 225; p=0.008). The study by Wood et al.[43] reported significant improvements in measurements of safety, but not in outcomes of efficacy (desensitisation and SU).

A pilot study with OIT in combination with OMB was planned, which has been accepted as a treatment for severe asthma, and reported successful desensitisation in a boy with severe CM allergy.[45] On the basis of these observations, a pilot study to evaluate the efficacy and safety of OIT combined with 24 weeks of OMB for inducing desensitisation in children with a CM allergy compared with an untreated group was conducted.[46] This study was a prospective, randomised, controlled trial in which 16 patients (aged 6–14 years) with high IgE levels and CM were enrolled. Patients were randomised 1:1 to receive OMB–OIT (treated group) or they were untreated (untreated group). The primary outcome was induction of desensitisation at 8 weeks after OMB was discontinued in the treated group and at 32 weeks after study entry. None of the 6 children in the

untreated group developed desensitisation to CM, but all of the 10 children in the treated group achieved desensitisation (p<0.001). A significantly decreased wheal diameter in response to a skin prick test using CM was found in the treated group (p<0.050). These data suggest that OIT combined with OMB using microwave-heated CM may help to induce desensitisation for children with a high-risk CM allergy. The results of this randomised trial suggest that patients with high specific milk IgE levels are more likely to develop allergic symptoms after stopping OMB than those whose IgE levels are not high. Future studies regarding the therapeutic duration and dosages of OMB administration are required.

Non-milk Allergies

A pilot trial of peanut OMB–OIT was reported in 2013 and its efficacy was reported in 12/13 patients.[47] In a recent study, MacGinnitie et al.[48] reported a randomised, controlled trial on OMB–OIT with peanuts. In the study, 37 subjects were randomised to OMB (n=29) or placebo (n=8). After 12 weeks of treatment, subjects underwent a rapid 1-day desensitisation of ≤250 mg of peanut protein, followed by weekly increases of ≤2,000 mg. OMB was then discontinued and subjects continued on 2,000 mg of peanut protein. The subjects underwent an open challenge of 4,000 mg of peanut protein 12 weeks after stopping the study drug. If tolerated, subjects continued on 4,000 mg of peanut protein daily. The median peanut dose that was tolerated on the initial desensitisation day was 250.0 mg for OMB-treated subjects versus 22.5 mg for placebo-treated subjects. Subsequently, 23/29 (79.0%) subjects who were randomised to OMB tolerated 2,000 mg of peanut protein 6 weeks after stopping OMB versus 1/8 (12.5%) subjects who received placebo (p<0.01). Furthermore, 23 subjects who received OMB versus 1 subject who received placebo passed the 4,000 mg food challenge. Overall reaction rates were not significantly lower in OMB-treated versus placebo-treated subjects (odds ratio: 0.57; p=0.15), although OMB-treated subjects were exposed to much higher peanut doses. OMB allows subjects with a peanut allergy to be rapidly desensitised over as little as 8 weeks of peanut OIT. In the majority of subjects, this desensitisation is sustained after OMB is discontinued.

Andorf et al.[36] reported anti-IgE treatment with OIT in multifood-allergic participants in a double-blind, randomised, controlled trial at the end of 2017. Enrolled in the study were participants who were aged 4–15 years with multifood allergies and validated by double-blind, placebo-controlled food challenges to their offending foods. Inclusion criteria included a positive skin prick test of ≥6 mm (wheal diameter, > the negative control), a food-specific serum IgE level >4 kU/L for each food, or both, and a positive DBPCFC at ≤500 mg of food protein. Exclusion criteria included eosinophilic oesophagitis and severe asthma. Participants were randomised 3:1 to receive multifood OIT for 2-5 foods, together with OMB (n=36) or placebo (n=12). Additionally, 12 individuals who fulfilled the same inclusion and exclusion criteria were included as controls. These individuals were not randomised and received neither OMB nor OIT. OMB or placebo was administered subcutaneously for 16 weeks once every 2 or 4 weeks and the doses administered were defined according to the manufacturer's instruction. OIT started at Week 8 and continued before the DBPCFC at Week 36. On the initial dose-escalation day, patients received an initial dose of 5 mg food protein (divided equally among the number of foods included), with increasing doses administered every 30 minutes until reaching 1,250 mg or a maximum-tolerated dose. The participants then continued self-administration of the combined OIT at the maximum-tolerated dose at home, returning every 2–4 weeks for an increase in their daily dose (build-up phase). When participants reached the maintenance dose of 2 g per food, this dose was maintained daily (maintenance phase) until the food challenge at Week 36. The primary endpoint was the proportion of participants who passed a double-blind, placebo-controlled food challenge to 2 g protein from ≥2 of their offending foods. A total of 165 participants were assessed for eligibility of whom 84 did not meet the inclusion criteria and 21 declined to participate. The authors enrolled and randomised 48 eligible participants and the remaining 12 patients were included as nonrandomised, untreated controls. At Week 36, a significantly greater proportion of participants in the OMB group (30/36 [83%]) than those in the placebo group (4/12 [33%]) passed DBPFC (odds ratio: 10.0; 95% CI: 1.8–58.3; p=0.0044). All participants completed the study and there were no serious or severe (Grade 3 or worse)

adverse events. Participants in the OMB group had a significantly lower median per-participant percentage of oral immunotherapy doses associated with any adverse events compared with the placebo group (27% versus 68%; p=0.0082). The most common adverse events in both groups were gastrointestinal events. In multifood-allergic patients, OMB enabled safe and rapid desensitisation. The above-described clinical trials are shown in Table 1. Randomised clinical trials and blinded trials were performed in 4/5 trials and 3/5, respectively; from these, 2/5 trials evaluated SU. In multifood and CM-allergic patients, OMB enabled safe and rapid desensitisation; whereas in multifood patients, OMB was efficacious, but not in CM patients. In the trial, OMB combined with OIT in patients with a CM allergy was efficacious in CM desensitisation compared with untreated patients with a CM allergy. Taken together, these five trials suggest that OMB is efficacious for desensitisation without severe adverse symptoms during OMB administration.

IMMUNOTHERAPIES WITHOUT ORAL IMMUNOTHERAPY FOR FOOD ALLERGIES

SLIT is generally used for allergic rhinitis provoked by an environmental allergen. SLIT for treating food allergies has largely concentrated on peanuts, whereas other foods studied include milk and hazelnuts, as well as peaches and kiwifruit. A double-blind, placebo-controlled study assessed peanut SLIT compared with placebo for 12 months in 18 children.[49] The treatment group allowed intake of 20 times more peanut protein than in the placebo group, with a median dose of 1,710 mg compared with 85 mg (p=0.011). SLIT appeared to be safe and relatively well tolerated, and its main side effects were largely oropharyngeal. A randomised, double-blind, placebo-controlled study evaluated 40 patients and compared peanut SLIT with placebo.[50] The initial active SLIT subjects were treated to Week 44 with ≤1,386 µg of peanut protein SLIT daily.

Table 1: Summaries of clinical trials.

Reference	Nadeau et al.,[40] 2011	Wood et al.,[43] 2016	Takahashi et al.,[46] 2017	MacGinnities et al.,[48] 2017	Andorf et al.,[36] 2018
Design	Milk OIT with OMB	Milk OIT with OMB or w/o OMB	Milk OIT with OMB or untreated	Peanut OIT with OMB or w/o OMB	Multifood OIT with OMB or w/o OMB
Allergen	Cow's milk	Cow's milk	Cow's milk	Peanut	Multiple (2–5)
Age (years)	7–17	7–32	6–14	7–25	4–15
Sample size (OMB–OIT; OIT or untreated)	(11)	(27;28)	(10;6)	(29;8)	(36;12)
Maintenance dose (g)	2.0	3.3	6.0	2.0	2.0 of each allergen
Duration (OMB)	16 W	28 M	24 W	20 W	16 W
Duration (OIT)	24 W	26 M	24 W	14 W	12 W
Results	82.0% achieved DS to 2 g	48.0% achieved SU (OMB–OIT); 37.0% achieved SU (OIT) to 10 g, but AE were significantly reduced in OMB–OIT	100.0% achieved DS SU (OMB–OIT); 0.0% achieved DS (untreated group) to 2 g	79.0% achieved SU (OMB–OIT); 12.5% achieved SU (w/o OMB-OIT) to 2 g	83.0% achieved SU (OMB–OIT); 33.0% achieved SU (OIT) to 2 g for ≥2 of their offending foods

DS: desensitisation; M: months; OIT: oral immunotherapy; OMB: omalizumab; OMB–OIT: oral immunotherapy combination with omalizumab; SU: sustained unresponsiveness; W: weeks; w/o: without.

At Week 44, peanut SLIT and placebo subjects completed a 5 g OFC and were unblinded, while placebo crossover subjects after unblinding at Week 44 were escalated to a higher dose peanut SLIT ≤3,696 µg daily (designated the high dose crossover group; the original peanut SLIT group maintained a maximum dose of 1,386 µg of peanut protein). After 44 weeks, 14/20 patients who received active treatment were considered responders. Of these patients, 3/20 patients who received placebo were considered responders. In the active treatment group, the median consumed dose increased from 3.5 to 496.0 mg at 44 weeks and this increased further to 996.0 mg at 65 weeks. Dose-related symptoms were reported for 18.3% of doses in the high-dose crossover subjects following 44 weeks of active therapy and for 18.1% doses received by peanut SLIT subjects following 44 weeks of active therapy. No subjects had severe dosing related symptoms and no dosing related reaction required treatment with epinephrine. A 3-year follow-up showed that 50% of patients had discontinued therapy[51] and 4/37 (10.8%) patients were desensitised to 10 g of peanut powder and achieved SU as measured by an OFC after 8 weeks off SLIT. Thus, peanut SLIT induced a modest level of desensitisation and had an excellent long-term safety profile. However, most patients discontinued therapy by the end of Year 3, and only 10.8% of subjects achieved sustained unresponsiveness. The reasons for discontinuation after 3 years might be explained by the difficulty of maintaining daily therapies, mild oral discomfort (17.8% of doses), and a lack of robust responses as measured during OFC.

Two randomised studies have compared SLIT with OIT. One double-blinded, placebo-controlled trial evaluated peanut SLIT compared with peanut OIT.[52] The SLIT maintenance dose was 3.7 mg and the OIT maintenance dose was 2,000 mg during a 12-month trial. The OIT group showed a much greater scale of change (141-fold increase) compared with the SLIT group (22-fold increase). The OIT group was more likely to have more severe reactions than the SLIT group.

Another study assessed milk SLIT with milk SLIT followed by OIT with 60 weeks of maintenance therapy in 30 patients.[53] In the study, 14/20 patients who received OIT passed an OFC with 8 g of milk compared with 1/10 patients who received SLIT (p=0.002). Patients who received OIT were more likely to have systemic adverse events compared with patients who received SLIT.

EPIT delivers even smaller dosages of the antigen than does SLIT. EPIT appears to be a relatively safe form of immunotherapy. A recent Phase III, multicentre, randomised, double-blind, placebo-controlled trial[54] conducted EPIT for the treatment of peanut allergy. Participants included peanut-allergic children (aged 4–11 years [n=356] without a history of severe anaphylactic reaction) developing objective symptoms during a DBPCFC at an eliciting dose of ≤300 mg peanut protein. Daily treatment was with a peanut patch containing 250 µg peanut protein (n=238) or placebo (n=118) for 12 months. In this randomised clinical trial of 356 peanut-allergic children, differences in the treatment response rate (percentage of participants meeting a defined eliciting dose to peanut challenge) after 12 months of treatment with peanut-patch therapy was statistically significant compared with placebo (35.3% versus 13.6%), but did not meet a prespecified criterion (15.0% lower bound of the CI) for a positive trial result. The EPIT study reported a statistically significant response in peanut-allergic children compared with placebo, but the study did not meet a component of the primary outcome.

CURRENT STATUS AND FUTURE PROSPECTS

The efficacy and risk of each immunotherapy is shown in Figure 1. OIT has shown the greatest promise for efficacy in terms of the amount of protein that can be ingested. However, OIT has less tolerability and a less favourable safety profile compared with SLIT and EPIT. EPIT offers the least protection but has the best safety and tolerability profile. Investigation is currently underway for modified antigens that may be used for immunotherapy and for adjuncts that may help facilitate immunotherapy, including biologics such as anti-IgE therapy. The combination of OIT with OMB has extremely high medical costs. SLIT and EPIT are extremely safe and highly effective, but there have only been a small number of clinical trials, and thus their effectiveness is controversial. Additionally, only two modalities (AR101 from Aimmune Therapeutics, California, USA,[19] and Viaskin Peanut from DBV Technologies, France)[54] have completed fully powered Phase III studies and only AR101 is being reviewed by regulatory authorities at this time.

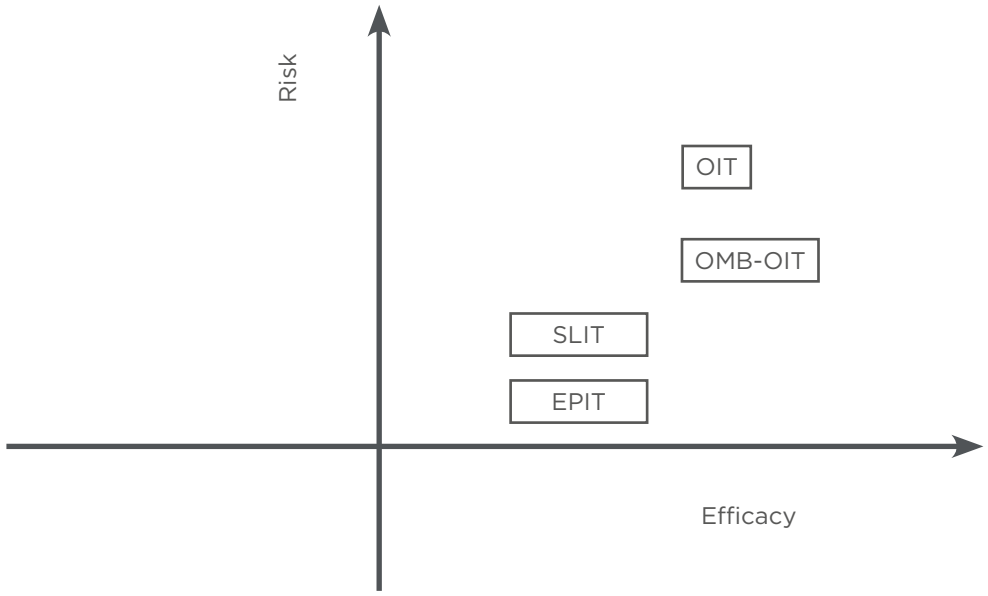


Figure 1: The efficacy and risk of oral immunotherapy, sublingual immunotherapy, epicutaneous immunotherapy, and oral immunotherapy combination with omalizumab.

EPIT: epicutaneous immunotherapy; OIT: oral immunotherapy; OMB: omalizumab; OMB–OIT: oral immunotherapy combination with omalizumab; SLIT: sublingual immunotherapy.

References

1. Hobbs CB et al. Food allergies affect growth in children. J Allergy Clin Immunol Pract. 2015;3(1):133-4.
2. Nowak-Wegrzyn A, Sampson HA. Future therapies for food allergies. J Allergy Clin Immunol. 2011;127(3):558-73.
3. Gupta RS et al. The prevalence, severity, and distribution of childhood food allergy in the United States. Pediatrics. 2011;128(1):e9-17.
4. Chafen JJ et al. Diagnosing and managing common food allergies: A systematic review. JAMA. 2010;303(18):1848-56.
5. Lieberman JA et al. Quality of life in food allergy. Curr Opin Allergy Clin Immunol. 2011;11(3):236-42.
6. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S116-25.
7. Kim J et al. The effects of elimination diet on nutritional status in subjects with atopic dermatitis. Nutr Res Pract. 2013;7(6):488-94.
8. Feuille E, Nowak-Wegrzyn A. Allergen-specific immunotherapies for food allergy. Allergy Asthma Immunol Res. 2018;10(3):189-206.
9. Taniuchi S et al. Immunotherapy for cow's milk allergy. Hum Vaccin Immunother. 2017;13(10):2443-51.
10. Sato S, et al. Nationwide questionnaire-based survey of oral immunotherapy in Japan. Allergol Int. 2018;67(3):399-404.
11. Wood RA. Food allergen immunotherapy: Current status and prospects for the future. J Allergy Clin Immunol. 2016;137(4):973-82.
12. Hussey Freeland DM et al. Advances in food allergy oral immunotherapy: Toward tolerance. Curr Opin Immunol. 2016;42:119-23.
13. MacGinnite A. Update on potential therapies for IgE-mediated food allergy. Curr Allergy Asthma Rep. 2017;17(1):4.
14. Albin S, Nowak-Wegrzyn A. Potential treatments for food allergy. Immunol Allergy Clin North Am. 2015;35(1):77-100.
15. Gernez Y, Nowak-Wegrzyn A. Immunotherapy for food allergy: Are we there yet? J Allergy Clin Immunol Pract. 2017;5(2):250-72.
16. Yee CSK, Rachid R. The heterogeneity of oral immunotherapy clinical trials: Implications and future directions. Curr Allergy Asthma Rep. 2016;16(4):25.
17. Jones SM et al. Long-term treatment with egg oral immunotherapy enhances sustained unresponsiveness that persists after cessation of therapy. J Allergy Clin Immunol. 2016;137(4):1117-27.
18. Vickery BP et al. Sustained unresponsiveness to peanut in subjects who have completed peanut oral immunotherapy. J Allergy Clin Immunol. 2014;133(2):468-75.
19. The PALISADE Group of Clinical Investigators. AR101 oral immunotherapy for peanut allergy. N Engl J Med. 2018;379:1991-2001.
20. Israel E, Reddel HK. Severe and difficult-to-treat asthma in adults. N Engl J Med. 2017;377:96576.
21. Pelaia C et al. Omalizumab, the first available antibody for biological treatment of severe asthma: More than a decade of real-life effectiveness. Ther Adv Respir Dis. 2018;12:1753466618810192.
22. Saini SS, Kaplan AP. Chronic spontaneous urticaria: The devil's itch. J Allergy Clin Immunol Pract. 2018;6(4):1097-106.
23. Maurer M et al. Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. N Engl J Med. 2013;368(10):924-35.

24. MacGlashan DW Jr et al. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol. 1997;158(3):1438-5.
25. Furuichi K et al. The receptor for immunoglobulin E on rat basophilic leukemia cells: Effect of ligand binding on receptor expression. Proc Natl Acad Sci USA. 1985;82(5):1522-5.
26. Lantz CS et al. IgE regulates mouse basophil Fc epsilon RI expression in vivo. J Immunol. 1997;158(6):2517-21.
27. Yamaguchi M et al. IgE enhances mouse mast cell Fc epsilon RI expression in vitro and in vivo: Evidence for a novel amplification mechanism in IgE-dependent reactions. J Exp Med. 1997;185(4):663-72.
28. Yamaguchi M et al. IgE enhances Fc epsilon receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: Synergistic effect of IL-4 and IgE on human mast cell Fc epsilon receptor I expression and mediator release. J Immunol. 1999;162(9):5455-65.
29. Gomez G et al. Omalizumab reverses the phenotypic and functional effects of IgE-enhanced FcεRI on human skin mast cells. J Immunol. 2007;179(2):1353-61.
30. Beck LA et al. Omalizumab-induced reductions in mast cell Fc epsilon RI expression and function. J Allergy Clin Immunol. 2004;114(3):527-30.
31. Prussin C et al. Omalizumab treatment downregulates dendritic cell Fc epsilon RI expression. J Allergy Clin Immunol. 2003;112(6):1147-54.
32. Eggel A et al. Accelerated dissociation of IgE-FcεRI complexes by disruptive inhibitors actively desensitizes allergic effector cells. J Allergy Clin Immunol. 2014;133(6):1709-19.e8.
33. Maggi L et al. Omalizumab dampens type 2 inflammation in a group of long-term treated asthma patients and detaches IgE from FcεRI. Eur J Immunol. 2018;48(12):2005-14.
34. Abdel-Gadir A et al. Oral immunotherapy with omalizumab reverses the Th2 cell-like programme of regulatory T cells and restores their function. Clin Exp Allergy. 2018;48(7):825-36.
35. Dantzer JA, Wood RA. The use of omalizumab in allergen immunotherapy. Clin Exp Allergy. 2018;48(3):232-40.
36. Andorf S et al. Anti- IgE treatment with oral immunotherapy in multifood allergic participants: A double-blind, randomised, controlled trial. Lancet Gastroenterol Hepatol. 2018;3(2):85-94.
37. Tsabouri S et al. Omalizumab for the treatment of inadequately controlled allergic rhinitis: A systematic review and meta-analysis of randomized clinical trials. J Allergy Clin Immunol Pract. 2014;2(3):332-40.e1.
38. Masieri S et al. Effects of omalizumab therapy on allergic rhinitis: A pilot study. Eur Rev Med Pharmacol Sci. 2016;20(24):5249-55.
39. Holm JG et al. Omalizumab for atopic dermatitis: Case series and a systematic review of the literature. Int J Dermatol. 2017;56(1):18-26.
40. Kim DH et al. Anti-immunoglobulin E in the treatment of refractory atopic dermatitis. Clin Exp Dermatol. 2013;38(5):496-500.
41. Lowe PJ, Renard D. Omalizumab decreases IgE production in patients with allergic (IgE-mediated) asthma; PKPD analysis of a biomarker, total IgE. Br J Clin Pharmacol. 2011;72(2):306-20.
42. Nadeau KC et al. Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy. J Allergy Clin Immunol.2011;127(6):1622-4.
43. Wood RA et al. A randomized, double-blind, placebo-controlled study of omalizumab combined with oral immunotherapy for the treatment of cow's milk allergy. J Allergy Clin Immunol. 2016;137(4):1103-10.
44. Burks AW et al. Oral immunotherapy for treatment of egg allergy in children. N Engl J Med. 2012;367(3):233-43.
45. Takahashi M et al. Successful desensitization in a boy with severe cow's milk allergy by a combination therapy using omalizumab and rush oral immunotherapy. Allergy Asthma Clin Immunol. 2015;11(1):18.
46. Takahashi M et al. Oral immunotherapy combined with omalizumab for high–risk cow's milk allergy: A randomized controlled trial. Sci Rep. 2017;7(1):17453.
47. Schneider LC et al. A pilot study of omalizumab to facilitate rapid oral desensitization in high-risk peanut-allergic patients. J Allergy Clin Immunol. 2013;132(6):1368-74.
48. MacGinnitie AJ et al. Omalizumab facilitates rapid oral desensitization for peanut allergy. J Allergy Clin Immunol. 2017;139(3):873-81.
49. Kim EH et al. Sublingual immunotherapy for peanut allergy: Clinical and immunologic evidence of desensitization. J Allergy Clin Immunol. 2011;127(3):640-6.e1.
50. Fleischer DM et al. Sublingual immunotherapy for peanut allergy: A randomized, double- blind, placebo-controlled multicenter trial. J Allergy Clin Immunol. 2013;131(1):119-27.e7.
51. Burks AW et al. Sublingual immunotherapy for peanut allergy: Long-term follow-up of a randomized multicenter trial. J Allergy Clin Immunol. 2015;135(5):1240-8.e1-3.
52. Narisety SD et al. A randomized, double-blind, placebo-controlled pilot study of sublingual versus oral immunotherapy for the treatment of peanut allergy. J Allergy Clin Immunol. 2015;135(5):1275-82.e1-6.
53. Keet CA et al. The safety and efficacy of sublingual and oral immunotherapy for milk allergy. J Allergy Clin Immunol. 2012;129(2):448-55.
54. Fleischer DM et al. Effect of epicutaneous immunotherapy vs placebo on reaction to peanut protein ingestion among children with peanut allergy: The PEPITES Randomized Clinical Trial. 2019;321(10):946-55.

A Case of Lance-Adams Syndrome Post Life-Threatening Bronchial Asthma

Author: Heng Shee Kim

Department of Medicine, Hospital Pakar Sultanah Fatimah, Muar, Johor, Malaysia
Correspondence to edwynkim@gmail.com

Disclosure: The author has declared no conflicts of interest.

Acknowledgement: The author would like to thank the Director-General of Health Malaysia for permission to publish this paper.

Received: 15.05.19

Accepted: 13.08.19

Keywords: Chronic post-hypoxic myoclonus, Lance-Adams syndrome (LAS), serotonin.

Citation: EMJ. 2019;4[4]:71-76.

Abstract

Lance-Adams syndrome (LAS) is a rare complication that occurs after successful cardiopulmonary resuscitation, in which myoclonus appears rapidly upon recovery from the severe hypoxic event. To date, <150 cases have been reported. The author reports a case of a 43-year-old male who presented with cardiorespiratory collapse as a result of life-threatening bronchial asthma that required 10 minutes of cardiopulmonary resuscitation. He was intubated and haemodynamically supported with inotropes for 4 days. Post-extubation, he developed progressively worsening action myoclonus involving limbs, trunk, and speech. His mini-mental state examination (MMSE) was 16/30. All his metabolic, infective, and neuroimaging screenings, including CT and MRI of the brain, were normal. He was diagnosed with LAS due to the classical onset and semiology. Clonazepam and sodium valproate were started, with piracetam added later. He showed marked functional improvement after the treatment. He was able to walk with walking-frame and MMSE improved to 26/30; however, minimal dysarthria persisted. Early and accurate diagnosis is of paramount importance for disability limitation; however, management of LAS can be challenging as high-quality, evidence-based treatment has not been established. This case highlights the importance of retaining high clinical suspicion in diagnosing LAS. Given its typical history and presentation, the diagnosis can be made confidently.

INTRODUCTION

It has been reported that in up to one-third of post resuscitated comatose patients experienced a seizure, with post-hypoxic myoclonus (PHM) as the most common type.[1]

PHM is defined as repetitive, generalised, focal or multifocal, myoclonic motor movements involving the face, limbs, or trunk that can occur at any time following cardiac arrest, and stems from increased neuronal excitability after brain injury.[2]

PHM can be classified by the time of onset, outcomes, response to treatment, characteristics of myoclonus, and neurological examination, with particular focus on the presence of coma. However, it is generally divided into acute PHM and chronic PHM or commonly known as Lance-Adams syndrome (LAS).

The author reports a case of LAS after a life-threatening bronchial asthma attack.

CASE REPORT

The author reports the case of a 43-year-old male who presented with life-threatening acute exacerbation of bronchial asthma precipitated by viral pneumonia. He had a case of partly controlled bronchial asthma on meter-dosed inhaler salbutamol and meter-dosed inhaler budesonide. On the day of presentation, he experienced worsening shortness of breath, purulent cough, and fever for 2 days.

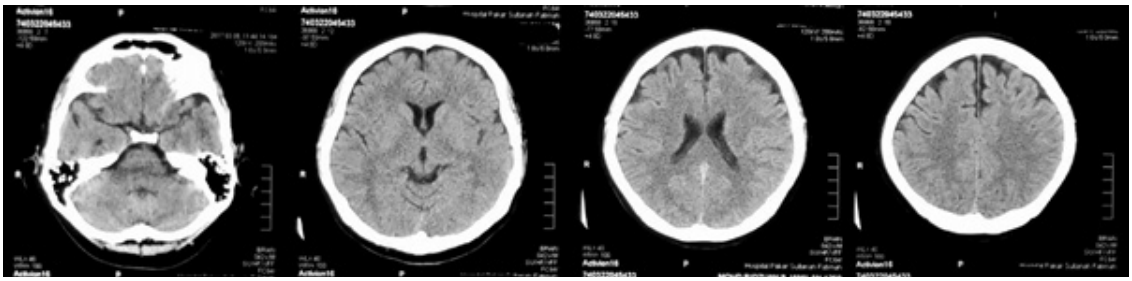


Figure 1: CT of the brain of a 43-year-old male with Lance-Adams syndrome.

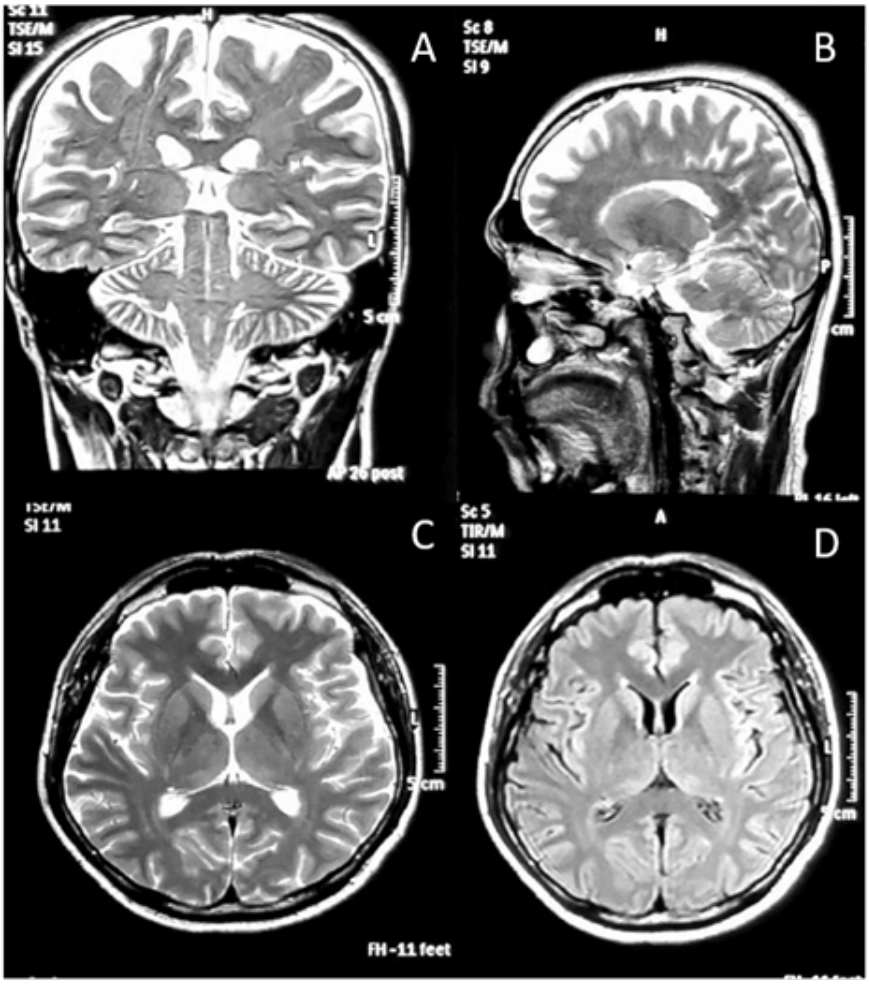


Figure 2: Magnetic resonance of the brain.

A) T2-weighted (coronal cut); B) T2-weighted (sagittal cut); C) T2-weighted (axial brain cut); D) FLAIR sequence.

He was brought to the emergency department with no sign of life and required 10 minutes of cardiopulmonary resuscitation. The patient was then intubated and haemodynamically supported with intravenous noradrenaline for 4 days in the intensive care unit. Post-extubation, he developed progressively worsening action myoclonus involving limbs, trunk, and speech. His mini-mental state examination (MMSE) was 16/30. All his metabolic, infective, and neuroimaging screenings, including CT (Figure 1) and MRI of the brain (Figure 2), were normal. Electroencephalogram (EEG) was not carried out due to the unavailability of the service. He was later diagnosed with LAS due to the classical onset of, and semiology of, myoclonus.

A titrating dose of clonazepam was started, from 0.5 mg 3 times a day and titrated up to a total of 8.0 mg per day. The patient could not tolerate 8.0 mg of clonazepam because of drowsiness and lethargy. Clonazepam was then reduced to 6.0 mg a day with added sodium valproate. With clonazepam (6.0 mg/day) and sodium valproate (1.2 g/day), his action myoclonus was partially controlled. After reviewing multiple case reports, piracetam 2.4 g/day was initiated as an add-on therapy. He showed marked functional improvement after the combination of clonazepam (6.0 mg/day), sodium valproate (1.2 g/day), and piracetam (2.4 g/day). He was then able to ambulate with a walking frame, self-dress with minimal aid, and MMSE had improved to 26/30. However, minimal dysarthria persisted upon discharge after 2 weeks of admission. During clinic follow-up, sodium valproate was further titrated up to 1.8 g/day with aggressive physiotherapy and occupational therapy. To date, functionally, he can walk without help, write, and hold a spoon and fork for food, but residual action myoclonus with fine movement persisted; for example, he was not able to use a screwdriver due to action myoclonus. The outpatient EEG was normal.

DISCUSSION

Post hypoxic myoclonus is defined as repetitive sudden muscle contraction or muscle tone lapses that are brief, involuntary, and shock-like, involving face, limbs, or trunk, and can occur at any time following cardiac arrest due to increased neuronal excitability after brain injury.[2,3] Multiple different classifications had been suggested, but it is predominantly divided into two main groups: acute PHM and chronic PHM. Reviews have suggested the importance of differentiating between acute and chronic PHM due to its prognostication value;[2,4-6] however, in a clinical setting, it could be challenging to classify between different post cardiac arrests due to the extensive use of sedation as a neuroprotective measure. Freund and Kaplan[2] suggested a summary of clinical features to differentiate between acute PHM and chronic PHM (Table 1).

This case was consistent with the diagnosis of chronic PHM, also known as LAS. The syndrome was first reported by Lance and Adams in 1963 and typically, after recovery of consciousness, patients exhibit muscle jerks, which are at first generalised, but later become more restricted to upper or lower limbs, and are characteristically made worse by voluntary action.[7] The severity of the myoclonus is proportionate to the precision of the task required and the patient may retain full consciousness during the episode of myoclonus. Other associated features include cerebral ataxia, dementia, spasticity, and incontinence.[8] The most commonly reported adverse event associated with LAS was respiratory arrest.[2] In this case, the patient showed classical onset and characteristic of action myoclonus post-cardiac arrest as a result of life-threatening bronchial asthma.

The pathophysiology of LAS is not entirely understood, although the literature has suggested that possible loss of serotonin (5-hydroxytryptamine or 5-HT) may be the cause of LAS.[3,8,9] This was based on an earlier report that showed relatively low levels of serotonin activity in cerebrospinal fluid and partial response toward serotonergic treatment. However, the further autopsy report showed multiple different areas of intracranial abnormalities such as in mammillary bodies, brainstem raphe nuclei, cuneiform, subcuneiform nuclei, supratrochlear nucleus, lateral subnucleus of mesencephalic grey matter, and midbrain periaqueductal grey matter.[2,3,6,7,9] The reason for multiple areas of abnormalities that have been suggested is different mechanisms of cardiac arrest involved.

Table 1: Summary of clinical and electroencephalogram findings in post hypoxic myoclonus.[2]

	Myoclonic status epilepticus	Lance-Adams syndrome
Clinical features		
Body parts involved	Generalised or multifocal; trunk, trapezius, sternocleidomastoid, face, limbs; proximal and distal limb involved	Generalised, multifocal, or focal; limb involvement depends on cortical versus subcortical sources
Timing of onset	Rarely after 72 hours post-cardiac arrest	From hours to years following cardiac arrest
Length of duration	Days to weeks	Days to years
Response to treatment	Usually poor	Variable
Neurologic examination	Usually comatose	Comatose if sedated; awake, alert, and cognition may be relatively preserved
Mortality rate	90–100% of cases	Unclear given selection and survivorship bias, but in one retrospective study 50% of patients with similar EEG findings survived
Good neurologic outcome	Rare, may be more likely with multifocal versus generalised MSE	Common
Circumstances of cardiac arrest	Longer time to CPR, less bystander resuscitation, higher rates of nonshockable cardiac rhythm on presentation	Often primary respiratory arrest
Stimulus-sensitivity of myoclonus	Yes	Yes
Spontaneous myoclonus	Yes	Occasionally
Intention myoclonus	No	Yes
Neurophysiological findings		
EEG findings	Generalised epileptiform discharges and burst suppression, status epilepticus noted by intermittent or continuous spike-waves; lateralised periodic discharges and focal discharges are less common; over time burst suppression can evolved into generalised periodic discharges; diffuse slowing less common; alpha coma, particularly later after cardiac arrest	Epileptiform activity in up to one-third of cases often maximally or primarily at the vertex especially within hours after cardiac arrest, can have normal background activity; diffuse or focal slowing; up to 20% are normal
SSEP	Normal or absent; giant SSEP not consistently demonstrated; lacking thorough evaluation of multifocal PHM	Can demonstrate both giant and normal-sized SSEP
EEG-EMG polygraphy	Typically lacks jerk-locking; a thorough evaluation of multifocal PHM is lacking	Jerk-locking has been noted in roughly 60% of cases
Localisation	Subcortical, possibly cortical	Both subcortical and cortical

CPR: cardiopulmonary resuscitation; EEG: electroencephalogram; EMG: electromyogram; PHM: post-hypoxic myoclonus; SSEP: somatosensory-evoked potential.

Imaging studies in diagnosing LAS have not yielded satisfactory results. Both brain CT and brain MRI show no disease-specific abnormality; however, with the advancement of medical technologies, functional neuroimaging such as brain single-photon emission CT or brain PET could provide additional information about the anatomical and pathophysiological basis of LAS.[10,11]

Unlike neuroimaging studies, neurophysiological movement studies can provide more useful information in guiding the decision making of the diagnosis and treatment modality. LAS typically shows focal EEG discharge over the sensorimotor cortical area, especially when using back-averaging of the EEG by jerk-locking or electromyogram discharge during the acute phase.[2,3] This could explain the semiology of action-induced myoclonus in LAS. Additionally, by identifying foci of myoclonus in EEG, area-specific medication can be prescribed more confidently. In this case, neurophysiological movement studies were not performed in the acute phase owing to unavailability of service, but subsequent EEG study was normal.

Given how rare this disease is and the lack of understanding of its exact pathophysiology, the treatment regime for LAS is frequently based on case reports or case series. Benzodiazepines, clonazepam, in particular, a chlorinated derivative of nitrazepam that was first introduced into clinical practice in 1966, has been used successfully to treat LAS since the 1970s.[12,13] It facilitates γ-Aminobutyric acid (GABA) transmission in the brain by directly affecting benzodiazepine receptors. Benzodiazepines do not affect 5-HT use in the brain and block the egress of 5-Hydroxyindoleacetic acid from the brain; however, these effects contradict the beneficial effect of 5-HT in human myoclonic features. It was postulated that the possibility of its benefit lies in benzodiazepine receptors.[14] Nevertheless, it is considered the first-line treatment in LAS. As LAS is associated with cortical origin myoclonus, the sodium valproate used has achieved a certain degree of success in treating LAS with the postulating mechanism through the elevation of brain GABA level in synaptic regions.[3] However, due to the side effects of sodium valproate such as tiredness, weight gain, tremor, and hair loss, it may render a higher dosage intolerable, similarly to the patient in the case reported herein.[15] Piracetam, a derivative of GABA, which postulated the effect of improved neuroplasticity, has shown to be beneficial in controlling myoclonus.[16] Recent studies also reported the efficacy of levetiracetam in treating LAS.[17,18] Clonazepam, sodium valproate, piracetam, and levetiracetam are the first-line therapy options in the management of LAS. Combination treatment may achieve the best possible outcome in these patients.

The prognosis had been reported to be good in LAS if early aggressive rehabilitation and appropriate drug treatment initiated.[3,5,6,9,15,18]

CONCLUSION

Early recognition and accurate diagnosis of LAS is of paramount importance to correctly predict post hypoxic myoclonus in the patient, to decide on the most effective treatment regimen, and for disability limitation. However, management of LAS can be challenging as high-quality, evidence-based treatment has not been established. This case report highlights the importance of retaining high clinical suspicion in diagnosing LAS. Given its typical history and presentation, the diagnosis can be made confidently.

References

1. Nolan JP et al. European Resuscitation Council and European Society of Intensive Care Medicine Guidelines for Post-resuscitation Care 2015: Section 5 of the European Resuscitation Council Guidelines for Resuscitation 2015. Resuscitation. 2015;95:202-22.
2. Freund B, Kaplan PW. Post-hypoxic myoclonus: Differentiating benign and malignant etiologies in diagnosis and prognosis. Clin Neurophysiol Pract. 2017;2:98-102.
3. Gupta HV, Caviness JN. Post-hypoxic Myoclonus: Current concepts, neurophysiology, and treatment. Tremor Other Hyperkinet Mov. 2016;6:409.
4. Datta S et al. Post-hypoxic myoclonic status: The prognosis is not always hopeless. Crit Care Resusc. 2009;11(1):39-41.
5. English WA et al. Myoclonus after cardiac arrest: Pitfalls in diagnosis and prognosis. Anaesthesia. 2009;64(8):908-11.
6. Werhahn KJ et al. The clinical features and prognosis of chronic posthypoxic myoclonus. Mov Disord. 1997;12(2):216-20.

7. Lance JW, Adams RD. The syndrome of intention or action myoclonus as a sequel to hypoxic encephalopathy. Brain. 1963;86(1):111-36.
8. Rustom R, Fisken RA. Action myoclonus after cerebral hypoxia: Treatment with clonazepam. J R Soc Med. 1992;85(12):761-2.
9. Shin JH et al. Lance-Adams syndrome. Ann Rehabil Med. 2012;36(4):561-4.
10. Zhang YX et al. Lance-Adams syndrome: A report of two cases. J Zhejiang Univ Sci B. 2007;8(10):715-20.
11. Frucht SJ et al. The metabolic topography of posthypoxic myoclonus. Neurology. 2004;62(10):1879-81.
12. Tassinari CA et al. The use of diazepam and clonazepam in epilepsy. Epilepsia. 1998;39:S7-14.
13. Goldberb MA, Dorman JD. Intention myoclonus: Successful treatment with clonazepam. Neurology. 1976;26:24-6.
14. Jenner P et al. Mechanism of action of clonazepam in myoclonus in relation to effects on GABA and 5-HT. Adv Neurol. 1986;43:629-43.
15. Malhotra S, Mohinder K. Lance-Adams syndrome: Difficulties surrounding diagnosis, prognostication, and treatment after cardiac arrest. Anesth Essays Res. 2012;6(2):218-22.
16. Winblad B. Piracetam: A review of pharmacological properties and clinical uses. CNS Drug Rev. 2005;11(2):169-82.
17. Krauss GL et al. Suppression of post-hypoxic and post-encephalitic myoclonus with levetiracetam. Neurology. 2001;56:411-2.
18. Ilik F et al. Levatiracetam for the management of Lance-Adams syndrome. Iran J Child Neurol. 2014;8(2):57-59.

Cardiovascular Complications in Pre-eclampsia: Can they be Predicted Electrocardiographically?

Authors: *Robert Adrianto Raharjo,[1] Yan Herry,[1] Udin Bahrudin,[1] M. Besari Adi Pramono,[2] Destrian Ekoputro Wismiyarso[1]

1. Department of Cardiology and Vascular Medicine, Faculty of Medicine, Diponegoro University, Semarang, Indonesia
2. Department of Obstetrics and Gynecology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

*Correspondence to robert.adriantoraharjo@gmail.com

Disclosure: The authors have declared no conflicts of interest.

Received: 18.09.2018

Accepted: 26.11.2018

Keywords: Cardiovascular complication, electrocardiography, pre-eclampsia.

Citation: EMJ. 2019;4[4]:77-83.

Abstract

Background: Pre-eclampsia is a common condition that causes significant morbidity and mortality in pregnant women; the occurrence of cardiovascular complications aggravates the disease. Efforts have been made to predict the complications of pre-eclampsia, but some modalities, such as echocardiography and biomarkers, are neither available nor widely feasible for use by healthcare providers, especially in developing countries. On the other hand, ECG is cheap, noninvasive, widely available, and already routinely performed for pre-eclampsia. The role of ECG in predicting cardiovascular complications in pre-eclampsia patients is not known.

Objective: This study aimed to investigate the role of ECG in pre-eclampsia diagnostics and simple clinical parameters in pre-eclampsia patients with and without cardiovascular complications.

Methods: This cross-sectional, analytical study used retrospective data from medical records of patients with pre-eclampsia from the Dr Kariadi General Hospital, Semarang, Indonesia, from January 2016–July 2017. Bivariate association between demographic, clinical, laboratory, and ECG results with the occurrence of cardiovascular complications was tested; this continued with logistic regression.

Results: Sixty-eight pre-eclampsia patients were identified, with a mean age of 30.2 years. Cardiovascular complications occurred in 16 patients (23.5%), with 14 patients exhibiting pulmonary oedema. In univariate analysis, haemoglobin level and heart rate showed a significant association with the occurrence of cardiovascular complications (p=0.035 and 0.033, respectively). No significant independent predictor was found in multivariate analysis.

Conclusion: This study showed that ECG parameters were not able to predict cardiovascular complications in pre-eclampsia patients. Nevertheless, there was a significant association between heart rate and haemoglobin level with cardiovascular complications in pre-eclampsia.

INTRODUCTION

High maternal mortality rate (MMR) is a health problem in many developing countries, and high MMR reflects the quality of healthcare during pregnancy and puerperium.[1] The MMR in Indonesia, with rates as high as 228 per 100,000 live births, is one of the highest in South East Asia, and pre-eclampsia is the major contributor to maternal and perinatal morbidity and mortality, with an incidence of 128,273 per year (5.3% of all pregnancies).[2,3] Furthermore, 99% of maternal deaths occur in low and middle-income countries.[4] This is associated with the occurrence of pre-eclampsia-related complications, including cardiovascular complications,[5] with a delay in diagnosis and treatment resulting in a higher rate of death.[6] Cardiovascular complications, such as pulmonary oedema, occur in 4.3%–15.0%[7-9] of pre-eclampsia patients and are the cause of 17.2% maternal deaths.[10] Women with pre-eclampsia or eclampsia experienced a 42% increased risk of cardiovascular disease, and those who developed cardiovascular disease experienced a 5-fold increase in healthcare-related costs during follow-up.[11]

These findings led to the development of a predictive model of pre-eclampsia's complications.[12-16] However, some examination techniques used in the prediction models, such as echocardiography and biomarkers, are not universally available, and are not feasible for use in developing countries. On the other hand, ECG has been used routinely in pre-eclampsia to identify cardiovascular complications that have already occurred, yet the role of ECG in predicting those occurrences is not yet clear. This study aimed to investigate the association between ECG and clinical parameters with the occurrence of cardiovascular complications in pre-eclampsia patients.

METHODS

This analytical cross-sectional study used retrospective samples from the medical records of patients who were diagnosed with pre-eclampsia at the Dr Kariadi General Hospital, Semarang, Indonesia, from January 2016–July 2017. Diagnosis of pre-eclampsia as the inclusion criteria was based on current guidelines,[17] and samples with cardiovascular complications upon admission, chronic hypertension (defined as blood pressure [BP] >140/90 mmHg before pregnancy or before 20 weeks' gestation), or heart disease (congenital, valvular, ischaemic, and/or arrhythmia) were excluded from the study. The study was approved by the Education and Research Board of Dr Kariadi Hospital.

The independent variables were demographic, clinical, laboratory, and ECG parameters, with the occurrence of cardiovascular complications manifesting as pulmonary oedema, heart failure, or myocardial ischaemia during the peripartum period as the dependent variables.[18]

The demographic data used were age and education level; the clinical data used were gestational age at diagnosis, weight, height, antenatal care frequency, systolic and diastolic BP, and nulliparous or not. Later, BMI and mean arterial pressure ([systolic BP+(2 x diastolic BP)]/3) were calculated. The laboratory data analysed were haemoglobin, ureum, creatinine, and lactate dehydrogenase.

Electrocardiography

The standard 12-lead ECG performed during admission was analysed both qualitatively and quantitatively. Heart rate (HR) was calculated by dividing 1,500 by the R wave-to-R wave small square interval. The electrical axis was determined using the method presented by Baltazar et al.[19] PR interval was measured as the time between the beginning of the P wave and the beginning of the QRS complex in milliseconds. QRS duration was measured from the start until the end of the QRS complex in milliseconds. Sokolow–Lyon voltage was taken from the bigger summation between S wave in V_1 and R wave in V_5 or V_6.[20] QT interval was measured as the time from the start of the Q wave to the end of the T wave and corrected by HR using Bazett's formula.[21]

The criteria for left atrial abnormality were 1) widely notched P wave (40 ms); 2) terminal negative component of the P wave in lead V_1 with duration ≥40 ms and amplitude ≥-0.1 mV; or 3) P wave duration ≥120 ms.[22]

Myocardial ischaemia was determined by either ST elevation at the J point in two contiguous leads, with the cut-points ≥0.1 mV in all leads other than leads V2–V3 where different cut

points applied: ≥0.2 mV in men ≥40 years, ≥0.25 mV in men <40 years, or ≥0.15 mV in women; or horizontal or down-sloping ST depression ≥0.05 mV in two contiguous leads and/or T inversion ≥0.1 mV in two contiguous leads with a prominent R wave or R:S ratio >1.[23]

Data Analysis

Statistical analysis was performed using SPSS version 23 (IBM SPSS Statistics, Armonk, New York, USA). Data are reported as a percentage or mean ± standard deviation according to the type of the data. Comparative tests between groups were performed using independent T-test and Chi-square tests, with Yates correction if the independent variable was numeric and nominal, respectively. If the data were not distributed normally and normalisation failed, Mann–Whitney and Fisher tests were used in place of the T-test and Chi-square tests, respectively; association would be considered significant if $p<0.05$. If the univariate analysis yielded more than one association with $p<0.25$, multivariate analysis was performed to determine the independent predictor(s).

RESULTS

During the study duration, 115 samples were obtained, and 47 of them were excluded; 30 were excluded due to chronic hypertension, 3 due to congenital heart disease (all of which were atrial septal defect), 9 due to valvular heart disease (3 rheumatic mitral stenosis, 6 mitral regurgitation), and 5 due to pre-existing arrhythmia (4 atrial fibrillation, 1 junctional bradycardia). Final analysis included 68 pregnant women with pre-eclampsia.

Characteristics and Outcome

Characteristics of the patient population are shown in Table 1. Cardiovascular complications occurred in 16 patients (23.5%). More specifically, heart failure with pulmonary oedema occurred in 14 patients, and heart failure without pulmonary oedema and heart failure with myocardial ischaemia occurred in 1 patient each, respectively.

Comparative Analysis

The results of the comparative analysis are reported in Table 1. Only haemoglobin ($p=0.035$) and HR ($p=0.033$) had a significant association with the occurrence of cardiovascular complication; nevertheless, in those with cardiovascular complications, left atrial abnormality and myocardial ischaemia were more prevalent and the corrected QT interval tended to be longer.

A multivariate analysis on weight, BMI, antenatal care frequency, systolic BP, mean arterial pressure, haemoglobin, and HR showed that no variable is a significant independent predictor of cardiovascular complications, as shown in Figure 1.

DISCUSSION

The occurrence of cardiovascular complications contributes to morbidity and mortality in pre-eclampsia patients. With prediction of the condition in mind, this study found that HR and haemoglobin levels were significantly associated with the occurrence of cardiovascular complications.

In this study, cardiovascular complications occurred in 23.5% of patients, a greater proportion than is reported in any of the published literature. The incidence of pulmonary oedema as the cardiovascular complication in the pre-eclampsia population varies, from 0.5% in all pregnancies[24] to 4.3–15.0% in patients with severe pre-eclampsia or HELLP syndrome,[5,8,9,25] and 5.0–33.0%[26,27] in patients with eclampsia. This discrepancy might be caused by the location of the study; the Dr Kariadi General Hospital is a national referral hospital and, thus, the patient population tends to suffer from more severe disease, while patients with less severe and non-complicated disease would be already treated in lower-tier hospitals.

Pulmonary oedema is the most common cardiovascular complication of pre-eclampsia in this study, in accordance with previous studies.[25,28] The physiological changes in the maternal cardiovascular system, including increased plasma blood volume, cardiac output, HR, and capillary permeability, and a decrease in plasma colloid osmotic pressure, are exaggerated in pre-eclampsia and so predispose patients to developing pulmonary oedema. Plasma colloid osmotic pressure decreases after delivery, which may be caused by excessive blood loss and fluid shifts secondary

to increased capillary permeability. These changes may explain why most occurrences of pulmonary oedema in pre-eclampsia develop after delivery.[25,29,30]

von Dadelszen et al.[12] developed the fullPIERS model, which aimed to identify risk factors for complications in pre-eclampsia patients, but HR and haemoglobin were not included in the study.

Table 1: Patients' characteristics and association between groups with and without cardiovascular complication.

		Cardiovascular complication		
Parameters	All n=68	No n=52	Yes n=16	p
Demography				
Age (years)	30.2±6.2	30.1±6.1	30.6±6.6	0.751†
Senior high school graduate or higher (%)	79.4	78.8	81.3	0.572‡
Weight (kg)	67.6±13.5	69.1±14.5	63.2±15.1	0.123†
Height (cm)	154.3±5.2	154.1±4.2	154.9±3.0	0.529§
BMI (kg/m^2)	28.4±5.2	29.0±5.6	26.3±6.3	0.108†
Clinical				
Gestational age at diagnosis, (week)s	34.6±3.5	34.6±3.4	34.4±3.9	0.789†
Antenatal care frequency	5.7±3.0	5.9±2.6	4.9±2.9	0.211§
Systolic blood pressure (mmHg)	161.6±23.2	163.5±22.1	155.4±17.5	0.184†
Diastolic blood pressure (mmHg)	100.5±12.8	101.6±12.8	97.1±8.5	0.295§
Mean arterial pressure (mmHg)	120.9±14.5	122.3±14.4	116.5±10.3	0.144†
Nulliparous (%)	32.4	34.6	25.0	0.679**
Laboratory				
Haemoglobin (g/dL)	11.41±2.17	11.77±2.21	10.34±2.24	0.035*†
Ureum (mg/dL)	27.36±28.00	25.60±15.50	32.20±33.20	0.747§
Creatinine (mg/dL)	0.89±0.32	0.86±0.31	0.97±0.54	0.795§
Lactate dehydrogenase (mg/dL)	638.5±493.7	660.2±484.4	562.8±310.7	0.676§
Electrocardiography				
Heart rate (bp)	96.2±19.0	93.2±19.6	106.3±25.4	0.033*†
Axis (degree)	45.00±24.6	43.85±35.7	48.75±35.8	0.633†
PR interval (ms)	147.2±23.8	149.0±29.1	141.3±22.5	0.409§
QRS complex duration (ms)	67.1±14.5	67.7±16.5	65.0±17.1	0.603§
Sokolow–Lyon voltage (mV)	1.87±0.6	1.88±0.7	1.84±0.4	0.824†
Corrected QT interval (ms)	445.2±39.1	442.9±43.8	452.9±57.3	0.463†
Left atrial abnormality (%)	45.6	42.3	56.3	0.489**
Myocardial ischaemia (%)	1.5	0.0	6.3	0.235‡

Values are shown as mean±standard deviation or percentage.

*p<0.05

†Independent T-test for same variance

‡Fisher test

§Mann–Whitney test

**Chi-square test with Yates correction.

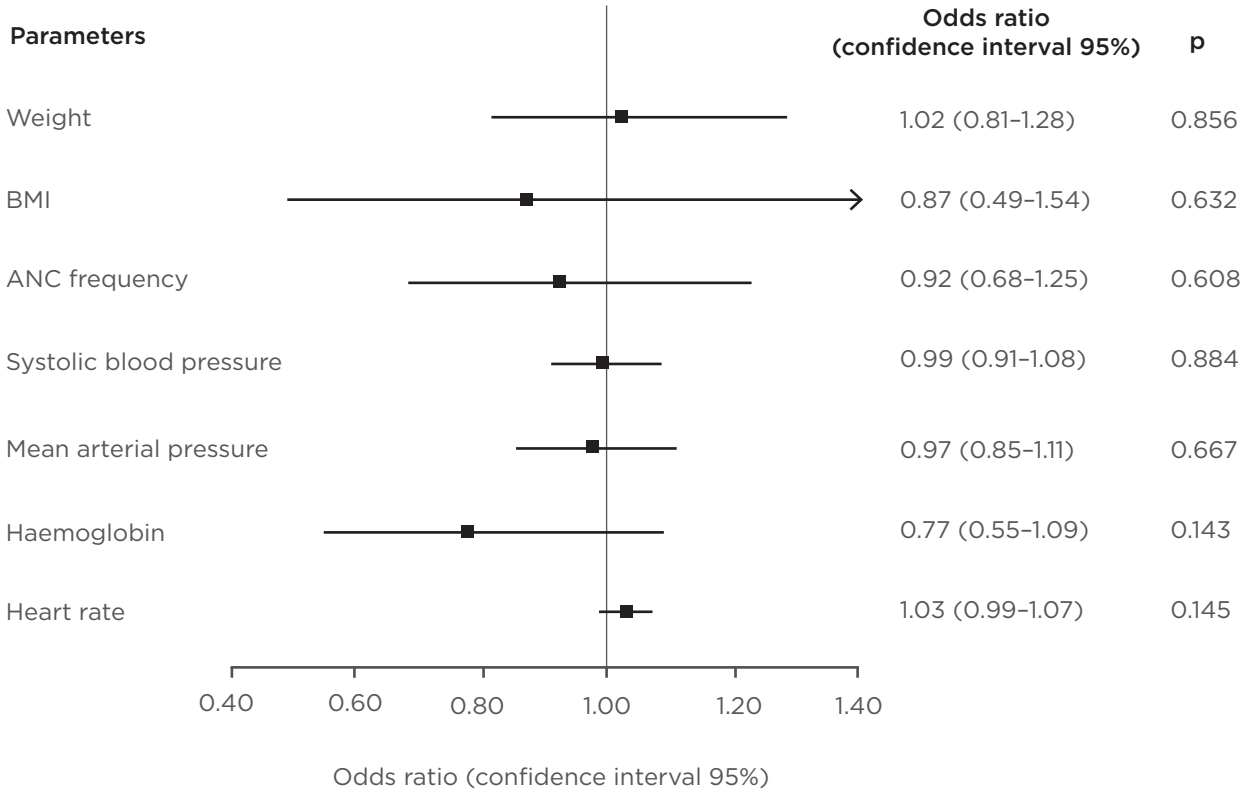


Figure 1: Forest plot of cardiovascular complication predictors in pre-eclampsia.

ANC: antenatal care.

Similarly, Thornton et al.[15] studied the incidence and causative factors of acute pulmonary oedema in patients with hypertensive disorders of pregnancy, and HR and haemoglobin levels were not considered in this study.

Angeli et al.[31] reported that HR was not able to predict the risk of hypertensive disorders during pregnancy; furthermore, Raffaelli et al.[32] also showed no association between HR and the cardiac effects of pre-eclampsia. It is possible that the increase in HR was not the cause or risk factor but a manifestation of cardiovascular complications.[33] This bias was minimised by using the initial ECG and excluding patients presenting with cardiovascular complications upon admission. The increase in HR is a compensatory mechanism to preserve cardiac output in this population and reflects the severity of pre-eclampsia; studies showed the development of left ventricular dysfunction,[34,35] increased aortic stiffness,[36] and decreased stroke volume.[37] As previously discussed, the incidence of cardiovascular complications is directly proportional to pre-eclampsia severity,[7-9] and this relation forms the basis of association between HR and cardiovascular complications.

Despite the limited research into ECG changes during pregnancy, there is evidence that pregnancy affects ECG and those changes are reversible at the end of or after pregnancy.[38] A similar scarcity of data also applies to women with hypertensive disease of pregnancy. Present evidence has demonstrated that hypertensive disorders of pregnancy was associated with changes of the P wave morphology and QT interval. Angeli et al.[31] reported that left atrial abnormality diagnosed by P wave changes in lead V_1 is an independent predictor of hypertensive disorder during pregnancy. On the other hand, Raffaelli et al.[32] reported that prolonged corrected QT interval and QT dispersion in pre-eclampsia patients increased the risk of cardiovascular events. However, this study did not yield a significant value of standard ECG parameters

for predicting the risk of cardiovascular complications. This might be caused by the inherent limitations of ECG. The left atrial abnormality criteria used has a sensitivity of 4–52% and specificity of 53–100%,[22] meanwhile Sokolow–Lyon voltage criteria for left ventricular hypertrophy has a sensitivity of 18–22% and specificity of 79–92%.[39] The Bazzett formula for correcting QT interval tends to overestimate the corrected result if the rate is high.[40] These inherent weakness, combined with the small sample size, might influence the ability of the study to achieve statistical significance. Nevertheless, the corrected QT interval was longer and left atrial abnormality and myocardial ischaemia was more prevalent in those who progressed to cardiovascular complication. HR, the only ECG parameter with significant univariate association, can be easily replaced with standard physical examination, hence further weakening the role of ECG in this context.

Haemoglobin level, the other significant variable in univariate analysis besides HR, is related to pre-eclampsia and/or eclampsia,[41-43] but there is no published literature currently available regarding the role of haemoglobin levels in cardiovascular complications. In their observational study, Patra et al.[43] reported on the incidence of heart failure in pregnant women with severe anaemia (haemoglobin <5 g/dL) to be as high as 18%. Low haemoglobin levels during pregnancy predisposes the patient to infection,[44] hypoxia,[45] oxidative stress,[46] endothelial dysfunction, and inflammatory response.[47] Furthermore, a decreased haemoglobin level accompanied by haemoconcentration can cause vasoconstriction and endothelial dysfunction, which later increased the vascular permeability.[41] Decreased haemoglobin and haemoconcentration were associated with the pathogenesis of pulmonary oedema,[33] hence the association between haemoglobin and cardiovascular complication.

LIMITATIONS AND FUTURE DIRECTIONS

This research was limited by a small sample size, the use of secondary retrospective data, not using a combination of ECG criteria to improve sensitivity and specificity, and manual interpretation of ECG, which allows for inter and intra-observer variability. Further research with a larger sample size, prospective research design, and use of more comprehensive parameters is needed. Further studies may allow for the development of a cheaper, non-invasive, and more widely available tool that can be used to predict cardiovascular complications in pre-eclampsia.

CONCLUSIONS

Even though HR and haemoglobin levels showed significant association with cardiovascular complication in univariate analysis, this study did not find ECG useful in predicting cardiovascular complication. The use of parameters, such as increased HR, prolonged corrected QT interval, and left atrial abnormality, for predictive purposes still requires further study.

References

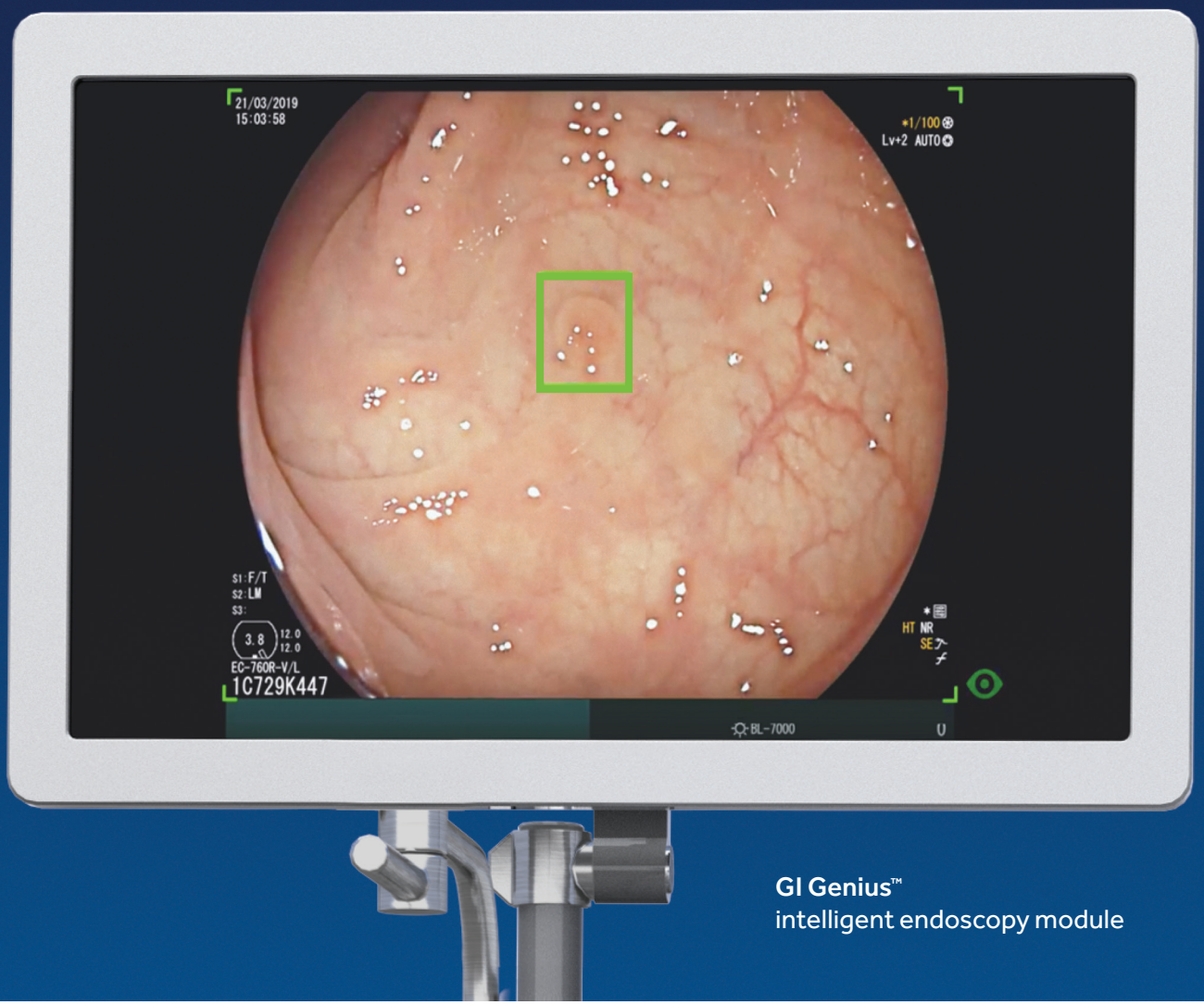
1. World Health Organization (WHO). The World Health Report 2005: Make every mother and child count. Available at: https://www.who.int/whr/2005/en/. Last accessed: 13 December 2018.
2. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol. 2009;33(3):130-7.
3. Ministry of National Development Planning/National Development Planning Agency (BAPPENAS). Report on the Achievement of Millennium Development Goals in Indonesia 2010. Available at:https://www.bappenas.go.id/files/1313/5229/9228/4-report-on-the-achievement-of-the-millennium-development-goals-in-indonesia-201020101 1181326210__20110812140730__0.pdf. Last accessed: 13 December 2018.
4. World Health Organization (WHO). The World Health Report 2005: Make every mother and child count. Available at: https://www.who.int/whr/2005/en/. Last accessed: 13 December 2018.
5. Angeli F et al. Hypertensive disorders during pregnancy: Clinical applicability of risk prediction models. J Hypertens. 2011;29(12):2320-3.
6. Khan KS et al. WHO analysis of causes of maternal death: A systematic review. Lancet. 2006;367(9516):1066-74.
7. Hogg B et al. Safety of labor epidural anesthesia for women with severe hypertensive disease. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Am J Obstet Gynecol. 1999;181(5 Pt 1):1096-101.
8. Gul A et al. Maternal and fetal outcomes in HELLP syndrome complicated with acute renal failure.

Ren Fail. 2004;26(5):557-62.

9. Gaugler-Senden IP et al. Maternal and perinatal outcomes of preeclampsia with an onset before 24 weeks gestation. Audit in a tertiary referral centre. Eur J Obstet Gynecol Reprod Biol. 2006;128(1-2):216-21.
10. Moodley J. Maternal deaths associated with hypertensive disorders of pregnancy: A population-based study. Hypertens Pregnan. 2004;23(3):247-56.
11. Cain MA et al. Pregnancy as a window to future health: Maternal placental syndromes and short-term cardiovascular outcomes. Am J Obstet Gynecol. 2016;215:484.e1-14.
12. von Dadelszen P et al.; PIERS Study Group. Prediction of adverse maternal outcomes in pre-eclampsia: Development and validation of the fullPIERS model. Lancet. 2011;377(9761):219-27.
13. Saleh L et al. Angiogenic markers predict pregnancy complications and prolongation in preeclampsia: Continuous versus cutoff values. Hypertension. 2017;70(5):1025-33.
14. Kim MJ et al. Echocardiographic assessment of structural and hemodynamic changes in hypertension-related pregnancy. J Cardiovasc Ultrasound. 2016;24(1):28-34.
15. Thornton CE et al. Acute pulmonary oedema as a complication of hypertension during pregnancy. Hypertens Pregnancy. 2011;30(2):169-79.
16. Solanki R, Nandita M. Echocardiographic assessment of cardiovascular hemodynamics in preeclampsia. J Obstet Gynecol India. 2011;61(5):519-22.
17. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gyneol. 2013;122(5):1122-31.
18. Magee LA et al.; Canadian Hypertensive Disorders of Pregnancy Working Group. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: Executive summary. J Obstet Gynaecol Can. 2014;36(5):416-41.
19. Baltazar RF, Basic and Bedside Electrocardiography 2009, Philadelphia: Lippincott Williams & Wilkins.
20. Sokolow M, Lyon TP. The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. Am Heart J. 1949;37(2):161-86.
21. Bazett H. An analysis of the time-relations of electrocardiograms. Heart. 1920;7:353-70.
22. Munuswamy K et al. Sensitivity and specificity of commonly used electrocardiographic criteria for left atrial enlargement determined by M-mode echocardiography. Am J Cardiol. 1984;53(6):829-32.
23. Thygesen K et al. Third universal definition of myocardial infarction. Circulation. 2012;126:2020-35.
24. DiFederico EM et al. Pulmonary oedema in obstetric patients is rapidly resolved except in the presence of infection or of nitroglycerin tocolysis after open fetal surgery. Am J Obstet Gynecol. 1998;179(4):925-33.
25. Bauer ST, Cleary KL. Cardiopulmonary complications of pre-eclampsia. Semin Perinatol. 2009;33(3):158-65.
26. Douglas KA, Redman CWG. Eclampsia in the United Kingdom. BMJ. 1994;309(6966):1395-400.
27. Tuffnell DJ et al.; Yorkshire Obstetric Critical Care Group. Outcomes in severe pre-eclampsia/eclampsia in Yorkshire 1999/2003. BJOG. 2005;112(7):875-80.
28. Dennis AT, Solnordal CB. Acute pulmonary oedema in pregnant women. Anaesthesia. 2012;67(6):646-59.
29. Benedetti TJ et al. Hemodynamic observations in severe preeclampsia complicated by pulmonary edema. Am J Obstet Gynecol. 1985;152(3):330-4.
30. Melchiorre K, Thilaganathan B. Maternal cardiac function in preeclampsia. Curr Opin Obstet Gynecol. 2011;23(6):440-7.
31. Angeli E et al. Additive value of standard ECG for the risk prediction of hypertensive disorders during pregnancy. Hypertens Res. 2011;34(6):707-13.
32. Raffaelli R et al. Pre-eclampsia: Evidence of altered ventricular repolarization by standard ECG parameters and QT dispersion. Hypertens Res. 2014;37(11):984-8.
33. Murray JF. Pulmonary edema: Pathophysiology and diagnosis. Int J Tuberc Lung Dis. 2011;15(2):155-60.
34. Shahul S et al. Subclinical left ventricular dysfunction in preeclamptic women with preserved left ventricular ejection fraction: A 2D speckle-tracking imaging study. Circ Cardiovasc Imaging. 2012;5(6):734-9.
35. Rafik Hamad R et al. Assessment of left ventricular structure and function in preeclampsia by echocardiography and cardiovascular biomarkers. J Hypertens. 2009;27(11):2257-64.
36. Hibbard JU et al. The arterial system in pre-eclampsia and chronic hypertension with superimposed pre-eclampsia. BJOG. 2005;112(7):897-903.
37. Tihtonen K et al. Maternal haemodynamics in pre-eclampsia compared with normal pregnancy during caesarean delivery. BJOG. 2006;113(6):657-63.
38. Carruth JE et al. The electrocardiogram in normal pregnancy. Am Heart J. 1981;102(6 Pt 1):1075-8.
39. Pewsner D et al. Accuracy of electrocardiography in diagnosis of left ventricular hypertrophy in arterial hypertension: Systematic review. BMJ. 2007;335(7622):711.
40. Vandenberk B et al. Which QT correction formulae to use for QT monitoring?. J Am Heart Assoc. 2016;5(6):e003264.
41. Khoigani MG et al. The relationship of hemoglobin and hematocrit in the first and second half of pregnancy with pregnancy outcome. Iran J Nurs Midwifery Res. 2012;17(2 Suppl 1):S165-70.
42. Karaşahin E et al. Maternal anemia and perinatal outcome. Perinatal Journal. 2007;15(3):127-30.
43. Patra S et al. Maternal and perinatal outcome in patients with severe anemia in pregnancy. Int J Gynaecol Obstet. 2005;91(2):164-5.
44. Ferguson SE et al. Preterm premature rupture of membranes. Nutritional and socioeconomic factors. Obstet Gynecol. 2002;100(6):1250-6.
45. Zhang Q et al. Maternal anaemia and preterm birth: A prospective cohort study. Int J Epidemiol. 2009;38(5):1380-9.
46. Yoo JH et al. Oxidative status in iron-deficiency anemia. J Clin Lab Anal. 2009;23(5):319-23.
47. Lu M, Lu JS. Maternal nutrition and infant mortality in the context of relationality. 2007. Available at: https://www.aucd.org/docs/resources/IM-MaternalNutrition.LU.pdf. Last accessed: 13 December 2018.

DETECT
THE UNDETECTED

Enhance your ability to detect colorectal cancer early - with Artificial Intelligence (AI).



GI Genius™
intelligent endoscopy module

The GI Genius™ intelligent endoscopy module - powered by AI - offers a transformative solution that:

- May offer a reliable and ever-vigilant second observer
- Works in real-time to automatically identify and mark abnormalities consistent with colorectal polyps, including those with flat (non-polypoid) morphology
- Seamlessly integrates with your existing colonoscopy equipment and workflow

Artificial Intelligence. Real Results.

Reference: 1. Corley DA, Jenson CD, Marks AR JR, et al. Adenoma Detection Rate and Risk of Colorectal Cancer and Death. NEJM 2014;370:1298-306.
IMPORTANT: Please refer to the package insert for complete instructions, contraindications, warnings and precautions.

Medtronic
Further, Together

Adverse Pregnancy Outcomes in Asthmatic Women According to Steps of Treatment: A Population-Based Study

Authors: *Bronislava Novotna,[1] Jiri Jarkovsky,[2,3] Klara Benesova[2,3]

1. Allergology Outpatient Department, Internal Gastroenterology Clinic University Hospital Brno, Masaryk University, Brno, Czech Republic
2. Institute of Biostatistic and Analysis, Faculty of Medicine, Masaryk University, Brno, Czech Republic
3. Institute of Health Information and Statistics of the Czech Republic, Prague, Czech Republic

*Correspondence to novotna.bronislava@fnbrno.cz

Disclosure: The authors have declared no conflicts of interest.

Recieved: 26.03.2019

Accepted: 27.06.2019

Keywords: Asthma, perinatal outcomes, pregnancy, step of treatment.

Citation: EMJ. 2019;4[4]:85-96.

Abstract

Bronchial asthma is the most common chronic disease in pregnancy associated with adverse pregnancy, obstetric, and perinatal outcomes. The aim of this study was to determine the influence of the steps of asthma treatment during pregnancy on adverse pregnancy, obstetric, and perinatal outcomes. The data of all women with singleton delivery in 2011–2017, including the diagnosis of asthma and its treatment for the same woman, were obtained from the National Registry of Reimbursed Health Services (NRRHS) of the Czech Republic. Relation of asthma and the steps of treatment to pregnancy, labour, and perinatal outcomes taken from the National Register of Reproduction Health (NRRH) for the period 2011–2015 were analysed using logistic regression and described by odds ratios, 95% confidence interval, and statistical significance. Of the total number of 752,000 women with singleton delivery, asthma and/or its treatment were found in 6.27% of deliveries. Data from 460,324 births, in which the combination of data sources was available, showed the association between asthma and pre-eclampsia, caesarean section, and birth weight ≤2,500 g, only for the fifth step of treatment ($p<0.001$). Caesarean section was more frequent in all evaluated groups of treatment compared with women without asthma ($p<0.001$). Gestational age of <37 weeks was found in children of mothers with asthma diagnosis and no treatment and for women at the fifth step of treatment ($p=0.003$). The incidence of birth defects and Apgar scores of <7 in 5 minutes were without statistical significance in all evaluated women. The authors concluded that pregnant women with asthma are at risk from adverse pregnancy, obstetric, and perinatal outcomes, especially upon the fifth stage of treatment.

INTRODUCTION

Asthma is one of the most common chronic diseases in pregnancy, with a reported prevalence of 3.7–12.0%.[1-7] Maternal asthma severity and control are often associated with pregnancy complications, adverse labour, and perinatal outcomes. A number of papers, meta-

analyses, and population-based studies have been published on this topic.[2,6] Hypertension, gestational diabetes mellitus, pre-eclampsia, vaginal bleeding, complicated delivery (increased birth rate by caesarean section), fetal growth restriction, low birth weight, premature birth (<37th week), neonatal hypoxia, and increased perinatal mortality have been observed more frequently in pregnant women with uncontrolled asthma.[1,3,6,8,9] Women with well controlled asthma have a much lower risk of these adverse complications.[8]

According to the Global Initiative for Asthma (GINA) guidelines, management of asthma in pregnancy is based on disease control and prevention of exacerbations that are achieved by pharmacological and non-pharmacological treatment. Pharmacological treatment uses three basic groups of drugs: relievers, controllers, and add-on therapy. In recent years, biological therapy has been recommended for patients with severe asthma.[10] The GINA guidelines use a stepwise approach to asthma treatment and recommend the use of short-acting β_2-agonists (SABA) as a relief treatment for all steps of asthma. Regular administration of low doses of inhaled corticosteroids (ICS) is recommended from the second step of treatment. In steps 3 and 4, moderate or high doses of ICS and long-acting β_2-agonists (LABA) are recommended. Steps 4 and 5 require add-on treatment with leukotriene receptor antagonists (LTRA), theophylline, and/or tiotropium bromide. The fifth step recommends the use of biological therapy. Adding low doses of oral corticosteroids is another option.[11] The disease severity is therefore determined by the steps of the treatment that are needed to control asthma and to prevent exacerbations.[2,5,6,10]

Additionally, pharmacological treatment of asthma in pregnancy is guided by categories of treatment safety, of which the U.S. Food and Drug Administration (FDA) pregnancy categories are the best known. The classification system uses five categories (A, B, C, D, and X) and is based on the degree of risk of teratogenic effects on the fetus.[11,12] In the case of asthma treatment, no drug group falls into the FDA category A; therefore, drugs are chosen from the FDA category B when the benefit outweighs the risk. The FDA category B includes budesonide, terbutaline, montelukast, ipratropium bromide, and omalizumab. The remaining asthma medications are included in the FDA category C: salbutamol, salmeterol, formoterol, vilanterol, and other ICS apart from budesonide (beclometasone dipropionate, fluticasone propionate, fluticasone furoate, mometasone furoate), theophylline, and systemic corticosteroids.[12]

The aim of this study was to show how the severity of the treatment, which is given by the number of medications needed to control asthma, affects complications during pregnancy and labour as well as perinatal outcomes. Unlike previous studies, which have worked with the severity of asthma defined by clinical symptoms, lung function, and the need to use relief medication, this study includes the severity of asthma determined by the steps of treatment used. Another aim of the study was to find out which drug groups were most commonly prescribed during pregnancy, and whether their selection respected the safety pregnancy categories.

METHODS

The analysis used data from national health registries of the Czech Republic. These registries contain data on the level of individual patients, with each patient being identified by a pseudonymous code that makes it possible to combine all records on a particular patient but not to identify the patient. The National Registry of Reimbursed Health Services (NRRHS) contains data on all healthcare paid by the public health insurance (almost 100% of healthcare in the Czech Republic) and the data is available for the period 2010–2017. It was adopted for the primary identification of deliveries using procedure codes for vaginal delivery, caesarean section, and other types of delivery, as well as for the identification of asthma and its treatment during pregnancy from its onset; the ICD10 code J45 was adopted for the identification of asthma and the Anatomical Therapeutic Chemical (ATC) classification code R03 (without CHOPN treatment codes) and codes H02AB04, H02AB07, and H02AB09 (in combination with the diagnosis) for the identification of its treatment. A combination of drugs according to asthma treatment guidelines was adopted for the identification of asthma treatment steps. The information on asthma in pregnant women from NRRHS was combined with detailed information

on singleton deliveries from the National Register of Reproduction Health (NRRH); the connections on patient level between NRRHS and NRRH are available for roughly 90–95% of deliveries each year (NRRHS and NRRH use different pseudonymous code and the links between them are missing in approximately 5–10% of patients). NRRH data are available until the year 2015 and the analysis was computed for the period 2011–2015; the year 2010 is not included due to the non-availability of treatment data in 2009, when some pregnancies started.

Standard descriptive statistics were applied in the analysis; absolute and relative frequencies were used for categorical variables. The relation of asthma to perinatal outcomes was analysed using logistic regression and described by odds ratio (OR), 95% confidence interval (CI), and statistical significance. The analysis was carried out using the SPSS 25.0.0.1 (IBM Corporation, 2018).

Using data from the national health registers of the Czech Republic, the authors evaluated 752,000 deliveries of women with singleton births in the period 2011–2017 and divided these deliveries into groups based on the presence of asthma and its treatment (Table 1). Variables on maternal age, educational level, marital status and parity, smoking, and gestational diabetes mellitus were retrieved from the NRRH (Table 2). Based on reported ACT codes, drug groups for asthma that had been prescribed to women during their pregnancy, and delivery in 2011–2017 were identified: relievers, controllers, and add-on therapy (Table 1).

Table 1: Asthma diagnosis and/or its treatment reported during pregnancy.

	2011[2]	2012	2013	2014	2015	2016	2017	Total
n (deliveries)	106,090	105,878	103,928	106,669	107,667	110,376	111,392	752,000
Without asthma during pregnancy	93.8%	93.9%	93.8%	93.6%	93.5%	93.7%	93.8%	93.73%
Asthma diagnosis without treatment	2.3%	2.2%	2.1%	2.2%	2.1%	2.0%	2.0%	2.13%
Treatment without asthma diagnosis	1.3%	1.3%	1.4%	1.5%	1.6%	1.5%	1.5%	1.44%
Asthma diagnosis with treatment, step of treatment	2.6%	2.5%	2.6%	2.8%	2.8%	2.8%	2.7%	2.69%
1	0.4%	0.5%	0.5%	0.5%	0.6%	0.6%	0.6%	0.53%
2	0.8%	0.7%	0.7%	0.8%	0.7%	0.7%	0.6%	0.71%
3	0.9%	0.9%	0.9%	1.0%	1.0%	1.0%	1.0%	0.96%
4	0.3%	0.2%	0.3%	0.3%	0.3%	0.3%	0.3%	0.29%
5	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.20%
ATC code[1] n (deliveries with asthma or its treatment)								
Reliever medication								
R03AC02: salbutamol	29.02%	29.53%	31.90%	33.58%	35.97%	37.09%	37.12%	33.46%
R03BB01: ipratropium bromide	3.20% 6,586	3.55% 6,448	4.23% 6,473	4.76% 6,802	5.09% 6,994	4.55% 6,987	5.28% 6,878	4.38% 47,148
R03AL01: fenoterol and ipratropium bromide	0.00%	0.00%	0.00%	2.28%	4.85%	4.61%	4.77%	2.36%
R03CC02: salbutamol	0.96%	1.15%	0.90%	0.97%	1.02%	0.66%	0.54%	0.89%
R03AC04: fenoterol	1.15%	1.23%	1.39%	1.06%	0.64%	0.55%	0.04%	0.87%

Table 1 continued.

	2011[2]	2012	2013	2014	2015	2016	2017	Total
R03CC03: terbutaline	0.20%	0.08%	0.05%	0.03%	0.00%	0.01%	0.01%	0.05%
R03AC03: terbutaline	2.29%	2.25%	1.98%	0.49%	0.00%	0.00%	0.00%	1.00%
Reliever medication in total	**35.55%**	**36.76%**	**39.15%**	**41.83%**	**45.74%**	**45.74%**	**46.35%**	**41.59%**
ICS+LABA								
R03AK07: formoterol and budesonide	16.79%	16.73%	18.21%	17.36%	16.93%	17.60%	16.92%	17.22%
R03AK06: salmeterol and fluticasone	6.47%	6.79%	6.81%	5.97%	5.68%	5.50%	5.12%	6.05%
R03AK08: formoterol and beclometasone	0.00%	0.00%	0.00%	0.96%	2.27%	2.76%	2.88%	1.27%
R03AK11: formoterol and fluticasone	0.00%	0.00%	0.00%	0.46%	1.47%	1.46%	2.11%	0.79%
R03AK10: vilanterol and fluticasone furoate	0.00%	0.00%	0.00%	0.00%	0.14%	0.72%	1.12%	0.28%
ICS+LABA in total	**22.81%**	**23.11%**	**24.55%**	**23.86%**	**25.41%**	**26.80%**	**27.12%**	**24.81%**
ICS								
R03BA02: budesonide	17.55%	15.91%	16.67%	17.02%	15.50%	14.83%	14.07%	15.94%
R03BA01: beclometasone	2.93%	2.96%	2.75%	3.06%	2.87%	2.60%	2.41%	2.80%
R03BA08: ciclesonide	1.91%	1.99%	2.02%	1.98%	2.27%	2.05%	1.92%	2.02%
R03BA05: fluticasone	0.65%	0.56%	0.48%	0.66%	0.53%	0.44%	0.55%	0.55%
R03BA07: mometasone	0.00%	0.08%	0.54%	0.72%	0.50%	0.30%	0.25%	0.34%
ICS in total	**22.37%**	**20.86%**	**21.69%**	**22.55%**	**20.99%**	**19.81%**	**18.84%**	**21.02%**
Add-on medication								
R03DC03: montelukast	2.92%	3.33%	3.55%	3.23%	3.57%	3.82%	3.55%	3.42%
R03DA04: theophylline	1.94%	1.67%	1.85%	1.53%	1.69%	1.33%	1.31%	1.62%
R03DA05: aminophylline	0.76%	1.01%	0.91%	0.71%	0.93%	0.70%	0.54%	0.79%
R03BC03: nedocromil	0.38%	0.20%	0.36%	0.28%	0.11%	0.37%	0.16%	0.27%
R03BB04: tiotropium bromide	0.05%	0.03%	0.05%	0.00%	0.07%	0.16%	0.15%	0.07%
R03DX05: omalizumab	0.00%	0.02%	0.00%	0.04%	0.03%	0.03%	0.03%	0.02%
Add-on medication in total	**5.68%**	**5.94%**	**6.43%**	**5.42%**	**6.09%**	**6.03%**	**5.45%**	**5.86%**
Corticosteroids- tablet, i.v.								
H02AB07: prednisone	2.93%	3.21%	3.26%	2.98%	3.29%	3.33%	2.92%	3.13%
H02AB04: methylprednisolone	1.25%	1.60%	1.62%	1.35%	1.82%	1.61%	1.60%	1.55%
H02AB09: hydrocortisone	0.38%	0.54%	0.62%	0.29%	0.46%	0.44%	0.36%	0.44%
Corticosteroids in total	**4.36%**	**4.93%**	**5.16%**	**4.45%**	**5.25%**	**4.95%**	4.67%	4.82%

Table 1 continued.

	2011[2]	2012	2013	2014	2015	2016	2017	Total
LABA								
R03AC13: formoterol	1.97%	2.11%	1.98%	1.54%	1.29%	1.21%	1.13%	1.60%
R03AC12: salmeterol	0.03%	0.05%	0.06%	0.01%	0.00%	0.03%	0.01%	0.03%
LABA in total	**2.00%**	**2.16%**	**2.04%**	**1.56%**	**1.29%**	**1.23%**	**1.14%**	**1.63%**
*R03DC01: zafirlukast	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

[1] Patients with asthma diagnosis or its treatment reported during pregnancy in National Registry of Reimbursed Health Services (NRRHS).

[2] Year 2010 not shown (some pregnancies started in 2009 and data of 2009 are not available).

*Drug is not available on the Czech market.

ICS: inhalded corticosteroids; iv: intravenous; LABA: long-acting β_2-agonists.

Statistical data on the perinatal outcomes of singleton deliveries were only available in the combined data of NRRH and NRRHS from the period 2011–2015. This group of 460,324 deliveries was divided into 4 subgroups: 1) deliveries by women without asthma (n= 431,116); 2) deliveries by women without asthma diagnosis and with asthma treatment (n=6,592); 3) deliveries by women with asthma diagnosis and with asthma treatment (n=12,502); and 4) women with an asthma diagnosis and without treatment (n=10,114) (Table 2). Statistical data on complications in pregnancy (pre-eclampsia or eclampsia), in the course of delivery (caesarean section) and the adverse perinatal outcomes (Apgar score in 5 minutes <7, birth weight <2,500 g, gestational age <37 weeks, and congenital malformations) were found for groups 1–3 of deliveries. The fourth group of deliveries, i.e., those by women with asthma diagnosis and without treatment, is not included in Table 3.

RESULTS

Two data sets were created from records on registered singleton deliveries. In the first data set, from the period 2011–2017, a total of 752,000 deliveries were evaluated. Based on the diagnosis and treatment, these deliveries were divided into three subgroups: 1) deliveries by women with diagnosed asthma and with asthma treatment (2.69% on average); 2) deliveries by women with diagnosed asthma and without treatment (2.13% on average); and 3) deliveries by women without the diagnosis of asthma and with asthma treatment (1.44% on average). On average, asthma diagnosis and/or its treatment were found in 6.27% of deliveries. Of the total number of deliveries evaluated, the group of women without the diagnosis of asthma and without asthma treatment accounted for 93.73% on average (Table 1).

The severity of asthma in pregnancy was determined by the number of drug groups (i.e., the step of treatment) used to control asthma and to prevent exacerbation. In this group of deliveries, most women with asthma were treated with the third step of treatment (0.96%), followed by women treated with the second step (0.71%), the fourth step (0.29%), and the fifth step (0.20%) of treatment.

The frequency prescription of individual drug groups for the treatment of asthma in deliveries in the period 2011–2017 are shown in Table 1. Reliever medications were the most commonly prescribed drugs (41.59%), with salbutamol as the most frequent (33.46%). ICS/LABA combination products were the second most commonly prescribed drugs (24.81%), mostly budesonide/formoterol (17.22%). ICS monotherapy was third (21.20%), with budesonide as the most

commonly prescribed drug from this group (15.94%). Montelukast (3.42%) was the most commonly prescribed drug from the add-on therapy group (3.42%).

When monitoring trends in prescribing asthma medications, an increase in salbutamol prescription was observed from 29.02% in 2011 to 37.12% in 2017. As for combination products, a slight increase in prescriptions was observed (from 22.81% in 2011 to 27.12% in 2017) at the expense of ICS monotherapy (from 22.37% in 2011 to 18.84% in 2017). The LTRA prescription increased from 2.92% in 2011 to 3.55% in 2017. An increase was also observed for tiotropium from 0.05% in 2011 to 0.15% in 2017. Prescription of systemic corticosteroids remained at approximately the same level for the entire 7-year period (from 4.36% in 2011 to 4.67% in 2017). Omalizumab was rarely prescribed (0.02% on average) and mepolizumab was not available on the Czech market until 2017.

The second data set available to evaluate the perinatal outcomes, from the years 2011–2015, involved 460,324 deliveries. The diagnosis of asthma without treatment was found in 10,114 (2.2%) deliveries. Asthma treatment without a previous diagnosis was found in 6,592 (1.4%) deliveries. Asthma diagnosis and its treatment according to the disease severity was found in 12,502 (2.7%) deliveries. Altogether, these groups involved 29,208 (6.4%) deliveries. Neither asthma nor its treatment were reported in 431,116 (93.7%) deliveries. Age (30–34 years) was almost the same for all of the aforementioned groups (Table 2). The proportion of smokers was lower in women with asthma diagnosis and treatment (5.4%) as compared to women with asthma treatment but without a previous diagnosis (6.4%) and also to women who did not suffer from asthma (6.8%). Education of only elementary level was more frequently seen in patients without asthma (9.0%). Marital status and parity were comparable for all groups. The incidence of gestational diabetes mellitus was higher in women with asthma treatment (5.0%), both with and without diagnosis, compared to women without asthma treatment (4.2%) (Table 2). Association between asthma in pregnancy and complications in pregnancy (pre-eclampsia/eclampsia) and adverse perinatal outcomes (birth weight <2,500 g) was statistically significant only for the fifth step of asthma treatment (OR: 4.908; 95% CI: 3.816–6.313; p<0.001, and OR: 1.513; 95% CI: 1.199–1.908; p<0.001, respectively). Gestational age <37 weeks was statistically more significant in women with asthma who were not treated (OR: 1.124; 95% CI: 1.041–1.213; p=0.003) and for women in the fifth step of asthma treatment (OR: 1.410; 95% CI: 1.122–1.772; p=0.003).

Table 2: Characteristics of analysed population according to bronchial asthma.

N=460,324[1]	**Without asthma** n=431,116	**Treatment without asthma diagnosis** n=6,592	**Asthma diagnosis with treatment** n=22,616
Age			
≤19	8,001 (1.9%)	144 (2.2%)	387 (1.7%)
20–24	48,397 (11.2%)	658 (10.0%)	2,361 (10.4%)
25–29	121,588 (28.2%)	1,660 (25.2%)	6,328 (28.0%)
30–34	158,885 (36.9%)	2,478 (37.6%)	8,169 (36.1%)
≥35	94,245 (21.9%)	1,652 (25.1%)	5,371 (23.7%)
Smoking	29,354 (6.8%)	419 (6.4%)	1,214 (5.4%)

Table 2 continued.

N=460,324[1]	Without asthma n=431,116	Treatment without asthma diagnosis n=6,592	Asthma diagnosis with treatment n=22,616
Education level			
Missing	36,044 (8.4%)	557 (8.4%)	1,933 (8.5%)
Elementary	38,909 (9.0%)	532 (8.1%)	1,675 (7.4%)
High school	243,320 (56.4%)	3,714 (56.3%)	13,109 (58.0%)
University	112,843 (26.2%)	1,789 (27.1%)	5,899 (26.1%)
Cohabitation/marital status			
Missing	8,644 (2.0%)	116 (1.8%)	441 (1.9%)
Unmarried	167,147 (38.8%)	2,407 (36.5%)	8,194 (36.2%)
Married	231,106 (53.6%)	3,638 (55.2%)	12,615 (55.8%)
Divorced	23,411 (5.4%)	415 (6.3%)	1,311 (5.8%)
Widow	808 (0.2%)	16 (0.2%)	55 (0.2%)
Diabetes	17,992 (4.2%)	331 (5.0%)	1,138 (5.0%)
Parity			
0	201,567 (46.8%)	3,007 (45.6%)	10,926 (48.3%)
1	159,412 (37.0%)	2,446 (37.1%)	8,224 (36.4%)
2–3	62,984 (14.6%)	1,018 (15.4%)	3,201 (14.2%)
≥4	7,153 (1.7%)	121 (1.8%)	265 (1.2%)

[1] Analysis computed for the singleton deliveries in period 2011–2015; year 2010 is not included due to non-availability of treatment data in 2009 when some pregnancies started; combined data of National Registry of Reimbursed Health Services (NRRHS) and National Register of Reproduction Health (NRRH) are available until 2015 only, connection between NRRHS and NRRH available for 90–95% of deliveries each year.

Table 3: Asthma occurrence and treatment as predictors of pregnancy, labour complications, and perinatal outcomes.

N=460,324	n	Endpoint (%)	OR (95% CI:)	p
Preeclampsia or eclampsia				
Without asthma during pregnancy	431,116	1.6%	reference	
Asthma without treatment	10,114	1.6%	1.031 (0.881–1.205)	0.706
Treatment without asthma diagnosis	6,592	1.6%	1.048 (0.865–1.269)	0.632
Asthma diagnosis with treatment step of treatment				

Table 3 continued.

N=460,324	n	Endpoint (%)	OR (95% CI:)	p
1	2,365	1.5%	0.945 (0.676–1.321)	0.741
2	3,514	1.7%	1.074 (0.830–1.391)	0.586
3	4,421	1.5%	0.983 (0.773–1.250)	0.888
4	1,290	1.3%	0.840 (0.520–1.357)	0.476
5	912	7.2%	4.908 (3.816–6.313)	<0.001
Cesarean section				
Without asthma during pregnancy	431,116	23.4%	reference	
Asthma without treatment	10,114	26.8%	1.194 (1.142–1.249)	<0.001
Treatment without asthma diagnosis	6,592	27.0%	1.210 (1.146–1.279)	<0.001
Asthma diagnosis with treatment step of treatment				
1	2,365	28.3%	1.290 (1.179–1.411)	<0.001
2	3,514	28.1%	1.281 (1.190–1.379)	<0.001
3	4,421	29.0%	1.334 (1.250–1.424)	<0.001
4	1,290	33.7%	1.664 (1.482–1.867)	<0.001
5	912	38.7%	2.065 (1.807–2.360)	<0.001
Apgar at 5 minutes <7				
Without asthma during pregnancy	431,116	1.2%	reference	
Asthma without treatment	10,114	1.2%	1.026 (0.856–1.229)	0.784
Treatment without asthma diagnosis	6,592	1.3%	1.123 (0.908–1.391)	0.285
Asthma diagnosis with treatment step of treatment				
1	2,365	1.1%	0.970 (0.663–1.419)	0.875
2	3,514	1.2%	1.016 (0.749–1.379)	0.918
3	4,421	1.1%	0.941 (0.709–1.249)	0.676
4	1,290	1.2%	0.988 (0.594–1.645)	0.964
5	912	1.1%	0.931 (0.499–1.738)	0.823
Delivery weight <2,500 g				
Without asthma during pregnancy	431,116	5.8%	reference	
Asthma without treatment	10,114	6.2%	1.067 (0.983–1.158)	0.120
Treatment without asthma diagnosis	6,592	5.9%	1.014 (0.915–1.124)	0.789
Asthma diagnosis with treatment step of treatment				
1	2,365	4.8%	0.811 (0.671–0.981)	0.031
2	3,514	5.7%	0.986 (0.855–1.138)	0.850
3	4,421	5.9%	1.006 (0.887–1.142)	0.921

Table 3 continued.

N=460,324	n	Endpoint (%)	OR (95% CI:)	p
4	1,290	5.4%	0.928 (0.729–1.181)	0.543
5	912	8.6%	1.513 (1.199–1.908)	<0.001
Gestational age <37 weeks				
Without asthma during pregnancy	431,116	6.5%	reference	
Asthma without treatment	10,114	7.2%	1.124 (1.041–1.213)	0.003
Treatment without asthma diagnosis	6,592	6.8%	1.047 (0.951–1.154)	0.350
Asthma diagnosis with treatment step of treatment				
1	2,365	5.6%	0.855 (0.717–1.020)	0.081
2	3,514	6.5%	1.009 (0.882–1.154)	0.902
3	4,421	5.9%	0.915 (0.807–1.037)	0.165
4	1,290	6.7%	1.046 (0.841–1.301)	0.684
5	912	8.9%	1.410 (1.122–1.772)	0.003
Birth defects				
Without asthma during pregnancy	431,116	3.8%	reference	
Asthma without treatment	10,114	4.1%	1.095 (0.992–1.210)	0.073
Treatment without asthma diagnosis	6,592	3.6%	0.961 (0.843–1.094)	0.546
Asthma diagnosis with treatment step of treatment				
1	2,365	4.0%	1.063 (0.864–1.307)	0.565
2	3,514	3.8%	1.002 (0.842–1.193)	0.981
3	4,421	4.0%	1.057 (0.908–1.230)	0.475
4	1,290	4.3%	1.143 (0.872–1.498)	0.334
5	912	3.8%	1.026 (0.731–1.438)	0.884

[1] Analysis computed for the period 2011–2015; year 2010 is not included due to non-availability of treatment data in 2009 when some pregnancies started; combined data of National Registry of Reimbursed Health Services (NRRHS) and National Register of Reproduction Health (NRRH) are available until 2015 only, connection between NRRHS and NRRH available for 90–95% of deliveries each year.

CI: confidence interval; OR: odds ratio.

Delivery complications (caesarean section) were statistically significantly more frequent in all groups of interest (i.e., in women with asthma diagnosis and treatment regardless of treatment steps, in women with asthma diagnosis who were not treated and in women without asthma diagnosis but with asthma treatment) when compared to women without asthma ($p<0.001$ in all cases; OR from 1.194 in asthma without treatment to 2.065 in the fifth step of treatment). The incidence of congenital malformations and Apgar score at 5 minutes <7 had no statistical significance for all of the above-mentioned groups of interest and did not differ between women with and without asthma and its treatment (Table 3).

DISCUSSION

This study evaluated two groups of deliveries. In the analysis of the first group, asthma diagnosis without asthma treatment was found in 2.13% deliveries, whereas asthma treatment without asthma diagnosis was reported in 1.44% of deliveries.

In a Finnish population-based study from 2018, Kemppainen et al.[13] reported that the diagnosis of asthma without asthma medication was found in 0.8 % of women. In a Swedish population-based study, Rejnö et al.[1] reported that 0.4 % of women were taking asthma medication without a diagnosis of asthma.

This present study also evaluated the steps of asthma treatment based on the findings of drug groups that were prescribed during pregnancy. The third step of treatment was most frequently used in the study's group of interest.

In a study from 2013, Charlton et al.[14] used longitudinal electronic medical records, which were associated with prescribing data, and assessed the degree of asthma treatment during pregnancy based on the prescription of asthma medications. Data were obtained from the General Practice Research Database (GPRD) in the UK. Most women were treated with the first step of treatment.[14]

In a Dutch population-based study, Zetstra-van der Woude et al.[15] reported that 8.1% of women took asthma medication with at least one drug. Of this group, 33.9% had prescription for SABA only.[15] According to Lim et al.,[16] almost one-third of pregnant women discontinue or reduce their asthma preventing drugs during pregnancy and overcompensate with SABA. In 2016, Carlton et al.[17] published a study to determine the prescription of asthma medications during pregnancy, which used data from seven electronic databases from Denmark, Norway, the Netherlands, Italy (Tuscany and Emilia-Romagna), Wales, and from a database of general practitioners from the rest of the UK.[17] SABA was prescribed to 90% of women in the UK, 75% of women in Denmark and Norway, and 26% of women in Italy. ICS during pregnancy was prescribed to 50% of women in Norway, 60% of women in the UK and Denmark, and 89% of women in Emilia-Romagna. In the UK (including Wales) and Italian databases, beclometasone was the most commonly prescribed ICS, whereas budesonide was most commonly prescribed in Denmark, and beclometasone and budesonide were equally prescribed in Norway. Norway was the only region where the prevalence of ICS prescription in a fixed-dose combination with a LABA was higher than the prescription of ICS products in monotherapy. Norway and Italy were the only countries, however, where the guidelines recommended budesonide as the ICS of choice, and Denmark was the only region where budesonide was found to be the most commonly prescribed ICS. In Italy, despite the guidelines recommending budesonide, beclometasone was by far the most commonly prescribed ICS. During pregnancy, evidence of a reduction in the prescription of LABA, both alone and as part of a fixed-dose combination with ICS, was observed in Norway, the Netherlands, and Italy. The percentage of LTRA prescription was low, from 0.04% in Norway to 0.41% in Wales. No prescription of anticholinergic drugs was reported in Denmark, whereas in the Netherlands, these were prescribed to 0.16% of women. Prescription of oral corticosteroids was highest in the UK and Italy. Prednisolone was the most commonly prescribed oral corticosteroid in all regions. During the 7-year study period, in Denmark, Norway, and the UK, the prevalence of prescripted LABA in fixed combination with ICS increased while pregnancy prescriped LABA decreased.[17]

This study respects the categories of drug safety in pregnancy, because ICS budesonide (FDA-B), which was used in ICS monotherapy and also in fixed combination with ICS (ICS/LABA), was still the most commonly prescribed drug. From the add-on therapy, montelukast (FDA-B) was the most commonly chosen drug, and an increasing trend in its use has been observed (Table 1). Several prescription trends were observed in the 7-year period. A decrease in the prescription of ICS monotherapy was reported, in favour of the use of fixed combinations (ICS and LABA), with a predominance of budenoside; moreover, an increase in the prescription of LTRA (FDA-B) was observed, as well as an increase in SABA prescription. The prescription of systemic corticosteroids (most commonly methylprednisolone) has not changed.

In the 7-year study period of prescribing habits in Europe, a similar increasing trend in the prescription of fixed combinations of ICS and LABA was found in Denmark, Norway, and the UK.[17]

In the second group, the incidence of pregnancy complications, childbirth, and adverse perinatal outcomes in relation to asthma treatment was assessed. In this group of deliveries by women with asthma, there was a statistically significant ($p<0.001$) incidence of some adverse outcomes (pre-eclampsia/eclampsia, caesarean section, birth weight <2,500 g, gestational age of <37 weeks) only in women treated with the fifth step of asthma treatment, i.e., those with severe asthma. The above-mentioned results are consistent with a number of studies, meta-analyses, population-based studies, and consensus studies.[1,2,6,8,9,12] In a Finnish study from 2018, it was shown that the number of used asthma medications increased the risk of low birth weight and fetal growth restriction. Each medication group increased the risk of low birth weight by 6% (OR: 1.06; 95% CI: 1.00–1.13; $p=0.0365$) and fetal growth restriction (SGA) by 13% (OR: 1.13; 95% CI: 1.07–1.19; $p<0.0001$).[13] The risk of perinatal mortality or preterm birth, however, was not increased in the logistic manner of evaluation.[13] A Swedish study showed that maternal asthma was associated with an increased risk of almost all pregnancy complications. There were also increased odds for adverse birth outcomes, including a low birth weight and a low gestational age.[1] In this study of all groups of deliveries (women with and without asthma diagnosis, with and without asthma treatment), there was no evidence of a higher incidence of congenital malformations, even for women with severe asthma, as compared to women without asthma. Systematic reviews and meta-analyses of >56,000 pregnancies with asthma and >1 milion pregnancies without asthma, from 14 studies, found a significant but very small increase of congenital malformations among women with asthma as compared with women without asthma (risk ratio: 1.30; 95% CI: 1.02–1.21).[9] However, the European case-malformed control study (EUROmediCAT) of 13 registers showed that cleft palate (OR: 1.63; 95% CI: 1.05–2.52) and gastroschisis (OR: 1.89; 95% CI: 1.12–3.20) were significantly more frequent in women who were treated in the first trimester by β_2-agonists.[7] An exploratory analysis found an association between renal dysplasia and exposure to the combination of ICS/LABA (OR: 3.95; 95% CI: 1.99–7.85). Administration of ICS during the first trimester of pregnancy appeared to be safe in relation to the risk of a range of specific major congenital anomalies.[7] In this present study group, deliveries led by caesarean section were significantly more frequent ($p<0.001$) in all groups of deliveries, as compared to women without asthma (Table 3).

A number of multicentre studies and meta-analyses have shown that women with asthma have a higher risk of caesarean delivery and even twice the risk of elective caesarean section (risk ratio: 2.14; 95% CI: 1.16–3.95) than women without asthma.[9]

A Swedish population-based study linked a higher incidence of caesarean delivery in women with asthma with the possibility that women with chronic conditions generally undergo this surgical procedure for medical reasons because doctors assume that vaginal delivery is associated with more complications.[1] Prof Michael Schatz, one of the leaders of international recommendations for the management of asthma during pregnancy, indicates that adverse perinatal outcomes in women with asthma might be explained by general pathogenetic factors that predispose the patients to bronchial, vascular, and uterine muscle hyperresponsiveness, as well as circulating mediators that cause abnormalities of smooth muscles and of the autonomic nervous system.[12]

CONCLUSION

Asthma during pregnancy is associated with increased risks of pregnancy, delivery, and perinatal complications. Severe asthma, defined by the number of drugs that a pregnant patient with asthma needs to control the disease and to prevent exacerbations, was more often associated with adverse outcomes. The prescription profile of drugs used in asthma during pregnancy should be consistent with international recommendations in which the choice of drugs is guided by safety categories. Appropriate asthma care during the preconception period, correct determination of treatment steps during pregnancy, as well as a proactive approach to treatment will help reduce potential adverse effects on pregnancy, delivery, and perinatal outcomes.

References

1. Rejnö G et al. Asthma during pregnancy in a population-based study - Pregnancy complications and adverse perinatal outcomes. PLoS ONE. 2014;9(8):e104755.
2. Murphy VE. Managing asthma in pregnancy. Breathe. 2015;11:258-67.
3. Rejnö G et al. Adverse pregnancy outcomes in asthmatic women: A population-based family design study. J Allergy Clin Immunol Pract. 2018;6(3):916-22.
4. Yland JJ et al. Fetal outcomes among women with asthma during pregnancy. Evidence from a large healthcare database in the United States. J Allergy Clin Immunol. Abstract No L5, AAAAI Annual Meeting, San Francisco, 22–25 February, 2019.
5. Goldie MH, Brightling CHE. Asthma in pregnancy. The Obstetrician & Gynaecologist. 2013;15:241-5.
6. Murphy VE, Schatz M. Asthma in pregnancy: A hit for two. Eur Resp Rev. 2014;23(131):64-8.
7. Garne E et al. Use of asthma medication during pregnancy and risk of specific congenital anomalies: A European case-malformed control study. J Allergy Clin Immunol. 2015;136(6):1496-502.
8. British Thoracic Society (BTS), Scottish Intercollegiate Guidelines Network (SIGN). British guideline on the management of asthma. 2016. Available at: https://www.brit-thoracic.org.uk/document-library/clinical-information/asthma/btssign-asthma-guideline-2016/. Last accessed: 12 August 2019.
9. Vanders RL, Murphy VE. Maternal complications and the management of asthma in pregnancy. Womens Health (Lond). 2015;11(2):183-91.
10. Global Initiative for Asthma (GINA). Pocket guide for asthma management and prevention. 2018. Available at: https://ginasthma.org/wp-content/uploads/2018/03/wms-GINA-main-pocket-guide_2018-v1.0.pdf. Last accessed: 12 August 2019.
11. Incaudo GA, Takach P. "The Diagnosis and Treatment of Allergic Rhinitis During Pregnancy and Lactation," Schatz M et al. (eds.), Asthma and Rhinitis During Pregnancy (2006), Philadelphia: Elsevier Sauders, pp.137-54.
12. Schatz M et al. "Asthma and Allergic Diseases during Pregnancy." Adkinson NF et al. (eds.), Middleton´s Allergy Principles and Practice (2014), 4th Edition, Philadelphia: Elsevier Saunders, pp.951-69.
13. Kemppainen M et al. Maternal asthma is associated with increased risk of perinatal mortality. PLoS ONE. 2018;13(5):e0197593.
14. Charlton RA et al. Asthma Management in Pregnancy. PLoS One. 2013;8(4):e60247.
15. Zetstra-van der Woude PA et al. A population analysis of prescriptions for asthma medications during pregnancy. J Allergy Clin Immunol. 2013;131(3):711-7.
16. Lim A et al. Asthma drugs in pregnancy and lactation. Aust Prescr. 2013;36:150-3.
17. Charlton RA et al. Asthma medication prescribing before, during and after pregnancy: A study in seven European regions. BMJ Open 2016;6:e009237.

The Role of the Gut Microbiome and its Derived Mediators in Nonalcoholic Fatty Liver Disease

Authors: *Cosmas Rinaldi A. Lesmana,[1,2] Meudia Syahidah,[2] Irsan Hasan,[2] Rino A. Gani[2]

1. Digestive Disease & GI Oncology Center, Medistra Hospital, Jakarta, Indonesia
2. Department of Internal Medicine, Hepatobiliary Division, Dr. Cipto Mangunkusumo National General Hospital, Universitas Indonesia, Jakarta, Indonesia

*Correspondence to medicaldr2001id@yahoo.com

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: Prof Laurentius A. Lesmana, Medistra Hospital, Jakarta, Indonesia, who gave the first insights on non-alcoholic fatty liver disease in Indonesia many years ago.

Received: 10.06.19

Accepted: 14.11.19

Keywords: Nonalcoholic fatty liver disease (NAFLD), gut microbiota, short-chain fatty acid (SCFA).

Citation: EMJ. 2019;4[4]:97-103.

Abstract

Nonalcoholic fatty liver disease (NAFLD) has become an emerging disease throughout the world. Metabolic comorbidities such as obesity (especially central obesity), diabetes, and dyslipidaemia have been established as risk factors not only for NAFLD development, but also for the disease progression. Dietary or genetic obesity has been hypothesised to induce alteration of gut microbiota, thereby causing the promotion of deoxycholic acid production in the intestinal tract. Elevated levels of deoxycholic acid can provoke senescence-associated secretory phenotype in hepatic stellate cells through enterohepatic circulation, which in turn leads to the secretion of various inflammatory and tumour-promoting factors in the liver and may further result in obesity-induced hepatocellular carcinoma. Short-chain fatty acids are mainly produced through the fermentation of indigestible carbohydrates by gut microbiota. Gut microbiota have been considered to play a role in NAFLD and its disease progression. The main end products resulting from the indigestible carbohydrate catabolism of intestinal microbes are short-chain fatty acids, constituting acetate, propionate, and butyrate. High concentrations of propionate can promote development of NAFLD, whereas acetate and butyrate can prevent the development of the disease.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is commonly associated with metabolic comorbidities such as obesity, diabetes, and dyslipidaemia. For diagnosis of NAFLD, the patient must have evidence of hepatic steatosis, either from imaging or histology, and lack of significant alcohol consumption (<20 g/day) or any other agents that contribute to liver fat accumulation. NAFLD is now considered as the third most common cause of hepatocellular carcinoma (HCC) in the USA.[1]

Gut dysbiosis is an imbalance condition of gut bacteria which has been hypothesised to have an important role in NAFLD. Short-chain fatty acids (SCFA) are mainly produced by the fermentation of indigestible carbohydrates (CHO) by gut microbiota.[2] The exact role of SCFA in the pathogenesis of NAFLD is still unclear. In this review, the nature of gut microbiota, the role of SCFA, and their implications on NAFLD are discussed.

NATURAL HISTORY, PREVALENCE, AND RISK FACTORS OF NONALCOHOLIC FATTY LIVER DISEASE

NAFLD is a wide spectrum of diseases developed from simple steatosis, potentially progressing to steatohepatitis and then to liver cirrhosis or HCC development. Based on its histopathology, NAFLD can be differentiated into two phenotypes: the usually stable condition, nonalcoholic fatty liver (NAFL), without hepatocellular injury evidence; or nonalcoholic steatohepatitis (NASH) with evidence of hepatocyte injury. Most patients with NAFLD will remain in a stable condition. If the liver histopathology only shows isolated steatosis or simple steatosis, patients will usually have a benign prognosis; however, some of the NAFLD population will progress to NASH. Prevalence of NASH ranges between 6% and 13%, and >50% attain condition stability or go into regression. Approximately 9–20% of the NASH population will progress to cryptogenic cirrhosis and 40–60% of them will experience cirrhosis-related complication, including HCC, over 5–7 years.[3,4]

Studies have shown that most of the metabolic parameters such as obesity, hyperlipidaemia, hypertension, and diabetes are strongly related to NAFLD.[5-10] Similar small studies in Asia conducted by Lesmana et al.[11] and Amarapurkar et al.[12] showed several metabolic components that were significantly associated with disease progression in NASH patients. The metabolic components strongly associated with NASH included obesity, hyperlipidaemia, and Type 2 diabetes mellitus (DM). Based on a World Health Organization (WHO) dataset, there was increased prevalence of overweight and obese individuals in 2016, and this is predicted to rise further in the future.[4] Based on follow-up research conducted in Indonesia, the prevalence of obesity (BMI >27.0 kg/m^2) in adults has significantly increased in the last 12 years, from 10.5% in 2007 to 21.8% in 2018.[13]

In line with this, there is an increased prevalence of NAFLD in the world, particularly in the USA where it has ranged between 15% and 25% for 5 years (Republic of Indonesia Ministry of Health, data on file). Indonesia, the largest country in south-east Asia, has also shown the clinical importance of NAFLD prevalence. The first Indonesian study conducted by Hasan et al.[14] in 2002 revealed that from 808 subjects in a community based in Depok, 30.6% experienced NAFLD. An additional study conducted by Lesmana et al.[15] in 2015, showed from a patient database that 51% of adult NAFLD cases are initially found in transabdominal ultrasound check-ups in hospitals.

These two studies also analysed the risk factors of NAFLD development. The first study conducted by Hasan et al.[14] identified the statistically significant risk factors for NAFLD as age, triglyceride level, DM, and obesity, whereas sex, fat intake, and physical activity were not associated with NAFLD. Lesmana et al.[15] conversely found that sex, age, blood pressure, BMI, fasting blood glucose, triglycerides, high-density lipoprotein, and serum alanine transaminase were statistically significant risk factors for NAFLD development. From these two studies, it can be concluded that NAFLD is a hepatic manifestation of the metabolic syndrome family, and obesity is considered as the most important factor for NAFLD based on the insulin resistance mechanism. This conclusion has been supported by many studies from Western countries.[16,17]

GUT MICROBIOME

The human microbiome exists in many parts of the body and outnumbers human cells by a ratio of 10:1. Gut microbiota play a key role in nutrition, fighting against pathogenic bacteria, and influencing developmental factors of the body, from body weight to brain development. Development of the gut microbiome begins at birth and continues to adulthood. The first phase of development of the gut microbiome is determined by whether a baby is born naturally or by caesarean section. In the next phase, the

baby's gut microbiome can be influenced by nutritional intake: especially by breast or formula milk. Breast feeding has an important role in increasing immunity, whereas formula milk has a role in increasing *Bacteroides*, *Clostridium*, and *Clostridium difficile* bacteria. Additionally, beneficial bacteria in the gut may decrease if the individual has a history of frequent antibiotic treatment.[18]

After the breastfeeding period, the baby will start to eat solid food. In this period, *Firmicutes* will develop as a sign of the adult gut microbiome ecosystem forming. The last stage of gut microbiome development is influenced by daily food intake. The traditional diets that contain high fibre and CHO will help develop more *Prevotella* in the human gut, whereas a Western diet which typically contains high fat will develop more gut *Bacteroides*. The imbalance of diversity and function of intestinal microbiota might contribute to disease development: either intestinally, as with inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and coeliac disease; or extraintestinally, with obesity, DM, and metabolic syndrome.[19-21]

Features of Gut Microbiota in Obesity

Some alterations of gut microbiota in obesity will be associated with many factors, such as satiety control in the brain; hormone release from the gut (shown as peptide YY and GLP-1); synthesis, storage, or metabolism of lipids in the adipose tissue, liver, and muscle; and intestinal permeability related to systemic inflammation and insulin resistance. Microbial molecules also increase intestinal permeability, leading to systemic inflammation and insulin resistance (Figure 1).[22]

The Role of Gut Microbiota in Nonalcoholic Fatty Liver Disease and Disease Progression

Gut microbiota are mostly belonging to phyla such as *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, and *Fusobacteria*. The main phyla dominating the gut are *Firmicutes* and *Bacteroidetes*. The disruption of the gut microbiota composition leads to the imbalance condition, termed gut dysbiosis.[23]

In high free fatty acid accumulation conditions such as obesity, obesity itself can cause gut dysbiosis and lead to insulin resistance, and it has been shown to have a strong association with NAFLD development. Gut dysbiosis has also been hypothesised to have a role in liver disease progression.[23] Studies have shown that small intestinal bacterial overgrowth (SIBO), which is a condition of gut dysbiosis, is strongly related to NAFLD development. There is, however, ongoing debate surrounding the data on this phenomenon.[24-26]

Research conducted by Yoshimoto et al.[27] analysed the gut microbiota in two groups of leptin-deficient mice (lean and obese mice) and treated them with a chemical carcinogen. After the intervention, the mice fed with a high-fat diet had more Gram-positive bacteria and significantly higher serum deoxycholic acid levels, a gut bacterial metabolite known to cause DNA damage. The obese mice eventually developed HCC. In the study, the model of obesity-induced HCC was proposed from the DCA-SASP (senescence-associated secretory phenotype) axis. Alterations of gut microbiota in mice were induced by dietary or genetic obesity, thus increasing the levels of DCA. DCA in enterohepatic circulation provokes SASP in hepatic stellate cells. This mechanism induces secretion of various inflammatory and tumour-promoting factors in the liver, which facilitate HCC development in mice after chemical carcinogen exposure. This hypothesis has also been supported by results from mice lacking a SASP inducer or depletion of senescent hepatic stellate cells which efficiently prevents development of HCC.[21] HCC development was also influenced by the activation of various cell signalling pathways, e.g., EGFR-pathway,[28] ERK1/2-pathway,[28] AKT-pathway,[29] and β-catenin pathway,[30] by DCA.

This hypothesis is also supported by the recent study by Fitriakusumah and Lesmana,[31] which shows that in NAFLD patients, the prevalence of metabolic factors was dominated by obesity (76.6%), followed by dyslipidemia (75.2%), central obesity (73.2%), metabolic syndrome (73.0%), and DM (70.0%), where SIBO was found to be higher in patients with NAFLD (65.5%) than those without NAFLD. Although there is no significant association between SIBO and NAFLD development, further analysis in the study showed that in the significant fibrosis group, there was a positive correlation to the presence of *Bacteroides* (p=0.037).[31]

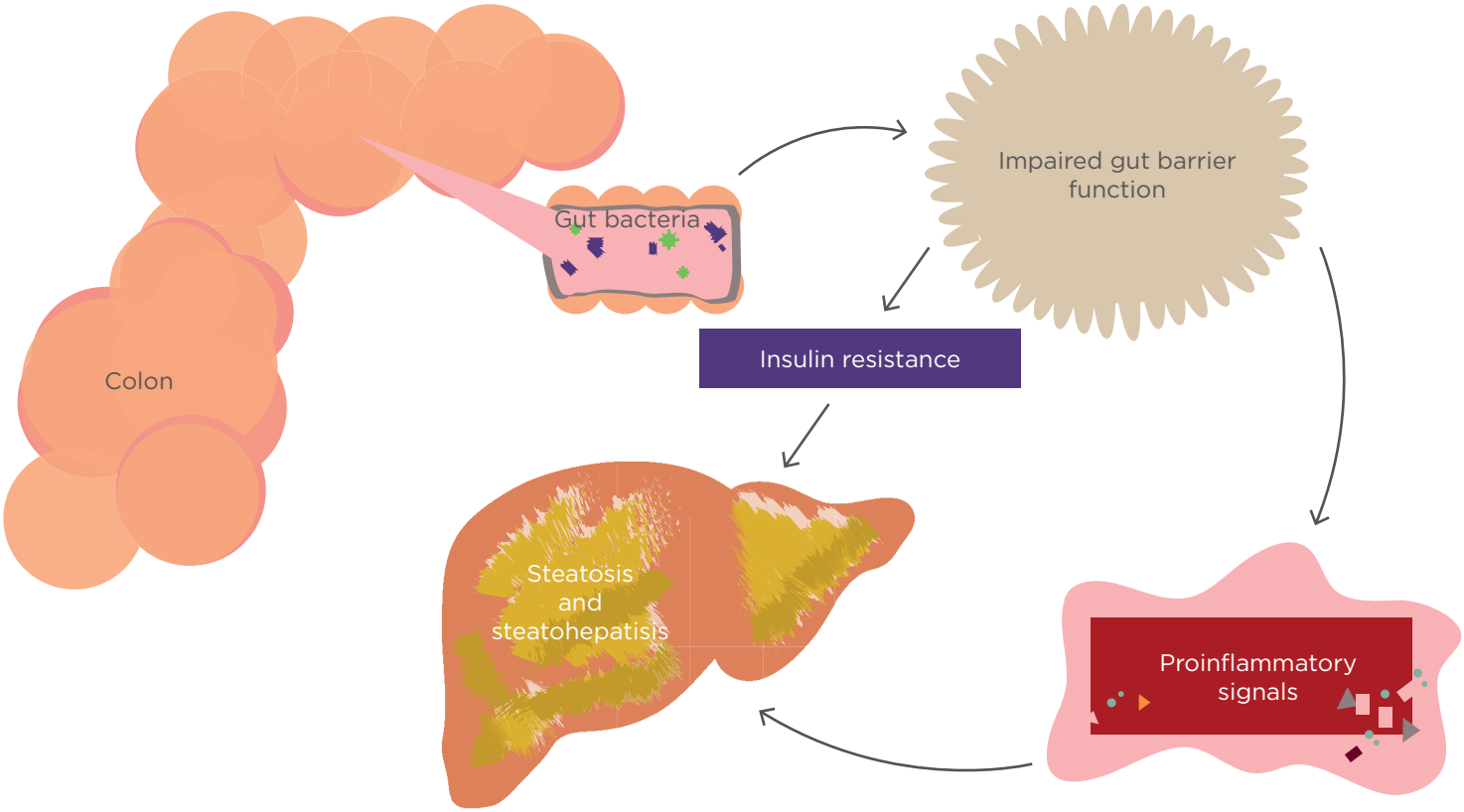


Figure 1: The role of gut microbiota in nonalcoholic fatty liver disease.

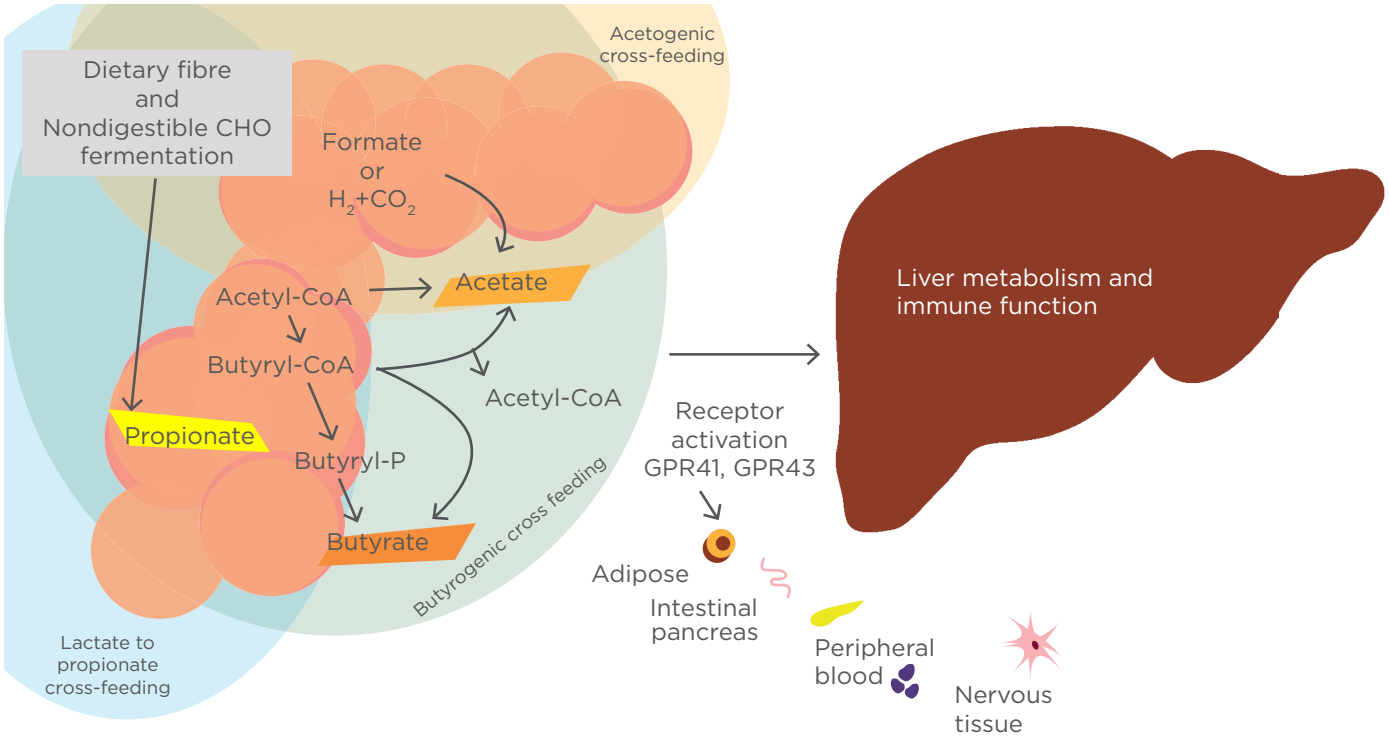


Figure 2: Microbial metabolic pathways to short-chain fatty acids in the human gut.

CHO: carbohydrate.

Based on the Asia-Pacific Working Party on NAFLD guidelines 2017,[32] it is stated that overnutrition, insulin resistance, and obesity are still the most important factors in NAFLD development. The most important risk factors for gut microbiota are largely still based on experimental data.

GUT MICROBIAL SHORT-CHAIN FATTY ACIDS AND THE METABOLIC PATHWAY

The main end products resulting from CHO catabolism of intestinal microbes are known as the faecal metabolites, specifically SCFA which include acetate, propionate, and butyrate. The gut microbiota are also the producers of these SCFA. The genus of gut microbiomes known as the primary source of dietary fermentation are *Roseburia*, *Ruminococcus*, *Salmonella*, *Blautia*, *Eubacterium*, *Anaerostipes*, *Coprococcus*, *Faecalibacterium*, *Marvinbryantia*, and *Megasphaera*. Most of these genera belong to the phyla *Firmicutes* or *Bacteroidetes*.[23,33]

Acetate and propionate are produced by *Bacteroidetes* phylum, whereas butyrate is mostly produced by the *Firmicutes* phylum. Acetate is the most abundant SCFA in the colon and has two main metabolic routes: from pyruvate via Acetyl-CoA and via the Wood–Ljungdahl pathway (reductive Acetyl-CoA pathway). Three different pathways are used by colonic bacteria for propionate formation: the succinate pathway, acrylate pathway, and propanediol pathway. In contrast, butyrate has two different pathways: the butyrate kinase and butyryl-CoA pathway. Each of these pathways have their own specific bacteria as producers of SCFA (Figure 2).[34]

THE NATURAL MECHANISM OF SHORT-CHAIN FATTY ACIDS AND FOOD RELATED ISSUES

The receptors for SCFA are of the G-protein-coupled class (GPCR). These receptors are found in various organs, meaning that SCFA can affect multiple parts of the body. Receptors for SCFA include GPR43, GPR41, and GPR109. GPR41 and GPR43 are activated by acetate, propionate, and butyrate. Meanwhile, GPR109 is activated by butyrate and niacin. The SCFA transporter is also found in the colonic epithelium. Transporters important for the handling of SCFA by the colon include monocarboxylate transporter 1 (MCT1) and sodium-coupled monocarboxylate transporter 1 (SMCT1) in the apical membrane, and MCT1 and MCT4 in the basolateral membrane. MCT1, a H^+-coupled low-affinity transporter, works under normal dietary conditions to allow SCFA entry into the colonic epithelium when the luminal fibre concentration is high. In contrast, SMCT1, a Na^+-coupled high-affinity transporter, plays a critical role under low luminal fibre conditions as a consequence of low fibre content in the diet. SCFA transporters, therefore, provide beneficial effects for colonic bacteria, allowing them to produce their fermentation product in the colon.[35]

Dietary CHO is usually absorbed in the intestine and delivered to the liver through the portal vein. Fast food and food containing high sugar (simple CHO) are not recommended for routine consumption because they are directly absorbed and induce *de novo* lipogenesis (DNL), causing liver fat accumulation which will disturb the balance of SCFA concentration. Fermented and high-fibre foods (complex CHO) are strongly recommended for consumption because gut microbiota use nonfermented CHO (fibre) as raw materials in their metabolism to produce SCFA. This is because dietary fibre cannot be digested in the intestines, and therefore the fermentation process is performed in the colon. Fermented food can also be used as a source of both probiotics and prebiotics.[36] Dietary fat, largely consumed as triglycerides, is also very important as it is usually coming after lipolysis. Saturated fat is more dangerous than unsaturated fat because it is more easily accumulated in the liver and can cause hepatocyte injury. It also has the potential to cause hepatic steatosis and an insulin resistance condition.[37]

EFFECT OF SHORT-CHAIN FATTY ACIDS ON NONALCOHOLIC FATTY LIVER DISEASE

SCFA has been considered as one of the key parameters in the pathogenesis of NAFLD. It is known that SCFA has some impact on adipose tissue, energy metabolism, the immune system, and also has a role as the gut microbiota-derived signalling mediators. The role of SCFA in NAFLD starts after its absorption process and travels

through the portal vein into the liver. It has been shown from experimental studies that T-cell regulation and neutrophil chemotaxis can be influenced by SCFA.[36,38]

Based on natural SCFA mechanism, liver fat is regulated mainly by receptor activation (GPR41, GPR43, GPR109A, and OLFR78). The GPR43 receptor is known to be related to the three SCFA: acetate, propionate, and butyrate. In studies this receptor has been shown to be increased in mice receiving a high-fat diet. High abundance of SCFA will lead to hepatic steatosis, meanwhile decreased SCFA reduces hepatic lipogenesis.[36,39]

Acetate is known to be involved in insulin sensitivity and plays a role in modulating gut hormones. However, butyrate and propionate are more important in preventing carcinogenesis, maintaining the intestinal barrier, preventing inflammation, and are also influential in satiety regulation. Acetate plays an important role in lipogenesis and cholesterol synthesis in the liver as well as in the peripheral tissue. Acetate can contribute to the accumulation of triglycerides, and propionate has been known to be involved in hepatic lipid metabolism. Both acetate and propionate are also responsible for low inflammation levels through the immune cellular system. In a probiotic study using the strain *Clostridium butyricum*, butyrate prevented liver disease progression by reducing lipid accumulation in the liver and improved insulin resistance.[36]

SCFA can also promote NAFLD development through secretion of peptide YY and GLP-1; however, butyrate is the only SCFA which is hypothesised to prevent NAFLD through decreasing both the immune response and insulin resistance.[36] An experimental study investigated the role of GLP-1 and showed that butyrate promoted this receptor, and that there was an apparent reduction of this receptor in NAFLD patients.[40] Another recent study in Europe used faecal collection analyses and showed that NAFLD patients with significant liver fibrosis had low levels of faecal acetate and propionate concentrations when compared to healthy controls. There was also a positive correlation between faecal SCFA (propionate/acetate) and peripheral proinflammatory T cells, supporting the evidence for the role of SCFA in liver disease progression in NAFLD/NASH.[41]

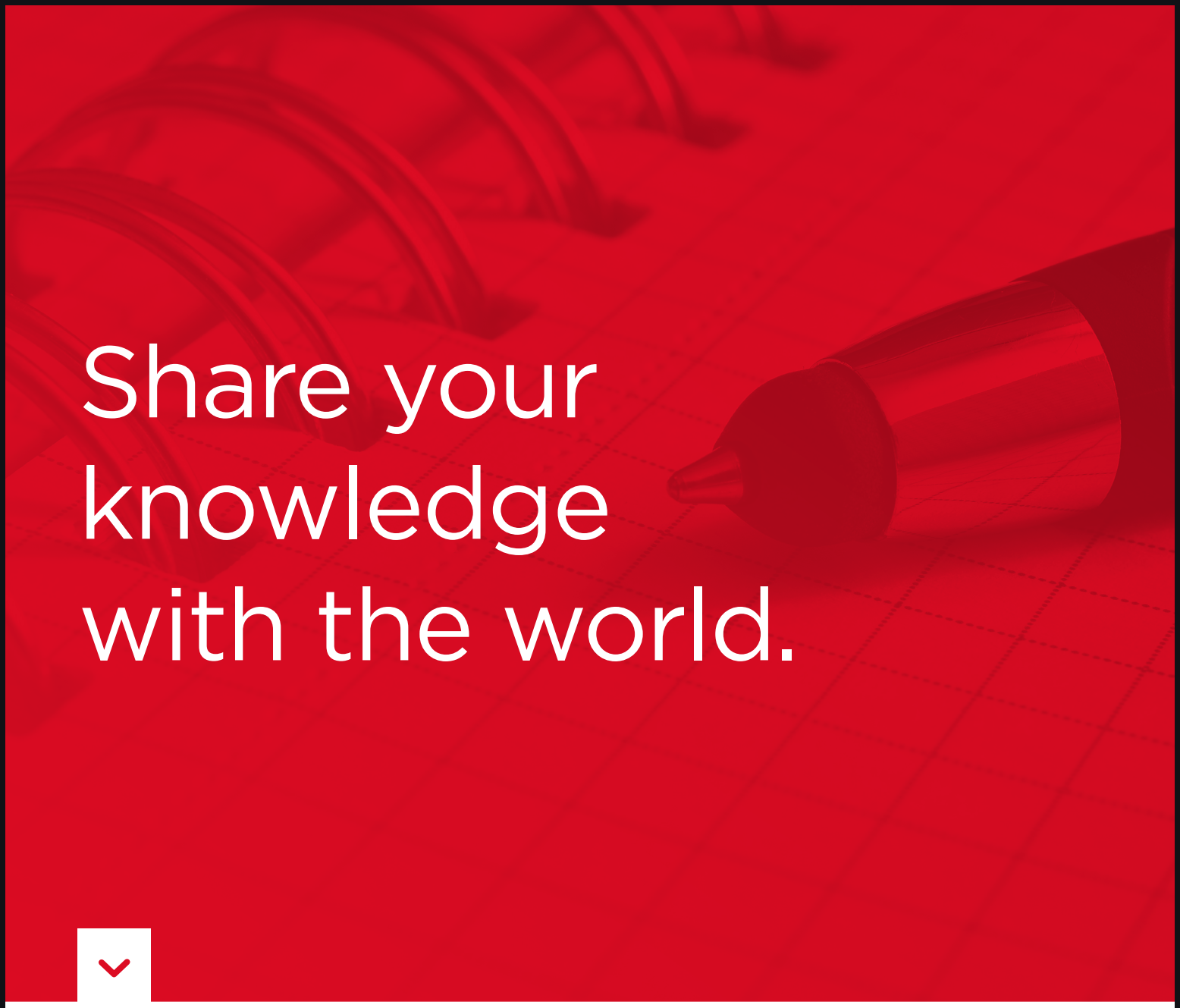
CONCLUSION

Gut microbiota plays an important role in NAFLD development and disease progression. SCFA faecal concentrations may be used as NAFLD biomarkers for disease prediction in the future. High concentration of propionate can promote the development of NAFLD, whereas acetate and butyrate can prevent the development of NAFLD.

References

1. Chalasani N et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American association for the study of liver diseases. Hepatol. 2018;67(1):328-57.
2. Aragonès G et al. Gut microbiota-derived mediators as potential markers in nonalcoholic fatty liver disease. Hindawi. 2019;2019:1-10.
3. Starley BQ et al. Nonalcoholic fatty liver disease and hepatocellular carcinoma: A weighty connection. Hepatol. 2010;51(5):1820-931.
4. Anstee QM et al. From NASH to HCC: Current concepts and future challenges. Nat. Rev Gastroenterol Hepatol. 2019;16(7):411-28.
5. Chavez-Tapia NC et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med. 2006;144(5):379.
6. Hamaguchi M et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med. 2005;143:722-8.
7. Kida Y, Sato T. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med. 2006;144:379-80 .
8. Marchesini G, Babini M. Nonalcoholic fatty liver disease and the metabolic syndrome. Minerva Cardioangiol. 2006;54(2):229-39.
9. Moseley RH. Progress in understanding the pathogenesis of nonalcoholic fatty liver disease. Hepatology. 2005;41:204-6.
10. Neuschwander-Tetri BA. Fatty liver and the metabolic syndrome. Curr Opin Gastroenterol. 2007;23:193-8.
11. Lesmana CR et al. Clinical picture, insulin resistance, and adipocytokines profiles of nonalcoholic steatohepatitis (NASH) patients in Indonesia. Acta Med Indones. 2009;41(1):6-10.
12. Amarapurkar DN, Patel ND. Prevalence of metabolic syndrome in non-diabetic and non-cirrhotic patients with non-alcoholic steatohepatitis. Trop Gastroenterol. 2004;25(3):125-9.
13. Perumpail BJ et al. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. World J Gastroenterol. 2017;23(47):8263-7

14. Hasan I et al. Prevalence and risk factors for nonalcoholic fatty liver in Indonesia (Abstract). J Gastroenterol Hepatol. 2002;17:A30.
15. Lesmana CRA et al. Development of non-alcoholic fatty liver disease scoring system among adult medical check-up patients: A large cross-sectional and prospective validation study. Diabetes Metab Syndr Obes. 2015;8:213-8.
16. Yki-Järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol. 2014;2(11):901-10.
17. Kim D et al. Nonalcoholic fatty liver disease and metabolic syndrome. Clin Liver Dis. 2018;22(1):133-40.
18. Tanaka M, Nakayama J. Development of the gut microbiota in infancy and its impact on health in later life. Allergol Int. 2017;66(4):515-22.
19. Roychowdhury S et al. The role of the gut microbiome in nonalcoholic fatty liver disease. Med Sci. 2018;6(2):47.
20. Valdés L et al. The relationship between phenolic compounds from diet and microbiota: Impact on human health. Food Funct. 2015;6(8):2424-39.
21. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012;489(7415):242-9.
22. Holmes E et al. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. Trends Microbiol. 2011;19(7):349-59.
23. Sharpton SR et al. Emerging role of the gut microbiome in nonalcoholic fatty liver disease: From composition to function. Clin Gastroenterol Hepatol. 2019;17(2):296-306.
24. Boursier J et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatology. 2016;63(3):764-75.
25. Dukowicz AC et al. Small intestinal bacterial overgrowth: A comprehensive review. Gastroenterol Hepatol (N Y). 2007;3(2):112-22.
26. Ponziani FR et al. Diagnosis and treatment of small intestinal bacterial overgrowth. Expert Rev Gastroenterol Hepatol. 2016;10(2):215-27.
27. Yoshimoto S et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013;499(7456):97-101.
28. Cheng K, Raufman JP. Bile acid-induced proliferation of a human colon cancer cell line is mediated by transactivation of epidermal growth factor receptors. Biochem Pharmacol. 2005;70(7):1035-47.
29. Fang Y et al. Bile acids induce mitochondrial ROS, which promote activation of receptor tyrosine kinases and signaling pathways in rat hepatocytes. Hepatology. 2004;40(4):961-71.
30. Pai R et al. Deoxycholic acid activates beta-catenin signaling pathway and increases colon cell cancer growth and invasiveness. Mol Biol Cell. 2004;15(5):2156-63.
31. Fitriakusumah Y et al. The role of small intestinal bacterial overgrowth (SIBO) in non-alcoholic fatty liver disease (NAFLD) patients evaluated using controlled attenuation parameter (CAP) transient elastography (TE): A tertiary referral center experience. BMC Gastroenterology. 2019;19:43.
32. Wong VWS et al. Asia-Pacific working party on non-alcoholic fatty liver disease guidelines 2017-Part 1: Definition, risk factors, and assessment. J Gastroenterol Hepatol. 2018;33(1):70-85.
33. Zhu L et al. Gut microbiome and nonalcoholic fatty liver disease. Pediatr Res. 2015;77(1-2):245-51.
34. Ríos-Covián DR et al. Intestinal short chain fatty acids and their link with diet and human health. Front Microbiol. 2016;7:185.
35. Sivaprakasam S et al. Short-chain fatty acid transporters: Role in colonic homeostasis. Compr Physiol. 2017;8(1):299-314.
36. Juárez-Hernández J et al. Role of bioactive fatty acids in nonalcoholic fatty liver disease. Nutrition J. 2016;15(1):72.
37. Duwaerts CC, Maher JJ. Macronutrients and the adipose-liver axis in obesity and fatty liver. Cell Mol Gastroenterol Hepatol. 2019;7(4):749-61.
38. Zhou D, Fan JG. Microbial metabolites in non-alcoholic fatty liver disease. World J Gastroenterol. 2019;25(17):2019-28.
39. Ji Y et al. Gut microbiota-derived components and metabolites in the progression of non-alcoholic fatty liver disease (NAFLD). Nutrients. 2019;11(8):e1712.
40. Zhou D et al. Sodium butyrate reduces high-fat diet-induced non-alcoholic steatohepatitis through upregulation of hepatic GLP-1R expression. Exp & Mol Med. 2018;50:157.
41. Rau M et al. Fecal SCFA and SCFA-producing bacteria in gut microbiome of human NAFLD as a putative link to systemic T-cell activation and advanced disease. United European Gastroenterol J. 2018;6(10):1496-507.



Share your knowledge with the world.

If you are interested in submitting your paper to EMJ, click here to contact us.

EUROPEANMEDICAL-JOURNAL.COM /AUTHORS

A Review of Liver Fibrosis and Emerging Therapies

Authors: Rooshi Nathwani, Benjamin H. Mullish, David Kockerling, Roberta Forlano, Pinelopi Manousou, *Ameet Dhar

Integrative Systems Medicine and Digestive Disease, Imperial College London, London, UK
*Correspondence to a.dhar@imperial.ac.uk

Disclosure: The authors have declared no conflicts of interest.

Received: 03.05.19

Accepted: 01.08.19

Keywords: Anticoagulation, antifibrotic therapy, fibrosis, hepatic fibrosis, hepatic stellate cell.

Citation: EMJ. 2019;4[4]:105-116.

Abstract

With the increasing burden of liver cirrhosis, the most advanced stage of hepatic fibrosis, there is a need to better understand the pathological processes and mechanisms to target specific treatments to reverse or cease fibrosis progression. Antiviral therapy for hepatitis B and C has effectively treated underlying causes of chronic liver disease and has induced fibrosis reversal in some; however, this has not been targeted for the majority of aetiologies for cirrhosis including alcohol or nonalcoholic steatohepatitis. Fibrosis, characterised by the accumulation of extracellular matrix proteins, is caused by chronic injury from toxic, infectious, or metabolic causes. The primary event of fibrogenesis is increased matrix production and scar formation mediated by the hepatic stellate cell, which is the principal cell type involved. Experimental models using rodent and human cell lines of liver injury have assisted in better understanding of fibrogenesis, especially in recognising the role of procoagulant factors. This has led to interventional studies using anticoagulants in animal models with reversal of fibrosis as the primary endpoint. Though these trials have been encouraging, no antifibrotic therapies are currently licenced for human use. This literature review discusses current knowledge in the pathophysiology of hepatic fibrosis, including characteristics of the extracellular matrix, signalling pathways, and hepatic stellate cells. Current types of experimental models used to induce fibrosis, as well as up-to-date anticoagulant therapies and agents targeting the hepatic stellate cell that have been trialled in animal and human studies with antifibrotic properties, are also reviewed.

INTRODUCTION

The burden of chronic liver disease continues to grow, with 0.1% of the European population affected by cirrhosis, the most advanced stage of hepatic fibrosis.[1] Although the aetiology of the disease varies between countries, fibrogenesis is the common pathological mechanism that causes cirrhosis. Fibrosis occurs following chronic liver injury from a range of insults including toxins (alcohol), infections (hepatitis B [HBV] and C viruses [HCV]), and metabolic disease (nonalcoholic fatty liver disease). Such insults drive inflammation, resulting in increased synthesis and altered deposition of extracellular matrix (ECM) components, and impaired regeneration and wound healing responses.[2] This is a complex, dynamic process, involving recruitment and activation of platelets, inflammatory cells, hepatic stellate cells (HSC), and other ECM-producing cells including portal fibroblasts, hepatocytes, cholangiocytes, and

bone marrow-derived cells.[3] The end result, cirrhosis, is defined by profound distortion of hepatic microarchitecture, ultimately resulting in the development of portal hypertension.[4] Histologically, cirrhosis is characterised by regenerative nodules of liver parenchyma separated by, and encapsulated in, fibrotic septa, with a clinical consequence of increased mortality, morbidity, complications of portal hypertension, and diminished quality of life.[2,5] The presence of hepatic fibrosis is a key predictor of prognosis in chronic liver disease, independent of aetiology.[6] Generally this process evolves over decades (usually 20–40 years), but it can be rapidly progressive, as seen in children affected by biliary atresia, drug-induced liver injury, HCV co-infection with HIV, or HCV infection post liver transplantation.[7]

Management of chronic liver disease has largely focussed on aetiology-specific treatments; however, significant progress has been made in understanding the pathophysiology of fibrosis, which has identified targets for potential antifibrotic agents to either halt progression or reverse fibrosis.

A search of the existing literature up to June 2019 was conducted using electronic databases PubMed, Medline, and the Cochrane library, as well as relevant guidelines, to present this literature review, an overview of the current understanding of the pathogenesis of hepatic fibrosis, *in vitro* and *in vivo* models used in exploring pathogenesis, and an update on proposed therapies.

PATHOPHYSIOLOGY

Extracellular Matrix

The ECM is a dynamic structural component of the normal liver.[8] It contains macromolecules that provide the scaffolding of the liver and act as transducers of extracellular signals. In normal liver tissue the ECM is composed of collagens, glycoproteins, fibronectin, laminin, tenascin, von Willebrand factor, and proteoglycans.[9] It is a component of Glisson's capsule, portal tracts, central veins, and the subendothelial space of Disse.[10] Various cellular sources of the ECM have been identified but the main source is the HSC. When liver injury is not severe, neighbouring hepatocytes regenerate and replace apoptotic and necrotic cells. However, in chronic insult, the mechanism contributing to fibrosis, this process fails and ECM proteins take on the role of hepatocytes.[11] The quantity and quality of the ECM changes, increasing up to 8-fold compared to a normal liver. There is significant increase in collagen content and proteoglycans, resulting in a higher density interstitial type matrix.[12] The ECM composition also transforms, from one predominantly made up of Type IV and VI collagen, glycoproteins, proteoglycans, and laminin, to a matrix consisting of Type I and III collagen and fibronectin.[10] These adaptations alter the local microenvironment leading to subsequent functional and physical restrictions on plasma flow between sinusoids and hepatocytes, causing impaired hepatic function.[9]

Signalling in Fibrosis

Fibrogenesis is a complex mechanism involving an array of cellular and extracellular signalling. Cytokine, chemokine, adipokine, neuroendocrine, angiogenic, and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) signalling have all been found to play important functions.[13] The release of cytokines in the context of fibrogenesis is controlled in part by activation of metalloproteinases. TGF-β1, platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), TNF-α, and vascular endothelial growth factor (VEGF) are all involved in key mechanisms of fibrosis.[9,14] TGF-α is particularly important in mediating stellate cell activation to myofibroblasts and stellate cell collagen production.[14] VEGF and PDGF control angiogenesis, which contributes to ECM production, portal hypertension, and regeneration postinjury.[15] During acute or chronic liver injury, platelets are the first cells recruited to the injury site. They form aggregates in damaged vasculature by converting fibrinogen into fibrin and platelet α-granules rich in PDGF, TGF-α, and VEGF.[16]

Chemokines, CCL2 (produced by Kupffer cells and HSC), and CCL5 are best recognised in fibrosis.[17] CCL2 promotes HSC activation, facilitating macrophage and monocyte recruitment into the liver. Inhibition of CCL2, or its receptor CCR2, is associated with reduced fibrosis in experimental models.[18] CCL5 and its receptors, CCR1 and CCR5, are associated with fibrogenesis promotion.[19]

Other signalling molecules involved in fibrogenesis include the adipokines, leptin, and adiponectin, synthesised by HSC, as well as cannabinoids and their respective receptors. Cannabinoid receptor 1 is a profibrotic mediator; however, cannabinoid receptor 2 has antifibrotic, but proinflammatory, properties.[13]

Reactive oxygen species mediate fibrogenesis by stimulating profibrogenic mediator release from Kupffer cells and activating HSC. Emerging evidence suggests that NADPH also plays a role in this process.[20]

Hepatic Stellate Cells

The HSC is the principal cell type involved in fibrogenesis[21] and its activation is the common pathway leading to fibrosis. Developing a clear understanding of factors that stimulate or inhibit activation of HSC has helped guide development of antifibrotic therapies. HSC lie in a quiescent state in the subendothelial space of Disse and comprise 15% of liver cells.[8] In the normal liver, they are the primary storage site for retinoids and are derived from neural crest tissue because of expression of neural crest markers, including glial fibrillary acidic protein and nestin.[22] These cells are activated by proinflammatory cytokines and protease activated receptor-1 ligation, resulting in a myofibroblastic phenotype which is proliferative, fibrogenic, and contractile.

Activation of HSC proceeds through an initiation and perpetuation phase.[13]

Initiation refers to early changes that occur in gene expression and phenotype, initiated by mediators that render quiescent HSC responsive to other stimuli. Characteristic to this phase is the production of a fibrogenic, contractile phenotype with induction of PDGF receptors.[22] Initiation is stimulated by oxidant stress signals, apoptotic hepatocytes, lipopolysaccharides, and paracrine stimuli from neighbouring cells including cholangiocytes, Kupffer cells, injured sinusoidal endothelial cells, or other stellate cells.[13] Initiation activates stellate cells, while perpetuation is the response to stimuli that maintains the activated stellate cell in its myofibroblastic state to allow fibrotic scar formation.[23] These changes include stellate cell proliferation, chemotaxis, fibrogenesis, increased contractility, altered matrix degeneration, and cytokine signalling.[22]

The release of a variety of mitogenic factors causes proliferation of stellate cells. PDGF is a potent mitogen upregulated during liver injury.[24] Other molecules with mitogenic activity include VEGF, thrombin, epidermal growth factor, TGF-α, keratinocyte growth factor, and fibroblast growth factor.[25] Matrix metalloproteinase-2 (MMP-2) acts as a stellate cell mitogen via activation by discoidin domain receptor-2 binding to fibrillar collagen.[26]

Activated stellate cells migrate towards areas of hepatic injury during chemotaxis. Growth factors within the liver which have chemoattractant properties include PDGF, insulin growth factor-1, endothelin-1, monocyte attractant protein-1, and the chemokine receptor CXCR3;[27] in contrast, adenosine inhibits chemotaxis, allowing cells to fix at injury sites.[28]

The primary event of fibrogenesis is increased matrix production and scar formation mediated by stellate cells.[10] Type I collagen, the major constituent of scar tissue, is upregulated post-transcriptionally in stellate cells by the actions of TGF-α via Smads, pivotal intracellular effector proteins that mediate TGF-α signalling.[29] CTGF also promotes fibrogenesis.[30] Other less potent mediation of Type I collagen production include angiotensin II, IL-1β, and TNF. [31]

On activation the HSC contracts, which is characterised by the increased expression of α-smooth muscle actin, a contractile filament protein.[32] This results in impendence of sinusoidal blood flow increasing portal resistance, and eventually increasing portal pressure once advanced fibrosis has developed. Progressive development of intrahepatic shunting occurs in conjunction with the constriction of hepatic sinusoids. The process is mediated by endothelin-1, as well as angiotensinogen II and atrial natriuretic peptide. Nitric oxide has an opposing effect, resulting in sinusoidal relaxation.[33]

The concept of matrix degradation evolved following evidence demonstrating that fibrosis is reversible. This propagated the theory that fibrosis is a dynamic balance between matrix production and degradation.[34] Initial matrix degradation may be termed pathological, corresponding to disruption of normal low-density matrix of the subendothelial basement

membrane, occurring in early stages of fibrosis. This allows for normal matrix to be replaced by a higher density pathological matrix, containing fibrils. Matrix degrading collagenases, including the MMP, break down Type IV collagen and are central to this paradigm.[35,36] Stellate cells and Kupffer cells are the primary sources of MMP-2 and MMP-9, respectively.[35] Conversely, MMP-1 degrades Type I collagen and is involved in the restorative degradation of the pathological matrix, thus, upregulation of this enzyme would favour resolution of scar tissue. Tissue inhibitors of metalloproteinases (TIMP) inhibit the actions of collagenases, resulting in reduced matrix degradation, and inhibit stellate cell apoptosis, which prevents fibrosis resolution and favours matrix accumulation.[36] TIMP-1 and 2 are upregulated in progressive fibrosis, and their sustained release from stellate cells is a key determinant of progressive fibrosis.[36,37]

HSC are not only effectors of fibrosis but also have a central role in amplifying inflammatory signaling via toll-like receptors and cytokines including monocyte chemotactic protein-1, CCL21, CCR5, and Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES). They demonstrate antigen presenting capabilities and produce neutrophil chemoattractants.[38,39]

EXPERIMENTAL MODELS OF LIVER INJURY

Techniques isolating and cultivating HSC have represented a major advance in exploring the complex mechanisms involved in fibrogenesis. Rodent stellate cell lines have been characterised but have now been superseded by human cell lines.[23] The most utilised human HSC lines include the LX1 and LX2 cell lines. LX1 cell line is generated by transformation with SV40 T antigen.[40] The LX2 cell line is generated by isolating stellate cells from normal human livers and immortalising them by culturing in low serum conditions. When activated, these cells bear close similarity to *in vivo* human activated HSC.[41] The LX2 cell line has been extensively validated and used in a number of studies exploring the effects of mediators on stellate cell activity.[25] Other human HSC lines are summarised in Table 1.[40] Utilising these cell lines therefore allows us the unique opportunity to study factors that both inhibit and activate HSC.

Animal models offer the opportunity to study interactions of different cell types, gene activation, protein-expressing profiles, and signalling pathways, but as yet no animal model exactly reproduces human hepatic fibrosis.[42]

Table 1: Summary table of human stellate cell lines used.

Human hepatic stellate cell line	Derivation
LI90	Human hepatic epithelioid haemangioendothelioma.
TWNT-1 TWNT-4	Retrovirally-induced human telomerase reverse transcriptase into LI90 cell line.
Human telomerase reverse transcriptase gene	Normal human liver.
HSC-Li	Normal human liver.
GREF-X	Cirrhotic human liver.
LX1	Transformation with SV40 T antigen.
LX2	Isolating stellate cells from normal human livers and immortalising them by culturing in low serum conditions.

A variety of agents, often carcinogenic, can be employed to chemically induce fibrosis. The most commonly used are carbon tetrachloride (CCl_4) and thioacetamide (TAA). Fibrosis generated in this manner has both a degree of reproducibility and similarity with human mechanisms and pathways involved in hepatic fibrosis.[43] They also have the added advantage that they can be used in conjunction with either transgenic or wild-type mice to explore underlying molecular mechanisms involved in hepatic fibrosis, or can be used to evaluate the potential of novel antifibrotic agents using strictly controlled environmental and genetic conditions.[43]

Carbon Tetrachloride

CCL_4 is the oldest and most common method of inducing liver fibrosis in rodent models.[44] It is a halothane and induces hepatic injury when given at repetitive low doses. It can be administered in a variety of ways, but is most commonly administered by intraperitoneal injection ≤3 times per week. CCl_4 is bioactivated by oxidases. This leads to a combination of effects including CCl_3 radical formation in the liver, which causes hepatocyte damage via lipid peroxidation; HSC activation; Kupffer cell activation; TGF-β-1 upregulation; and increased oxidative stress.[45,46] Histological alterations result in fatty change with necrosis and intense necroinflammation in centrilobular areas. This progresses to both septal and nonseptal fibrosis resulting in extensive fibrosis and cirrhosis. The fibrous septa are classically thin and can regress with CCl_4 withdrawal. The reproducibility and ease of induction of fibrosis are its main advantages. Disadvantages include heterogeneity in the amounts of fibrosis produced between animals.[32]

Thioacetamide

TAA is a selective hepatotoxin. Chronic administration not only induces liver fibrosis, but can result in carcinogenesis, including cholangiocarcinoma and hepatocellular carcinoma. TAA is usually given in drinking water over a period of 8–18 weeks to induce liver fibrosis.[47] Histologically, mild to moderate amounts of fibrosis develop by 8 weeks with elevated transaminases. By 12 weeks, parenchymal damage occurs, with hepatocyte swelling, necrosis, and proliferation. This results in fibrous enlargement of the portal tracts, with portal–portal and portal–central septa developing resulting in cirrhosis. Histological similarities with human viral hepatitis have led to its use as an indirect model of both fibrosis secondary to HBV and HCV infection. Withdrawal of TAA results in resolution of fibrosis over an 8-week period. A longer period of resolution, compared with CCl_4 models, makes it a more suitable model when evaluating potential antifibrotic therapies, and the occurrence of regenerative nodules make it more comparable to human cirrhosis.[48]

Dimethylnitrosamine

Dimethylnitrosamine (DMN) is a hepatotoxin and a carcinogenic and mutagenic agent. DMN is typically administered intraperitoneally three times a week, with centrilobular and periportal fibrosis characteristically developing after 3 weeks. Microscopically, this pattern of fibrosis is seen in cirrhosis.[49] DMN models induce HSC and Kupffer cells to express profibrotic cytokines resulting in deposition of excessive ECM, the primary pathogenesis of fibrosis, making it a potentially useful animal model.[50] However, DMN has mutagenic and carcinogenic properties and exposure can cause hepatocellular carcinoma. This makes understanding fibrosis pathways difficult to interpret but does allow the pathogenesis of fibrosis to hepatocellular carcinoma to be better understood.[51]

BILE DUCT LIGATION

Surgical ligation of the common bile duct causes cholestasis and periportal inflammation. This technique causes proliferation of biliary epithelial cells and increases expression of fibrogenic markers such as TIMP-1, Alpha-SMA, Type I collagen, and TGF-β1.[52] Although this model aids portal fibrosis and portal myofibroblast interrogation, bile duct ligation model use is restricted due to the high frequency of gall bladder perforation and bilio-peritoneum in mice.[53] Mortality risk is therefore significant and this model is more suitable for short-term studies investigating cholestasis-induced fibrosis.[52,53]

Table 2: Summary table of anti-fibrotic strategies targeting hepatic stellate cell.

Agent	Target	Mechanisms
1. Reducing inflammation and immune responses before HSC activation.		
Silymarin	Oxidative stress mechanisms, NFκB pathways, and PDGF signalling.	Inhibits oxidative stress and subsequently stellate cell activation.
Glucocorticoids	Immune mediators	Reduction of inflammation.
Caffeine	A2A adenosine receptor	Inhibition of A2A adenosine reception expressed by myofibroblasts and linked to matrix production.
Curcumin	Cannabinoid receptors	Downregulation of cannabinoid 1 receptor, a profibrotic mediator.
Ursodeoxycholic acid	Cholangiocytes	Reduction in the cytotoxic effects of bile acids and protects hepatocytes against apoptosis.
2. Inhibition of HSC activation		
Vitamin E	Oxidative stress mechanisms	Prevention of oxidative stress implicated in fibrogenesis.
Thiazolindinediones	PPAR	Anti-inflammatory and antifibrotic effects by inhibiting PDGF expression. Antifibrotic effect has been demonstrated in patients with nonalcoholic fatty liver disease.
Oleoylethanolamide	PPAR	Same mechanism as thiazolidinediones and but also initiation of α-smooth muscle expression.
Imatinib mesylate	PDGF	Suppression of PDGF and HSC activation.
ACE inhibitors	Renin–angiotensin system	Downregulation of angiotensin II receptors on HSC, responsible for proliferation and contraction. Though readily available, no large randomised trials have been conducted in humans.
Recombinant IL-22	Th22 receptors	Inhibit HSC activation and suppresses inflammatory cytokine release.
Thrombin and FXa	PAR1 and 2 stellate cell activation	Inhibition of PAR 1 and 2 stellate mediated activation.
ROCK inhibitor	GTP-binding protein Rho	Inhibition of stellate cell activation.
3. Inhibiting response after HSC activation		
GW6604	TGF-α	Inhibit TGF-β1 signalling pathways.
Cytosporone B *NR4A1* gene agonist	TGF-α	Inhibit TGF-β1 signalling pathways.
Bosentan	Endothelin	Endothelin antagonism reduces stellate cell activation and extracellular matrix production.
Halofuginone	Collagen synthesis	Inhibition of collagen Type I synthesis by inhibition of SMAD3 phosphorylation.

Table 2 continued.

Agent	Target	Mechanisms
Caspase inhibitors	Caspase	Inhibits effectors of apoptosis signalling in hepatocytes.
Obeticholic acid	Farnesoid-X receptor	Improved integrity of hepatocytes, reduction in HSC contractility and reduction of collagen.
4. Promoting activated HSC into apoptosis		
Gliotoxin	NFκB	Inhibition of NFκB pathways, suppressing chronic hepatic inflammation.
Sulfasalazine	NFκB	Inhibition of NFκB pathways, suppressing chronic hepatic inflammation.
Thalidomide	NFκB	Inhibition of NFκB pathways, suppressing chronic hepatic inflammation.
Melatonin	NFκB	Inhibition of NFκB pathways, suppressing chronic hepatic inflammation.
Cannabinoid 1 antagonist	Cannabinoid 1 receptor	Inhibition of collagen Type I synthesis by inhibition of SMAD3 phosphorylation. Reduces cellular proliferation and promotes myofibroblast apoptosis.
Cannabinoid 2 agonist	Cannabinoid 2 receptor	Inhibits myofibroblast proliferation and induces apoptosis.
Interferon	NK Cells	Promotes NK cell activity and promotes HSC death.
Hepatocyte growth factor	Myofibroblast	Inhibits extracellular matrix producing myofibroblasts.

ACE: angiotensin-converting enzyme; FXa: Factor Xa; GTP: guanosine-5′-triphosphate; HSC: hepatic stellate cell; NK: natural killer; NR4A1: nuclear receptor subfamily 4 group A member 1; PAR1: protease-activated receptor 1; PAR2: protease-activated receptor 2; PDGF: platelet-derived growth factor; PPAR: peroxisome proliferator activated receptors; ROCK: Rho kinase; SMAD3: SMAD Family Member 3.

Adapted from Ebrahimi H et al.[58]

ATP-Binding Cassette Subfamily B Member 4

The ATP-binding cassette subfamily B member 4 (*Abcb4*$^{-/-}$) gene encodes multidrug resistance 3 (MDR3) protein, the canalicular phosphatidylcholine lipid transporter. Altering this gene by rendering it deficient causes intrahepatic cholestasis and disease similar to that of primary biliary cirrhosis. It functions to protect cellular membranes facing the biliary tree against bile acids and without phosphatidylcholine there is biliary epithelial and ductular damage, portal inflammation and proliferation, and progressive portal fibrosis.[54] *Abcb4*$^{-/-}$knockout mice develop fibrosis between 4 and 8 weeks with TGF-β1 expression, HSC activation at 4 weeks with collagen deposition, and scarring at 8 weeks.[55]

These models have been used to study primary biliary cirrhosis, cholestasis of

pregnancy drug-induced cholestasis, and therapeutic interventions.[56]

ANTIFIBROTIC THERAPY

Trials of antifibrotic therapies in humans have been limited compared to experimental models in animals. This is partly due to the long duration antifibrotic agents would need to be administered to demonstrate a treatment effect because of the time taken for the agent to systemically accumulate. Trials are limited by funding and concerns over side effects. Furthermore, the historical need for histological endpoints can limit recruitment. An ideal trial would be one that has a short duration of treatment, with a pre-existing clinical need for routine liver histology.

Though significant progress has been made in understanding the pathogenesis of fibrosis, particularly the role of the HSC, no antifibrotic therapy specifically targeting this cell type responsible for increased matrix production and scar formation is currently licensed for human use. Several potential agents and antifibrotic molecules such as silymarin, caffeine, and curcumin, summarised in Table 2, have shown antifibrotic properties via targeting the HSC, but are not routinely used in clinical practice.[57,58]

Silymarin, mainly consisting of silibinin, is a flavonoid complex extracted from milk thistle. It has been shown to diminish the following: oxidative stress, lysis of hepatocytes, activation of Kupffer cells, and expression of α-SMA and TGF-β1, all implicated in the pathogenesis of fibrosis in rats treated with CCl_4 to induce fibrosis.[59] Despite showing promise in animal models with improved liver function tests, in humans there is little evidence demonstrating its clinical benefit. The De Avelar et al.[60] meta-analysis of six human studies demonstrated that although alanine transaminase and aspartate transaminase levels were reduced with silymarin use, there was no clinically significant benefit associated with this. To date, only one randomised, double-blinded, placebo-controlled study with biopsy-proven fibrosis investigating the effect of silymarin has been conducted. This showed that silymarin did not significantly reduce nonalcoholic fatty liver disease activity scores, although some improvement of fibrosis was histologically seen compared to placebo. This study was underpowered and did not include other aetiologies of fibrosis.[61]

Several studies have reported the beneficial effect of caffeine against liver disease.[62] The major mechanism by which caffeine exerts its antifibrotic effect is largely attributable to caffeine being a pan antagonist of the adenosine receptor. Liver myofibroblasts are profibrogenic and express the A2A adenosine receptor; therefore, blocking this receptor inhibits fibrogenesis.[63] A second proposed mechanism is that caffeine alters signalling and inflammation pathways in fibrogenesis by reducing TGF-β expression, as suggested in rodent models of fibrosis.[64]

Curcumin, a monomer extract from turmeric, has not only shown anti-inflammatory and antiproliferative properties but also antifibrotic actions, as evidenced by its ability to protect against fibrosis in CCl_4 treated rats.[65,66] Curcumin also interferes with TGF-β signalling pathways and PDGF receptors, leading to inhibition of HSC activity. This is further achieved through modulation of the cannabinoid receptor system with downregulation of the cannabinoid receptor 1, inhibiting ECM expression by HSC.[67]

Anticoagulation

Evidence suggests that hepatic fibrogenesis is associated with prothrombotic tendencies, including factor V Leiden (FVL); this is demonstrated by Wright et al.,[68] who found that FVL mutation increased the rate of fibrosis in HCV infection. The coagulation proteins thrombin and factor Xa (FXa) are also implicated through their activation of HSC.[69] Activation of the coagulation system generates FXa, which in turn results in the production of thrombin from its precursor protein, prothrombin. Both thrombin and FXa activate stellate cells via G-protein-coupled receptors known as protease activated receptors (PAR).[70] Duplantier et al.[71] evaluated, in a rat model of CCl_4-induced chronic liver injury, the effect of thrombin inhibition using a synthetic thrombin antagonist SSR182289. The study demonstrated a reduction in liver fibrosis and α-smooth muscle actin expression, a marker of stellate cell activation. Dhar et al.[72] demonstrated that administration of rivaroxaban, an FXa antagonist, to mice that had been exposed to TAA for 8 weeks induced milder fibrosis, especially around

central veins, compared with control mice. The overall mean percentage area of fibrosis was significantly reduced, as was α-smooth muscle actin expression. This effect is likely because of FXa antagonists blocking PAR1 and 2-mediated stellate cell activation.[72] Prolonged administration of enoxaparin in a rat model of cirrhosis, (induced using CCL_4 or TAA), resulted in an improvement in both portal hypertension and liver fibrosis, possibly by potentiating fibrosis regression, resulting in reduction of hepatic vascular resistance and portal pressures.[73] The use of warfarin anticoagulation to reduce liver fibrosis induced by CCl_4 has been tested using a transgenic mouse model of FVL-activated protein C resistance. The progression of fibrosis in FVL mice was compared to the control C57BL/6 mice which had been anticoagulated with warfarin. The results confirmed that the thrombophilic mouse developed fibrosis at a faster rate than the control mice, but warfarin anticoagulation significantly reduced the rate of fibrosis seen in both strains.[74]

A small study has suggested an efficacy of low-molecular-weight heparin as an antifibrotic in humans. Patients with HBV infection who were treated with 3 weeks of heparin showed improved serum levels of alanine aminotransferase and bilirubin, with a reduction in serum hyaluronic acid and Type IV collagen concentrations.[75] Long-term use of heparin as an antifibrotic agent in humans may not be practical because of side effects including osteopaenia, thrombocytopenia, and idiopathic hepatitis. A multicentre Phase II study evaluated the antifibrotic effect of warfarin anticoagulation in transplanted HCV patients with cirrhosis. Interim results demonstrated a reduction in fibrosis scores at 1-year post-transplantation in warfarinised patients compared to those who were not.[76]

Angiotensin Converting Enzyme Inhibitors

The role of angiotensin converting enzyme (ACE) inhibitors as antifibrotic agents has been explored with some success. Angiotensinogen and angiotensin-1 are present in hepatocytes and are highly activated in chronic liver disease. Specifically, angiotensin II receptors are upregulated during chronic liver injury resulting in HSC activation. Activation of the renin–angiotensin–aldosterone system also occurs within the liver, triggering oxidative stress and inflammatory cell release contributing to fibrogenesis.[77] The use of ACE inhibitors in preventing HSC, and renin–angiotensin system activation in preventing fibrogenesis, is a promising treatment option. This has been supported in animal studies, which demonstrated that ACE inhibitor use in CCl_4 treated rats ameliorated levels of oxidative stress, hepatic inflammation, and hepatic fibrosis.[78] A meta-analysis by Kim et al.[78] confirmed the benefits of their use in reducing hepatic fibrosis in humans. This meta-analysis only included studies in which intervention groups used ACE inhibitors or angiotensin receptor blockers and compared their effect to placebo. Histological changes in fibrosis were the primary outcome. ACE inhibitors lowered fibrosis scores, serum fibrosis markers including TGF-β-1, collagen Types I and IV, TIMP-1, and MMP-2. Significantly, they were shown to be safe with no significant differences in renal function in those who received ACE inhibitors versus those who did not.[78]

Farnesoid X Receptor Agonists

The potent farnesoid X receptor (FXR) agonist obeticholic acid has been shown to have antifibrotic effects, especially in those with primary biliary cirrhosis and nonalcoholic steatohepatitis, histologically characterised by the presence of fibrosis. FXR is present on HSC and is involved in cellular regulation and activation. FXR agonists, therefore, have the ability to inhibit HSC activation and hepatic fibrogenesis.[79] Obeticholic acid use in TAA-treated rats decreased hepatic inflammation and fibrogenesis, as well as portal pressure and intrahepatic vascular resistance. There was also decreased profibrotic cytokine activity, assessed by TGF-β-1, CTGF, and PDGF.[80] The FLINT trial, a Phase IIb nonalcoholic steatohepatitis study, compared those treated with 72 weeks of obeticholic acid versus placebo and fibrosis improvement was the primary outcome of the study. It assessed for statistically significant improvement in hepatic fibrosis and fibrosis scores observed in the treatment arm compared to those not being treated.[79] Although the antifibrotic properties of FXR agonists are significant, mild-to-moderate side effects including pruritis, dyslipidaemia, fatigue, headache, and gall stone disease have been reported, which may limit their use in clinical practice.[79]

Thiazolidinediones

Thiazolidinediones, including pioglitazone and rosiglitazone, are peroxisome proliferator-activated receptors (PPAR) agonists and are routinely used in the treatment of diabetes. They are selective ligands for nuclear transcription factor PPAR, expressed in HSC. PPAR activation downregulates the ability to proliferate and migrate in response to PDGF and reduces expression of TGF-β-1, procollagen 1, fibronectin, and TIMP, inhibiting the fibrogenic process. Thiazolidinediones have been shown to be effective antifibrotic agents in CCl_4 treated rats, with positive improvements in histology and profibrotic cytokine profiling.[81] A meta-analysis of 8 randomised clinical trials, including 516 patients diagnosed radiologically or histologically, concluded that thiazolidinedione use for 2 years reversed advanced fibrosis and improved fibrosis stage, even in those without diabetes.[82]

CONCLUSION

Our understanding of the pathophysiology of liver fibrosis continues to grow. Numerous experimental studies have demonstrated agents that may have therapeutic potential as antifibrotic agents. Despite this, there are still few candidates that have been successfully trialled in human clinical trials that have demonstrated a clear and significant antifibrotic effect. More recently, potential therapies have shown increasing promise and further studies are required to ascertain whether these therapies will be translated to clinical practice. At present, removal of the insult resulting in fibrosis remains a key strategy in its reduction and prevention.

References

1. Pimpin L et al. Burden of liver disease in Europe: Epidemiology and analysis of risk factors to identify prevention policies. J Hepatol. 2018;69(3):718-35.
2. Pinzani M. Pathophysiology of Liver Fibrosis. Dig Dis. 2015;33(4):492-7.
3. Calvaruso V et al. Coagulation and fibrosis in chronic liver disease. Gut. 2008;57(12):1722-7.
4. Wallace K et al. Liver Fibrosis. Biochem. 2008;411:1-18.
5. Younossi ZA et al. Assessment of utilities and health-related quality of life in patients with chronic liver disease. Am J Gastroenterol. 2001;96(2):579-83.
6. Lackner C, Tiniakos D. Fibrosis and alcohol-related liver disease. J Hepatol. 2019;70(2):294-304.
7. Bonnard P et al. Documented rapid course of hepatic fibrosis between two biopsies in patients coinfected by HIV and HCV despite high CD4 cell count. J Viral Hepat. 2007;14(11):806-11.
8. Wells RG. Cellular sources of extracellular matrix in hepatic fibrosis. Clin Liver Dis. 2008;12(4):759-68.
9. Schuppan D et al. Matrix as a modulator of hepatic fibrogenesis. Semin Liver Dis. 2001;21(3):351-72.
10. Hernandez-Gea V and Friendman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol. 2010;6(1):425-56.
11. Yanguas SC et al. Experimental models of liver fibrosis. Arch Toxicol. 2016;90(5):1025-48.
12. Gressner AM. The cell biology of liver fibrogenesis – An imbalance of proliferation, growth arrest and apoptosis of myofibroblasts. Cell Tissue Res. 1998;292(3):447-52.
13. Freidman SL. Evolving challenges in hepatic fibrosis. Nat Rev Gastroenterol Hepatol. 2010;7(8):425-36.
14. Higashi T. Hepatic stellate cells as key target in liver fibrosis. Adv Drug Deliv Rev. 2017;121:27-42.
15. Fernández M. Angiogenesis in liver disease. J Hepatol. 2009;50(3):604-20.
16. Henderson NC and Iredale JP. Liver fibrosis: Cellular mechanisms of progression and resolution. Clin Sci. 2007;112(5):265-80.
17. Seki E et al. Hepatic inflammation and fibrosis: Functional links and key pathways. Hepatology. 2015;61(3):1066-79.
18. Baeck C et al. Pharmacological inhibition of the chemokine C-C motif chemokine ligand 2 (monocyte chemoattractant protein 1) accelerates liver fibrosis regression by suppressing Ly-6C(+) macrophage infiltration in mice. Hepatology. 2014;59:1060-72.
19. Seki E et al. CCR1 and CCR5 promote hepatic fibrosis in mice. J Clin Invest. 2009;119:1858-70.
20. Aoyama T et al. Nicotinamide adenine dinucleotide phosphate oxidase in experimental liver fibrosis: GKT137831 as a novel potential therapeutic agent. Hepatology. 2012;56(6):2316-27.
21. Carpino G et al. Alpha-SMA expression in hepatic stellate cells and quantitative analysis of hepatic fibrosis in cirrhosis and in recurrent chronic hepatitis after liver transplantation. Dig Liver Dis. 2005;37(5):349-56.
22. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem. 2000;275(4):2247-50.
23. Friedman SL. Mechanisms of Hepatic Fibrogenesis. Gastroenterology. 2008;134(6):1655-69.
24. Ying HZ et al. PDGF signalling pathway in hepatic fibrosis pathogenesis and therapeutics (review). Mol Med Rep. 2017;16(6):7879-89.
25. Friedman SL. Hepatic stellate cells: Protean, multifunctional, and enigmatic cells of the liver. Physiol Rev. 2008;88(1):125-72.
26. Olaso E et al. Discoidin domain receptor 2 regulates fibroblast proliferation and migration through the extracellular matrix in association with transcriptional activation of matrix metalloproteinase-2. J Biol Chem. 2002;277(5):3606-13.
27. Tangkijvanich P et al. Wound-induced migration of rat hepatic stellate cells

is modulated by endothelin-1 through rho-kinase-mediated alterations in the acto-myosin cytoskeleton. Hepatology. 2001;33(1):74-80.

28. Hashmi AZ et al. Adenosine inhibits cytosolic signals and chemotaxis in hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol. 2007;292(1):G395-401.

29. Xu F et al. TGF-β/SMAD pathway and its regulation in hepatic fibrosis. J Histochem Cytochem. 2016;64(3):157-67.

30. Schuppan D et al. Liver fibrosis: Direct antifibrotic agents and targeted therapies. Matrix Biol. 2018;68-69:435-51.

31. Bataller R et al. NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis. J Clin Invest. 2003;112(9):1383-94.

32. Rockey DC et al. Rat hepatic lipocytes express smooth muscle actin upon activation in vivo and in culture. J Submicrosc Cytol Pathol. 1992;24(2):193-203.

33. Iwakiri Y et al. Vascular pathobiology in chronic liver disease and cirrhosis – Current status and future directions. J Hepatol. 2014;61(4):912-24.

34. 34. Benyon RC et al. Extracellular matrix degradation and the role of hepatic stellate cells. Semin Liver Dis 2001;21:373-84.

35. Duarte S et al. Matrix metalloproteinases in liver injury, repair and fibrosis. Matrix Biol. 2015;0:147-56.

36. Iredale JP. Hepatic stellate cell behavior during resolution of liver injury. Semin Liver Dis. 2001;21(3):427-36.

37. Herbst H et al. Tissue inhibitor of metalloproteinase-1 and -2 RNA expression in rat and human liver fibrosis. Am J Pathol. 1997;150:1647-59.

38. 38. Bonacchi A et al. The chemokine CCL21 modulates lymphocyte recruitment and fibrosis in chronic hepatitis C. Gastroenterology. 2003;125(4):1060-76.

39. Schwabe RF et al. Human hepatic stellate cells express CCR5 and RANTES to induce proliferation and migration. Am J Physiol Gastrointest Liver Physiol. 2003;285(5):G949-58.

40. Shang L. Human hepatic stellate cell isolation and characterisation. J Gastroenterol. 2018;53(1):6-17.

41. 41. Xu L. Human hepatic stellate cell lines, LX-1 and LX-2: New tools for analysis of hepatic fibrosis. Gut. 2005;54(1):142-51.

42. Yanguas SC et al. Experimental models of liver fibrosis. Arch Toxicol. 2016;90(5):1025-48.

43. Weiler-Normann C et al. Mouse models of liver fibrosis. Z Gastroenterol. 2007;45(1):43-50.

44. Morrione TG. Factors influencing collagen content in experimental cirrhosis. Am J Pathol. 1949;25(2):273-85.

45. Hillebrandt S et al. Genome-wide analysis of hepatic fibrosis in inbred mice identifies the susceptibility locus Hfib1 on chromosome 15. Gastroenterology. 2002;123(6):2041-51.

46. Kim KH. The antifibrotic effect of TGF-beta1 siRNAs in murine model of liver cirrhosis. Biochem Biophys Res Commun. 2006;343(4):1072-78.

47. Delire B et al. Animal Models for Fibrotic Liver Diseases: What We Have, What We Need, and What Is under Development. J Clin Transl Hepatol. 2015;3(1):53-66.

48. Zimmerman T et al. Biochemical and morphological studies on production and regression of experimental liver cirrhosis induced by thioacetamide in Uje: WIST rats. Z Versuchstierkd. 1987;30(5-6):165-80.

49. Jenkins SA et al. A dimethylnitrosamine-induced model of cirrhosis and portal hypertension in the rat. J Hepatol. 1985;1:489-99.

50. Kitamura K et al. Pathogenic roles of tumor necrosis factor receptor p55-mediated signals in dimethylnitrosamine-induced murine liver fibrosis. Lab Invest. 2002;82(5):571-83.

51. Magee PN et al. The production of malignant primary hepatic tumours in the rat by feeding Dimethylnitrosamine. Br J Cancer. 1956;10(1):114-22.

52. Georgiev P et al. Characterization of time-related changes after experimental bile duct ligation. Br J Surg. 2008;95(5):646-56.

53. Geerts AM et al. Comparison of three research models of portal hypertension in mice: Macroscopic, histological and portal pressure elevation. Int J Exp Pathol. 2008;89(4):251-63.

54. Oude Elferink RP and Paulusma CC. Function and pathophysiological importance of ABCB4 (MDR3 P-glycoprotein). Pflugers Arch. 2007;452(5):601-10.

55. Ikenaga N et al. A new Mdr2(-/-) mouse model of sclerosing cholangitis with rapid fibrosis progression, early-onset portal hypertension, and liver cancer. Am J Pathol. 2015;185(2):325-34.

56. Trauner M et al. MDR3 (ABCB4) defects: A paradigm for the genetics of adult cholestatic syndromes. Semin Liver Dis. 2007;27(1):77-98.

57. Bansal MB and Chamroonkul N. Antifibrotics in liver disease: Are we getting closer to clinical use? Hepatol Int. 2019;13(1):25-39.

58. Ebrahimi H et al. New concepts on reversibility and targeting of liver fibrosis; A review article. Middle East J Dig Dis. 2018;10(3):133-48.

59. Clichici S et al. Silymarin inhibits the progression of fibrosis in the early stages of liver injury in CCl_4-treated rats. J Med Food. 2015;18(3):290-8.

60. De Avelar CR et al. Effect of silymarin on biochemical indicators in patients with liver disease: Systematic review with meta-analysis. World J Gastroenterol. 2017;23(27):5004-17.

61. Kheong W et al. A randomized trial of silymarin for the treatment of nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol. 2017;15(12):1940-9.

62. Modi AA et al. Increased caffeine consumption is associated with reduced hepatic fibrosis. Hepatology. 2010;51(1):201-9.

63. Dranoff JA et al. How does coffee prevent liver fibrosis? Biological plausibility for recent epidemiological observations. Hepatology. 2014;60(2):464-7.

64. Shim SG et al. Caffeine attenuates liver fibrosis via defective adhesion of hepatic stellate cells in cirrhotic model. J Gastroenterol Hepatol. 2013;28(12):1877-84.

65. Goel A et al. Curcumin as "Curecumin:" From kitchen to clinic. Biochem Pharmacol. 2008:75(4):787-809.

66. 66. Fu Y et al. Curcumin protects the rat liver from CCl4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. Mol Pharmocol. 2008;73(2):399-409.

67. Zhang Z et al. Curcumin modulates cannabinoid receptors in liver fibrosis in vivo and inhibits extracellular matrix expression in hepatic stellate cells by suppressing cannabinoid receptor Type-1 in vitro. Eur J Pharmacol. 2013:721(1-3):133-40.

68. Wright M et al. Factor V Leiden polymorphism and the rate of fibrosis development in chronic hepatitis C virus infection. Gut. 2003;52(8):1206-10.

69. Cheng JYK and Wong GLH. Advances in the diagnosis and treatment of liver fibrosis. Hepatoma Res. 2017;3:156-69.

70. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature. 2000;407(6801):258-64.

71. Duplantier JG et al. A role of thrombin in liver fibrosis. Gut. 2004;53(11):1682-7.

72. Dhar A et al. Thrombin and factor Xa link the coagulation system with liver fibrosis. BMC Gastroenterol. 2018;18:60.

73. Cerini F et al. Enoxaparin reduces hepatic vascular resistance and portal pressure in cirrhotic rats. J Hepatol. 2016;64(4):834-42.
74. Anstee QM et al. Coagulation status modulates murine hepatic fibrogenesis: Implications for the development of novel therapies. J Thromb Haemost. 2008;6(8):1336-43.
75. Shi J et al. Effects of heparin on liver fibrosis in patients with chronic hepatitis B. World J Gastroenterol. 2003;9(7):1611-4.
76. Dhar et al. LP11: Warfarin anticoagulation for liver fibrosis in patients transplanted for hepatitis C (WAFT-C): Results at one year. J Hepatol. 2015;62(2):S268-9.
77. Reza HM et al. Angiotensin-converting enzyme inhibitor prevents oxidative stress, inflammation, and fibrosis in carbon tetrachloride-treated rat liver. Toxicol Mech Methods. 2016;26(1):46-53.
78. Kim G et al. Renin-angiotensin system inhibitors and fibrosis in chronic liver disease: A systematic review. Hepatol Int. 2016;10(5):819-28.
79. Abenavoli L et al. Obeticholic acid: A new era in the treatment of nonalcoholic fatty liver disease. Pharmaceuticals (Basel). 2018;11(4):104.
80. Verbeke L et al. FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis. 2016;6:33453.
81. Marra F. Thiazolidinediones and hepatic fibrosis: don't wait too long. Gut. 2006;55(7):917-9.
82. Musso G et al. Thiazolidinediones and advanced liver fibrosis in nonalcoholic steatohepatitis: A meta-analysis. JAMA Intern Med. 2017;177(5):633-40.

National Analyses on Survival in Maltese Adult Patients on Renal Replacement Therapy Started During 2009–2012

Authors: *Baldacchino Ian,[1] Debattista Sarah,[2] Debattista Daniel,[2] Balzan Gabriella,[3] Abdilla Stefania,[1] Baldacchino Lisa,[3] Borg Gabriel,[1] Buttigieg Sacha,[1] Calleja Stafrace Nadine,[1] Cutajar Karl,[1] Galea Marica,[1] Sciberras Whitney,[1] Xerri Thelma,[2] Camilleri Liberato,[4] Farrugia Emanuel[2]

1. Department of Primary Healthcare, Floriana, Malta
2. Mater Dei Hospital, Msida, Malta
3. Karin Grech Rehabilitation Hospital, Pieta, Malta
4. Full time Associate Professor, University of Malta, Msida, Malta,

*Correspondence to ian.baldacchino@gov.mt

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: We would like to thank the renal unit supporting staff from Mater Dei hospital involved in providing data.

Received: 26.05.2019

Accepted: 07.10.2019

Keywords: Diabetic nephropathies, Malta, prognosis, regression analysis, renal dialysis.

Citation: EMJ. 2019;4[4]:117-123.

Abstract

Chronic kidney disease patients on maintenance dialysis (CKD 5D) experience major morbidity and mortality. No data on survival in Maltese dialysis patients exist; therefore, the aim of this study was to rigorously examine survival statistics in a complete cohort of Maltese CKD 5D patients.

The study population was comprised of all incident chronic patients (N=328) starting dialysis at the renal unit, Mater Dei hospital, Msida, Malta, for 4 consecutive years (2009–2012). Each yearly cohort was analysed in detail up to 31st December 2017, providing up to 8 years follow-up. Demographics (male 65%; female 35%), aetiology of renal failure (diabetic kidney disease: n=191; 58.2%), comorbidities, transplant status, and death were documented. Data collection and follow up were completed and statistical analysis was performed on the aggregated cohorts with SPSS version 23 with censoring up to 31st December 2017.

The cumulative adjusted 5-year overall survival in Maltese CKD 5D patients was 0.36 and 0.25 at 8 years. No statistical difference was observed according to the year of starting dialysis. Cox regression analysis showed that age and transplant status influenced survival. The unadjusted hazard of death increased by 3% for every 1-year increase in age and was increased by 7% if the patient did not receive a transplant, and overall 22% (n=72) of the entire cohort eventually received transplants.

This study reports an approximate 65% mortality at 5 years in Maltese haemodialysis patients, a poor prognosis that, despite optimal medical management, is consistent with worldwide reports.

INTRODUCTION

Chronic kidney disease (CKD) requiring dialysis carries a significantly high mortality rate.[1] To date there have been no studies in the Maltese islands that have described the incidence and mortality of patients requiring renal replacement therapy (RRT). In this 8-year study, the survival of patients suffering from CKD-5D (estimated glomerular filtration rate [eGFR] <15mL/min/1.73m^2) requiring RRT was measured. All incident dialysis cases of CKD requiring dialysis were initially reviewed at a single clinical centre providing a complete source of data for the Maltese archipelago.

Aims

- To create an electronic register containing details and comorbidities of Maltese patients who started dialysis in view of CKD-stage 5D between 1st January 2009 and 31st December 2012 at the renal unit, Mater Dei Hospital.
- To note the survival duration, comorbidities, demographics, and transplant status of patients in the register.
- To follow up survival outcomes till the 31st January 2017.
- To determine survival outcomes and mortality indicators for patients on RRT, including renal transplant recipients.

Ethics Approval

Permission was granted by the clinical chairman, central executive officer, data protection officer, and consultant nephrologists at Mater Dei Hospital; the Department of Health Information Research (DHIR); and the Faculty Research Ethics Committee, University of Malta (FREC). Patient consent was deemed unnecessary by the FREC as data was gathered from secondary sources and interpreted in a nonidentifiable manner.

Manual records kept at the renal unit, Mater Dei Hospital, detailing information on name, identification number, and date of start dialysis from the 1st January 2008 to the 31st December 2012 were reviewed and transferred to an electronic medium (Microsoft® Excel 2007, Redmond, Washington, USA). Information on demographics, age of start dialysis, duration of dialysis, comorbidities, transplant status, aetiology of renal failure, cause of death, and survival were gathered from hospital electronic and manual records (Isoft Clinical Manager®, Sydney, Australia; Electronic Case Summaries) and DHIR databases. Electronic records for the year 2008 were incomplete as they were not yet established. The final data set contained nonidentifiable data.

MATERIALS AND METHODS

Methodology of data collection and interpretation were reviewed by a consultant nephrologist, statistician, and trainee in general practice. A pilot study was carried out involving ten patients from each year reviewing survival from 1st January 2009 to 31st December 2012. Criteria for inclusion and exclusion were modified accordingly. The eGFR was calculated using the 'Modification of Diet in Renal Disease' formula.

Inclusion criteria:

- Starting dialysis between 1st January 2009 and 31st January 2012 or receiving a new kidney transplant.
- Maltese national.
- History of prior CKD stage 5 (eGFR ≤15mL/min/1.73m^2 >3 months)

Exclusion criteria:

- Starting dialysis outside the timeframe of 1st January 2009 and 31st January 2012.
- Prior history of transplantation before starting dialysis.
- Foreign national.
- Acute kidney injury requiring dialysis eGFR >15mL/min/1.73m^2

Defining Renal Replacement Therapy Start Date

Survival figures were taken from the first day of RRT with dialysis or pre-emptive transplant.

Sample Size

At the renal unit, Mater Dei Hospital, 613 patients were started on dialysis from 1st January 2009 to 31st January 2012. Of these patients, 328 cases fitted the inclusion criteria for the study.

Statistical Methods

The age-adjusted survival probabilities (with 95% confidence intervals [CI]) of all incident end-stage renal disease patients receiving RRT and patients receiving a first transplant between 1st January 2009 and 31st December 2012 were calculated using the Kaplan-Meier method. However, this method can only investigate the effect of a single categorical confounding factor on survival duration. In order to adjust for several continuous and categorical confounding factors, the proportional hazards Cox regression model was used.

From the electronic case summaries, 60 explanatory variables were identified as possible predictors of the survival duration of renal patients. To identify the significant predictors of survival duration a Cox regression model was fitted using a forward Wald procedure. The parsimonious model identified nine significant predictors which included for example the age of the patient and transplant status (Table 1).

For each covariate the model provides a hazard ratio (HR), which is the ratio of the hazard rates corresponding to the conditions described by two categories of the covariate. Age was adjusted initially to <60 years (n=109), 60–70 years of age (n=97), 71–95 years (n=122), and then in a second analysis at 40 years of age: <40 years (n=28); >40 years (n=300). Multivariate logistic regression analysis was performed to identify independent predictors of mortality which revealed seven factors that did not show variation in responses. A 95% confidence level was used when computing CI and a 0.05 level of significance was used when conducting statistical tests.

Patients were followed up to 31st December 2017 providing an 8-year time frame. For the analysis of patient survival on RRT, the first day of RRT was taken as the starting point and the event studied was death. Adjustments were made for age, sex, cause of renal failure, transplant status, and confounding variables on mortality (Table 1). SPSS version 25 was used for statistical analysis.

Table 1: Cox regression analysis of hazard ratios and 95% confidence interval for renal replacement therapy: Specific subgroups adjusted to age.

Covariates	Sample size (n)	B	SE	Wald	Df	p-value	HR	95% CI for hazard ratio	
								Lower	Upper
Transplanted[1]	71	-2.334	0.347	45.258	1	0.000	0.097	0.049	0.191
Age (overall)[1]	328	0.029	0.006	20.543	1	0.000	1.029	1.016	1.042
Age group >70[1]	108						aliased		
Age group <60[1]	97	-0.614	0.184	11.148	1	0.001	0.541	0.378	0.776
Age group 60-70[1]	123	-0.375	0.155	5.897	1	0.015	0.687	0.508	0.930
Alcoholism[1]	3	1.290	0.604	4.556	1	0.033	3.632	1.111	11.869
Gout[1]	18	-0.956	0.359	7.100	1	0.008	0.384	0.190	0.777
Liver disease[1]	3	1.617	0.646	6.261	1	0.012	5.037	1.420	17.872
Nephrolithiasis[1]	3	1.476	0.593	6.195	1	0.013	4.374	1.368	13.979
Renal artery stenosis[1]	1	2.463	1.030	5.725	1	0.017	11.744	1.561	88.343
Transplant failure[1]	8	1.562	0.561	7.747	1	0.005	4.766	1.587	14.313
COPD[2]	31	0.540	0.220	6.100	1	0.01	1.71	1.120	2.630

B: constant coefficient; COPD: chronic obstructive pulmonary disease; Df: degrees of freedom; HR: hazard ratio; SE: standard error.

Methodology for Unadjusted Yearly Incidences per Million Population Rates of Dialysis Dependent Chronic Kidney Disease Based on the Maltese Population

Data on the number of the Maltese population was gathered from the National Statistics Office (NSO) website and the unadjusted incident yearly incidence rate of CKD requiring dialysis per million population (pmp) was calculated (number of incident dialysis patients ÷ total population × 1,000,000).[2]

End of year population estimates for the Maltese population were; 2009: 395,075; 2010: 395,850; 2011: 397,244; 2012: 398,898.[2]

Methodology for Incident Patient Survival and Censoring

The dialysis patient population included all patients who started dialysis between 1st January 2009 and 31st December 2017. Patients who undertook or attempted a renal transplant were not censored from the study and the 4-year incident data was combined (2009–2012). Measured outcomes were mortality at 1 month, 1 year after 1 month, 5 years, and 8 years after starting dialysis. Data was adjusted according to age (<60 years: n=108; 32.9%, 60–70 years: n=29.6%, >70 years: n=123; 37.5%) and gender did not influence survival outcomes in this group.

Methodology of Median Life Expectancy

Several Cox regression models were fitted to relate survival probabilities to dialysis duration for different age groups (<60 years, 60-70 years, >70 age group) for incident patients starting RRT from 2009–2012. The patient inclusion criteria are the same as those of the incident patient cohort described above. Patients were followed until death and those who survived till the end of the study period were right censored. Median life expectancy is the age when the survival function reaches a survival probability of 0.5.

RESULTS

Demographics

The mean age of patients starting RRT was 63.6 ranging from 19 to 91 years. Of these patients, 58.2% cases had diabetes as their primary renal diagnosis (n=191). The majority of patients starting RRT were men (65.2%; n=214) with an average age of 63, of which 56.5% (n=121) were diabetic (Table 2). Causes of renal failure included: diabetes (58.2%); unknown (20.4%); polycystic kidney disease (PCKD) (6.7%); and other (17.6%).

Table 2: Baseline characteristics.

Categorical variables		
	Yes	**No**
Diabetes	57.3% (n=188)	42.7% (n=140)
Gender (male)	65.2% (n=214)	34.8% (n=34)
Renal transplant recipient	21.6% (n=71)	78.4% (n=257)
Continuous variables		
	Mean	**Standard deviation**
Age (years)	63.57	14.85
Survival Duration (days)	1286.66	994.88

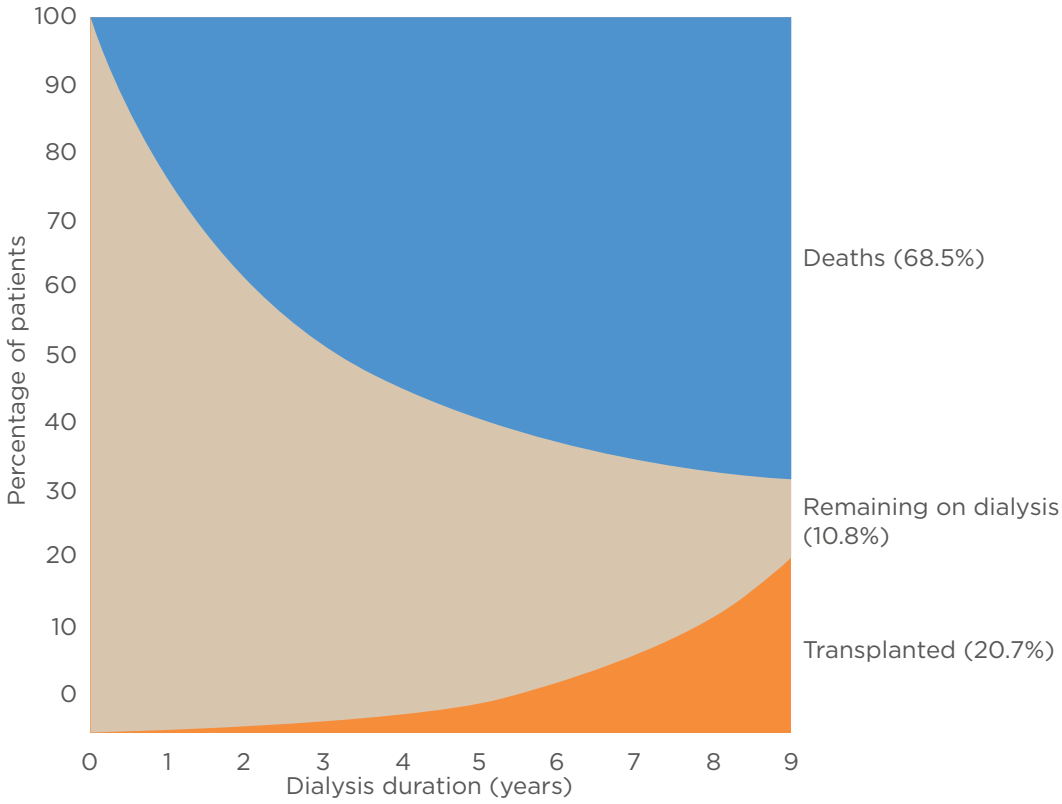


Figure 1: Adjusted cumulative incidence of death and receiving a kidney transplant according to age: incident dialysis patients from Day 1.

Other included: focal segmental glomerulosclerosis: n=22; obstructive uropathy: n=13; urinary tract infections: n=3; chronic lymphocytic leukaemia infiltration: n=1; IgA nephropathy: n=3; membranous glomerulonephritis: n=2; microscopic polyangiitis: n=1; multiple myeloma: n=3; hypertension: n=1; unspecified glomerulonephritis: n=3; lupus nephritis: n=3; and unspecified vasculitis: n=3.

Incidence (New Renal Replacement Therapy) Rates per Year

The unadjusted yearly incidences pmp rates based on the Maltese population were:[2]

- 2009 (n=90): 228 pmp.
- 2010 (n=84): 212 pmp.
- 2011 (n=72): 181 pmp.
- 2012 (n=82): 206 pmp.

Across the 4 years, there were on average two males for every female requiring dialysis. The Chi square test showed that these percentages (65.2% for males and 34.8% for females) vary marginally between the age groups because the p-value (0.213) exceeds the 0.05 level of significance.

Incidence of Renal Transplants per Year

The unadjusted incident renal transplant rates pmp years rates were:[2]

- 2009 (n=24): 61 pmp.
- 2010 (n=15): 38 pmp.
- 2011 (n=20): 50 pmp.
- 2012 (n=12): 30 pmp.

Overall the average rate of transplantation for the Maltese population was 44.75 pmp over the 4-year period totalling 71 transplants.

Overall Survival

The unadjusted survival rates following start of RRT was 94% (standard error 0.013) at 1 month; 72% (standard error 0.025) at 1 year after 1 month; 36% (standard error 0.027) at 5 years; and 25% at 8 years (standard error 0.028). At the end of the study almost 70% of the cohort passed away, 21% were transplanted, and 11% remained on dialysis (Figure 1).

Median Life Expectancy

In the >70 age group, 50% of the patients starting RRT survived 1,040 days (2.84 years). Median life expectancy for the 60–70 age group was 1,450 days (3.96 years), whereas for the <60-year age group this was 1,850 days (5.06 years).

Covariates Influencing Survival

Seventy-two variables were included into the Cox regression model. On comparing covariates age, transplant status (p<0.001, 95% CI: 0.049–0.191), transplant failure (p=0.005, 95% CI: 1.59–14.31), nephrolithiasis (p=0.013, 95% CI: 1.37–13.98), renal artery stenosis (p=0.017; 95% CI: 1.56–88.34), gout (p=0.008; 95% CI: 0.19–0.78), liver disease (p=0.012; 95%: 1.42–17.87), and alcoholism (p=0.033; 95% CI: 1.11–11.87) influenced survival (Table 1). On adjusting survival to age 40, chronic obstructive pulmonary disease (p=0.014; 95% CI: 1.12–2.63) was also significant (Table 1).

Age and Hazard of Death

Cox regression analysis demonstrated that for every 1 year increase, the hazard of death increased by 3% (p<0.0001%; 95% CI: 1.016–1.042) (Table 1).

Hazard Ratio According to Year of Dialysis

The HR varies marginally from 1, indicating that the hazard of death is not changing significantly throughout the 4 years.

Survival by Gender

The log-rank test showed that the survival probability functions did not vary significantly between the two genders (χ^2=0.018; degrees of freedom (df)=1; p=0.894).

DISCUSSION

The Maltese population has a high incidence of dialysis when compared to other countries in the European Renal Association - European Dialysis and Transplant Association (ERA-EDTA) registry 2012 annual report, with unadjusted rates of dialysis surpassed only by Greece (210.0 pmp) and Portugal (220.0 pmp), reflecting a higher trend for southern European countries.[1] Rates of incident dialysis cases remained relatively stable over the 4 years. Compared to the average unadjusted transplant rates in Europe, Malta's annual rates (30.0 pmp) were slightly above average (28.2 pmp), and comparable to Slovenia (30.6 pmp) and Poland (30.3 pmp).[1]

The overall increase in the hazard of death by 3% (HR: 1.03), and the hazard of death for a patient who received a kidney transplant is 0.097 times the hazard of a patient not receiving a kidney transplant. This implies that the hazard of death for a patient not receiving a kidney transplant is 10.31 times that of a patient receiving a kidney transplant. This result does not eliminate the bias of eligibility for renal transplantation. Wolfe et al.,[3] established that eligibility for renal transplantation confers a 50% reduced risk of death; however, this study has shown that transplant failure confers an increased risk of mortality as well.

European data has identified an increase in PCKD requiring RRT, attributable to a decrease in cardiovascular mortality in this group of patients. In the Maltese population, PCKD was classified as the second most common cause of end-stage kidney disease requiring RRT, while in European data PCKD ranks fourth.[4] This may be attributable to a significant proportion (24%) of cases for which the cause remains unknown. Chronic obstructive pulmonary disease has shown reproducibility as a factor that increases mortality in patients with advanced kidney disease.[5,6]

Diabetic nephropathy has been identified as the number one cause of end-stage kidney disease in Europe.[1] The latter has also been implicated as the major contributor to CKD requiring dialysis in this study. Malta has a predominantly overweight population with a 10% national prevalence of diabetes.[7,8] Interestingly, diabetes did not influence mortality in this cohort possibly due to

relatively small numbers, although this has been established in other studies.[9,10]

The emergence of gout as a protective marker (HR: 0.34; 95% CI: 0.19–0.78) may reflect better nutritional status in these patients, but since neither serum albumin nor anthropometric data were studied, this observation could just be a statistical aberration. Gout is associated with a higher cardiovascular risk in the general population; however, further studies are needed.[11,12,13]

LIMITATIONS

Limitations included BMI, smoking status, socio-economic status, and biomarkers that influence survival and treatment which were not included in the regression analysis. Seven comorbidities were not included in the regression analysis in view of lack of variability. Data was collected from secondary sources and was not adjusted to RRT modality (haemodialysis versus peritoneal dialysis). Cause of death was not included due to variability in reporting.

CONCLUSION

Malta had the third highest unadjusted incident rate of dialysis dependent CKD compared to other European Union (EU) countries in 2012. The largest contributor leading to dialysis is diabetes and should be the focus of prevention and management. CKD requiring RRT incident rates appeared stable with 22% of the cases receiving a transplant. Renal transplant rates were comparable to the EU average in 2012 but on the decrease in trend. Although transplantation is associated with increased survival it is accompanied by an increased mortality when failure occurs. Diabetes did not appear to influence survival in this group.

References

1. Pippias M et al. Renal replacement therapy in Europe: A summary of the 2012 ERA-EDTA registry annual report. Clin Kidney J. 2015;8(3):248-61.
2. National Statistics Office (NSO) Malta. Population statistics (revisions): 2012-2016. Available at: https://nso.gov.mt/en/News_Releases/View_by_Unit/Unit_C5/Population_and_Migration_Statistics/Documents/2018/News2018_022.pdf. Last accessed: 30 August 2018.
3. Wolfe RA et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N Engl J Med. 1999;341(23):1725-30.
4. Spithoven EM et al. Renal replacement therapy for autosomal dominant polycystic kidney disease (ADPKD) in Europe: Prevalence and survival-an analysis of data from the ERA-EDTA Registry. Nephrol Dial Transplant. 2014;29(Suppl 4):iv15-25.
5. Navaneethan S et al. Mortality outcomes of patients with chronic kidney disease and chronic obstructive pulmonary disease. Am J Nephrol. 2016;43(1):39-46.
6. Lai C et al. The association between COPD and outcomes of patients with advanced chronic kidney disease. Int J Chron Obstruct Pulmon Dis. 2018;13:2899-905.
7. Cuschieri S et al. Prevalence of obesity in Malta. Obes Sci Pract. 2016;2(4):466-7.
8. International Diabetes Federation (IDF). International Diabetes Federation Atlas 6th edition. Available at: https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/19-atlas-6th-edition.html. Last accessed: 30 April 2019.
9. Soleymanian T et al. Clinical outcomes and quality of life in hemodialysis diabetic patients versus non-diabetics. J Nephropathol. 2017;6(2):81-9.
10. Nitsch D et al. Patients with diabetic nephropathy on renal replacement therapy in England and Wales. QJM. 2007;100(9):551-60.
11. Feig DI et al. Uric acid and cardiovascular risk. N Engl J Med. 2008;359(17):1811-21.
12. Latif W et al. Uric acid levels and all-cause and cardiovascular mortality in the hemodialysis population. Clin J Am Soc Nephrol. 2011;6(10):2470-7.
13. Park C et al. Serum uric acid, protein intake and mortality in hemodialysis patients. Nephrol Dial Transplant. 2017;32(10):1750-7.

Boosting Cancer Immunotherapies with Optical Biosensor Nanotechnologies

Authors: *Maria Soler,[1] Laura M. Lechuga[2]

1. Networking Research Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain
2. Nanobiosensors and Bioanalytical Applications Group, Catalan Institute of Nanoscience and Nanotechnology (ICN2), UAB Campus, Bellaterra, Barcelona, Spain

*Correspondence to maria.soler@icn2.cat

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors acknowledge the financial support from PreDICT project (Programa estatal de investigación, desarrollo e innovación orientada a los Retos de la Sociedad, TEC2016-78515-R). ICN2 is the recipient of Grant SEV-2017-0706 from the "Severo Ochoa Centers of Excellence" Program of Spanish Ministry of Science.

Received: 28.06.19

Accepted: 06.08.19

Keywords: Adoptive cell transfer, biomedical engineering, biosensors, cancer, cell analysis, immunotherapy, nanoplasmonics.

Citation: EMJ. 2019;4[4]:124-132.

Abstract

An increasing number of cancer patients have found new hope in the form of recently discovered cell immunotherapies. These novel treatments empower our own immune system to fight and eradicate the malignant tumours, even in metastatic stages. Unprecedented outcomes have been demonstrated with bioengineered cells especially modified in laboratories to trigger a highly specific and active anti-tumour function; however, a main bottleneck for the implementation of these promising therapies resides in the old-fashioned and limited techniques employed for the *in vitro* analysis and study of cells during manufacturing and testing processes. Optical label-free biosensors are currently arising as promising analytical technologies for a simplified and accurate cell analysis. In particular, biosensors based on evanescent field principles enable direct, non-invasive, and real-time monitoring of biological events, showing outstanding sensitivities and excellent robustness. Furthermore, these biosensors are easily incorporated in lab-on-a-chip platforms for simple operation and implementation in clinical laboratories. In this review, the authors present and discuss the more recent research aimed to develop label-free optical biosensors for the analysis of live cells and the direct benefits offered to cell immunotherapy production allowing for its widespread implementation.

THE REVOLUTION OF CANCER IMMUNOTHERAPIES

Cancer immunotherapy has been profiled as one of the major breakthroughs of the decade and will probably remain a paramount biomedical landmark in decades to come. While it has long been a pursued goal in immunology and oncology, only now are the first clinical trials showing significant and impressive results in the treatment of cancer patients.[1] This relatively new therapy seeks the activation, inhibition,

or improvement of the immune system to fight cancer. Instead of directly targeting tumour cells, such as conventional radiation therapy or chemotherapy, immunotherapy approaches empower the patient's natural immune system to attack them. Therefore, the benefits promised by cancer immunotherapies include not only a remarkable increase of treatment efficiencies, but also fewer side effects, and the possibility to treat certain cancer types that do not respond to other therapies or are in advanced stages.

Immunotherapy strategies can be categorised into three broad groups: passive immunomodulation, active immunisation (i.e., cancer vaccines), and the adoptive cell transfer approaches. The first passive immunomodulatory treatments were approved by the U.S. Food and Drug Administration (FDA) in the 1990s and are widely available for cancer patients. This type of immunotherapy consists of the administration of cytokines and other molecules that boost the general immune response of the body to attack the malignant cells. Among them, the administration of IL-2, a T cell growth factor, is used to stimulate endogenous tumour-reactive cells *in vivo* and has shown reproducible regression of solid human cancers. More recently, a new immunomodulatory approach has emerged, which makes use of monoclonal antibodies to inhibit the immune checkpoints that prevent T cells to identify and eliminate the tumour cells.[2] The blockade of specific checkpoint molecules, such as cytotoxic T-lymphocyte-associated 4 (CTLA4) or programmed-death 1 (PD1), have shown impressive results for the treatment of different malignancies, such as bladder cancer, non-small cell lung cancer, and cutaneous melanoma. In fact, the innovation of these approaches was awarded with the Nobel Prize in Medicine in 2018. However, because these drugs interfere with the patient immune system in a non-specific manner, the treatment is far from reaching optimum efficacy and probably causes severe side effects.

Instead, a more active immunotherapy could provide better outcomes. Therapeutic cancer vaccines are generally intended to treat growing tumours by strengthening the immune response of the patient to selectively attack the malignant cells.[3] For that purpose, the vaccines are designed to activate the T cells by presenting specific tumour-associated antigens, which are proteins or peptides solely or over-expressed in cancer cells. Several vaccination strategies are currently under development or being evaluated in clinical and preclinical trials, including protein and peptide vaccines, genetic vaccines (DNA, RNA, and viral), and cell vaccines (tumour or immune cells). This therapeutic immunisation has demonstrated minimal toxicity in all clinical studies and also offers a long-lasting protection against tumour appearance and progression. The main challenge to cancer vaccines relies on the immunosuppressive tumour microenvironment that prevents the immune cells to act and proliferate and, therefore, suggests that vaccines should be administered in combination with other agents or approaches to synergistically engage the anti-tumour response.

Finally, one of the most attractive and encouraging immunotherapies is the so-called adoptive cell therapy.[4] This strategy consists of identifying and/or engineering the patient's own T lymphocytes to enhance the anti-tumour activity, expand them *ex vivo*, and reinfuse them again into the patient with cancer (Figure 1). The massive transfer of these powerful cells into the patient's body has demonstrated an almost immediate tumour regression and eradication while offering a long-lasting protection against cancer recurrence. Furthermore, because the treatment employs the patient's own cells, the associated side effects and toxicity could be minimal.

This article briefly reviews the current biomedical challenges associated with cell immunotherapies, especially during the production and evaluation processes, and how they can limit their widespread administration to cancer patients. The authors also introduce optical biosensor nanotechnologies as pioneering and powerful platforms to boost and facilitate the manufacturing and implementation of such personalised medicine, enabling a highly accurate, faster, and simpler analysis of live cells and immune system activity.

CELL IMMUNOTHERAPIES: PROMISES AND CHALLENGES

There are two main types of cell-based immunotherapies depending on whether they use the patient's own tumour infiltrating

lymphocytes (TIL) or artificially bioengineered T cells carrying a chimeric antigen receptor (CAR) on the cell surface.

Autologous TIL are immune cells that have recognised and infiltrated the tumour to eradicate it but have been inactivated by the effect of the tumour microenvironment and cancer immunosuppressive strategies. These lymphocytes can be extracted from the tumour peripheral blood, expanded *ex vivo*, and stimulated to reinforce their anti-tumour immune activity.[5] Prior to reinfusion, the patient undergoes a previous lymphodepletion procedure to eliminate endogenous lymphocytes that can compete with activated TIL. Additionally, the cell transfer is usually aided by co-adjuvants, such as IL-2, to enhance the anti-tumour performance. The adoptive TIL therapy has so far demonstrated outstanding response rates in melanoma, even in metastatic stages. Ongoing trials for breast, ovarian, or colorectal cancer are showing promising results when combined with costimulatory ligands or other immunotherapeutic strategies; however, the broad applicability of TIL therapy is limited because not all tumours yield to immunologically active T cells and not all cancer patients have TIL. Identifying antigen-specific T cells with effective anti-tumour function in blood samples is an arduous task in clinical and biomedical laboratories. Another important limitation relates to the manufacturing process of the therapy. TIL are usually grown with multiple rounds of expansion using cytokines or other stimuli, and it has been shown that their immune activity and anti-tumour efficiency is seriously reduced after the long-term ex vivo cultures.

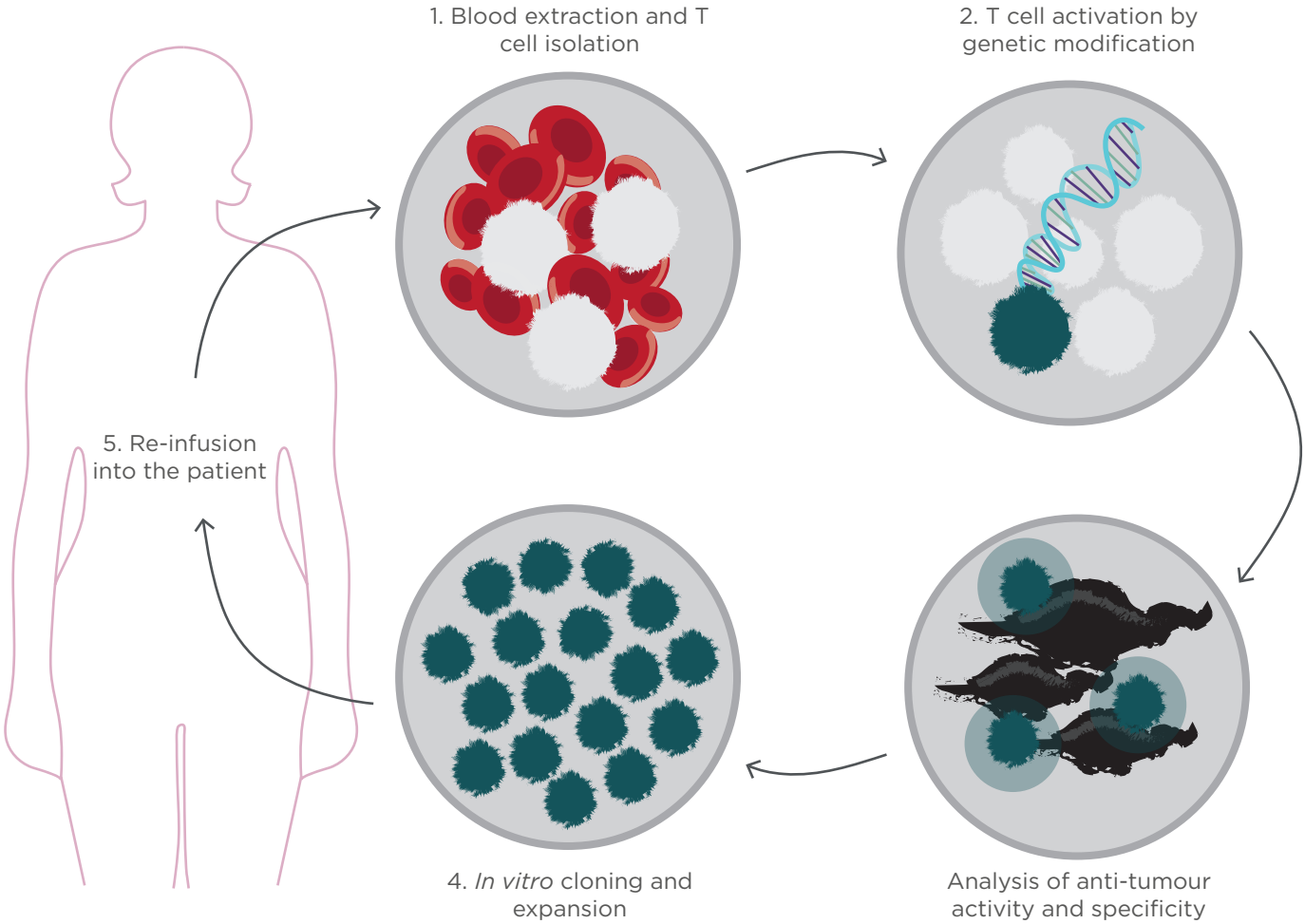


Figure 1. Schematic representation of the production process of cell immunotherapies based on adoptive cell transfer.

Reducing the number of expansion cycles during therapy production is a key factor to enhance the final effectivity and performance of the immune treatment.

An alternative strategy is providing the most exciting results for cell immunotherapies. In the so-called 'CAR-T' cell therapy, T lymphocytes from the patients are modified with artificial surface receptors (i.e., CAR) that consist of an antibody-derived ligand fused with the T cell receptor signalling machinery.[6] A CAR moiety therefore combines a specific antigen binding domain that confers recognition of a tumour-specific antigen, with intracellular signalling motifs that promote T cell activation. The main advantage of CAR-T cell therapy in comparison with the TIL strategy is that the CAR-T cells can be bioengineered and produced from a small number of T lymphocytes, independently of their native anti-tumour avidity. Two of these immunotherapies have been already approved by the FDA for treating blood cancers (i.e., acute lymphoblastic leukaemia and aggressive B-cell non-Hodgkin lymphoma). The European Medicines Agency (EMA) has also started the approval procedure for these CAR-T cell therapies. In parallel, many laboratories worldwide are intensively working on developing, optimising, and evaluating new CAR-T cell therapies for attacking solid tumours; however, relevant success has not yet been accomplished, probably due to the multiple immunosuppressive mechanisms that T cells find within the tumour microenvironment. As well as this, CAR-T cell therapy has been associated with cytokine release syndrome in patients. The rapid activation and expansion of these T cells upon infusion may trigger an immune system reaction that can yield to fever, organ dysfunction, neurologic symptoms, and other toxicities. To manage these serious side effects, the use of corticosteroids or cytokine receptor blockers along with the immunotherapy is being tested, with promising results so far.

Beyond the particular biomedical challenges, cell immunotherapies need to face important obstacles that hamper their widespread implementation and administration.[7,8] The manufacturing and evaluation process of cell immunotherapies involves multiple and highly specialised procedures, including labour-intensive cultures and routine performance of cell analyses to verify immune responses and anti-tumour activity before re-infusion into the patient (Figure 1). Currently, the analysis of the live cell functionality in clinical and research laboratories is based on fluorescent or colorimetric techniques, such as flow cytometry and enzyme-linked immunosorbent assays (ELISA). These methodologies have demonstrated outstanding sensitivities for analysing and sorting cell phenotypes according to protein expression and secretion; however, they experience important constraints in terms of automation, time resolution, cost efficiency, and cell viability. The need for fluorescent and colorimetric labels for the detection not only compromises cell activity and recovery after the study, but also limits the analysis to endpoint results and impedes the integration in appropriate culture environments. Techniques such as flow cytometry require initial large amounts of cells, which usually imply previous *in vitro* expansion procedures that reduce the eventual anti-tumour efficiency of the cells and inevitably delay the therapy administration to the patient.

Herein, the introduction of optical biosensor nanotechnologies to the field of cell analysis could bring an opportunity for accelerating the production process of cell immunotherapies, improving the evaluation accuracies, and also reducing the final costs of the treatment.

OPTICAL BIOSENSORS FOR LABEL-FREE AND REAL-TIME ANALYSIS

Optical biosensors exploit the electromagnetic properties of light for the detection, quantification, and analysis of biochemical interactions with a high sensitivity, and with capabilities for label-free and real-time format assays.[9,10] Optical label-free biosensors are generally based on the evanescent wave techniques, which enables the detection of minute refractive index variations caused by changes of mass within an evanescent electromagnetic field that is generated at the interface between the sensor and the sample (100–500 nm). The two most common configurations are the surface plasmon resonance (SPR)-based biosensors and the waveguide-based technologies, such as resonators or interferometers (Figure 2). SPR systems employ a nanometre thin layer of gold (45–50 nm) as sensor surface. Upon illumination at certain angle and wavelength, the free

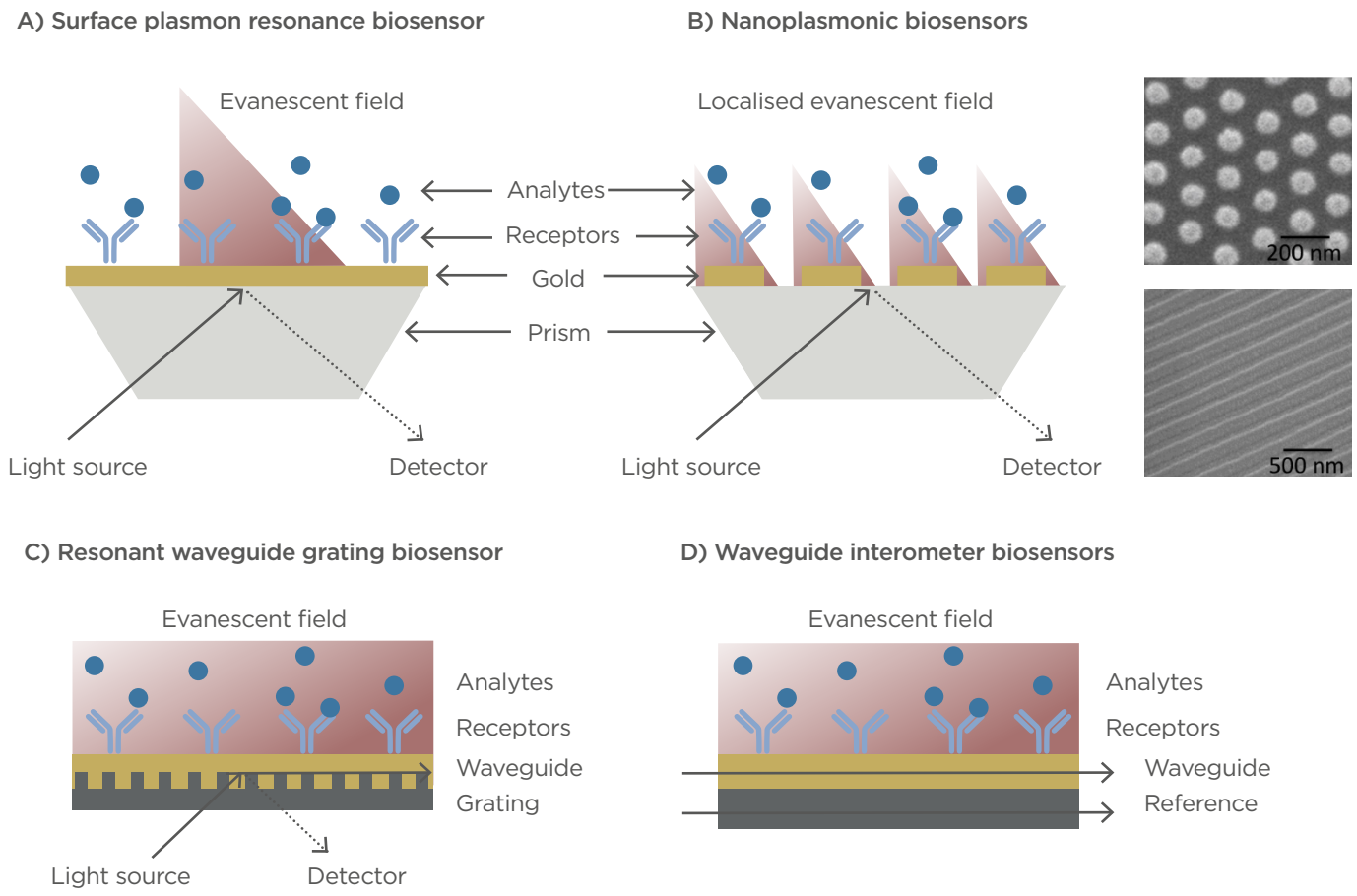


Figure 2: (A) Illustration of a surface plasmon resonance biosensor working in prism-coupling configuration. (B) Illustration of a localised surface plasmon resonance biosensor working in prism-coupling configuration. Right panels show scanning electron microscopy images of different nanoplasmonic structures. (C) Illustration of a resonant waveguide grating biosensor in angular configuration. (D) Illustration of a waveguide interferometer biosensor.

electrons of the metal start oscillating collectively creating the so-called plasmon resonance, which in turn generates an evanescent wave that penetrates in the dielectric medium and propagates a small distance along the surface.

The plasmon resonance is characterised by the appearance of an absorption dip in the reflected light. By measuring variations in the angle, wavelength, or intensity of the SPR dip, it is possible to monitor in real time biological events occurring on the sensor surface.[11] SPR biosensors are widely commercially available and are employed in many research laboratories and pharmaceutical industries to carry out routine analysis of biomolecular interactions, kinetic analysis, or drug discovery and evaluation assays.[11–14] Accompanied by the progress in nanotechnology in the last decade, more sophisticated versions of plasmonic biosensors have appeared and exponentially arose. Nanoplasmonic biosensors incorporate nanostructured surfaces such as nanodiscs, nanorods, nanoslits, and nanoholes that exhibit a localised surface plasmon resonance and provide exceptional features that can be translated in enhanced sensitivities, multiplexing capabilities, or higher miniaturisation and integration adaptability.[10,15,16] On the other hand, waveguide-based technologies are based on the leaky electromagnetic mode of light travelling along a waveguide material. The resonant waveguide grating (RWG) biosensors are commonly used and commercially available. In this platform, the light is coupled to a waveguide via grating structures, where it propagates through generating an evanescent field and resulting in a narrowband of reflected or transmitted wavelengths detected at the output.[17] RWG biosensors have been successfully integrated in

multiwell plates and imaging configuration for high-throughput and multiplexed screening.[18] In parallel to RWG systems, waveguide-based interferometers have also been realised, achieving outstanding sensitivities for label-free detection. Here, the measuring guided light mode, in contact with the sample, is combined to a reference light creating an interference. The differences in phase are used to detect and quantify biochemical interactions occurring at the sensor area.[19] Some examples of waveguide-based sensors are the well-known Mach-Zehnder interferometers,[20] the bimodal waveguide interferometers,[21] or the grating-coupled interferometers.[22]

Over the last years, there has been a steady increase in the number of scientific publications and patents in the area of optical label-free biosensors for the detection of numerous molecules or pathogens, such as bacteria, viruses, DNA or RNA, proteins, peptides, or small molecules like drugs or neurotransmitters.[10,11,19,23] Surprisingly, the application of optical label-free platforms for live cell biology remains relatively unexplored.[24,25] The predominate challenges are related to the lack of an appropriate biochemical design for providing clinically relevant information; the use of conventional microfluidic systems being unsuitable for cell culture and long-term analysis; and the inherent limitations of mass-related sensing of optical label-free methods, which commonly yields to insufficient sensitivity for small biomolecule detection and selectivity issues when evaluating complex fluids, such as culture media or blood serum and plasma. Nevertheless, a few studies have investigated the use of label-free optical sensing technology for the analysis of cell functionality in real time, showing promising potential, especially for the implementation in cell immunotherapy development.

OPTICAL LABEL-FREE BIOSENSORS FOR CELL IMMUNOTHERAPY APPLICATIONS

Two critical steps in the cell immunotherapy development process could be greatly improved with the introduction of optical sensing technologies: the detection and identification of relevant immune cells from human samples, and the evaluation of the immune cell activity and tumour response. The high sensitivity, label-free format, and real-time analysis capabilities of optical biosensors might enable the rapid isolation of specific T lymphocytes with anti-tumour reactivity directly from tumour peripheral blood samples, without the need for previous expansion steps and avoiding cell staining with fluorescent markers. On the other hand, retrieving real-time and quantitative information of cell activity events, such as membrane receptor activity or protein secretion, provides direct insights in cell status and functionality.

For example, cancer cells secrete cytokines to the tumour microenvironment in order to grow and proliferate, or to inhibit the action of immune cells trying to attack them. Likewise, immune T cells can release different types of proteins (e.g., cytokines, interferons, necrosis factors, and enzymes) for either killing tumour cells or as a signalling tool for recruiting other immune cells and enhancing the immune response. Thus, the label-free and real-time analysis of cell secretion can facilitate the evaluation of immune cell activation upon interaction with tumour antigens, the cancer cell response, or the study of immunosuppressive strategies in tumour microenvironments (Figure 3A). For the study of cancer cell activity and tumour progression, Liu et al.[26] developed a SPR biosensor able to monitor, in real time, the secretion of vascular endothelial growth factor (VEGF), a cytokine responsible for promoting the formation of blood vessels and tumour spread. The SPR system incorporated a mini cell culture module within the microfluidics that was placed close to the sensor surface. Ovarian carcinoma cells were stimulated with a calcium ionophore and the secretion of VEGF was readily detected by specific antibodies immobilised on the sensor surface. Li et al.[27] developed a more advanced lab-on-a-chip nanoplasmonic system for VEGF secretion analysis from live HeLa cells. The platform consisted of two interconnected modules: a microfluidic cell module, where cells could be cultured with appropriate temperature and humidity conditions, and the optical sensor module based on gold nanohole arrays. The nanoplasmonic sensor with multiplexing adaptability monitored the culture cell supernatant for up to 10 hours and was able to detect VEGF secretion from cancer cells at approximately 6 hours after stimulation.

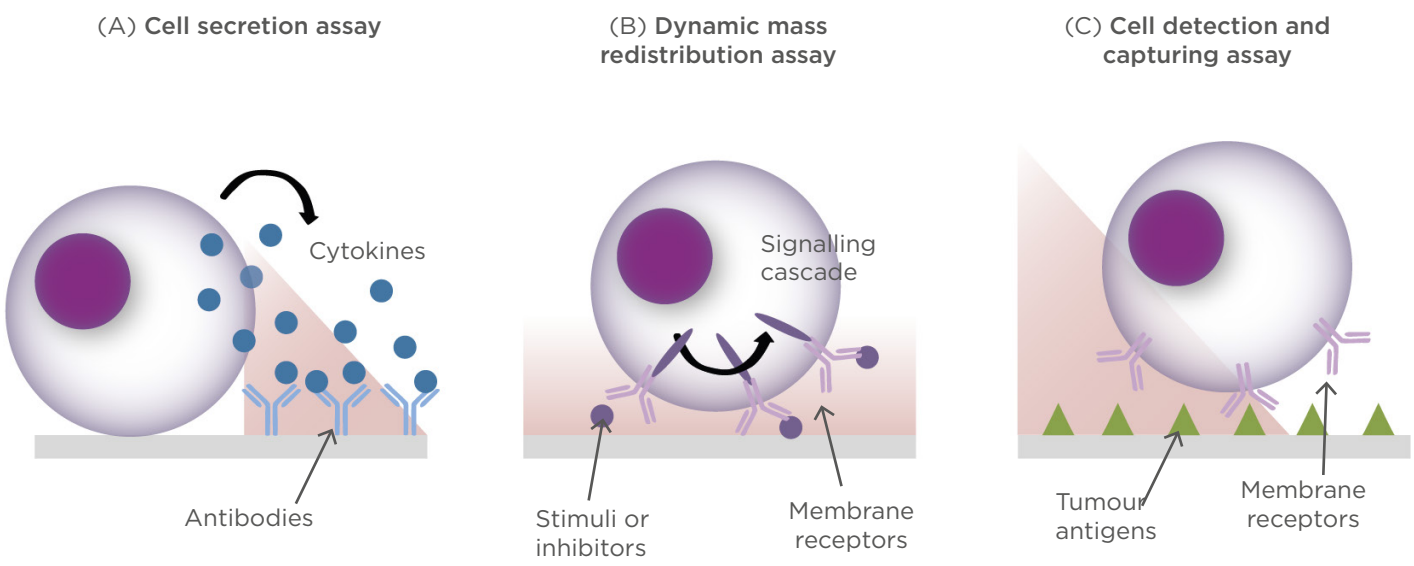


Figure 3: Conceptual illustrations of different cell assays performed with optical label-free biosensors.

(A) Detection of protein secretion; (B) Detection of cytosolic dynamic mass redistribution (DMR); (C) Detection and capture of specific anti-tumour cells.

For the analysis of immune cell activity, some promising approaches have also been proposed. Oh et al.[28] demonstrated for the first time the detection of cytokine secretion from immune T cells directly isolated from human blood. Their system was based on a nanoplasmonic surface surrounded by a microfluidic cell chamber only separated by a micropillar array. After cell incubation and stimulation, they could monitor the detection of TNF-α directly secreted by CD45 cells. With this platform, they could perform a cellular function immunoanalysis with minimal blood sample volume (3 µL) and a total assay time three times shorter than conventional ELISA. Later, the same group demonstrated the multiplexed analysis of different cytokines (IL-2, IFN-γ, and TNF-α) employing a gold nanorod-based biosensor.[29] Although in this system the cells were not cultured *in situ*, they proved the potential of nanoplasmonic biosensors for immune cell profiling in a rapid and accurate mode. Pursuing the sensitivity limits of nanoplasmonics, researchers have also attempted single-cell secretion analysis. Raphael et al.[30] developed a gold nanodisc biosensor and showed real-time detection of antibody secretion directly from an individual hybridoma cell. More recently, Li et al.[31] employed a gold nanohole array sensor integrated with a valve-gated microfluidic chamber to monitor IL-2 secretion from isolated T cells. The optofluidic system enabled not only the ultrasensitive detection of the cytokine secretion, but also the spatio-temporal profiling of the secretion event, revealing large heterogeneity and behaviour differences between various cells from the same cell line.

Another approach for elucidating cell activity is by measuring the triggering of membrane receptor signalling. When live cells are placed on the sensor surface, the evanescent field is able to probe changes of mass distribution inside the cytosol of the cell (i.e., dynamic mass redistribution), such as those occurring when the signalling is activated (Figure 3B). This strategy, however, requires the evanescent field to propagate over large areas ensuring maximum coverage of the cell entity; therefore, waveguide-based biosensors are better suited for this application. Fang et al.[32,33] employed a RWG biosensor with angular modulation to monitor the EGF-receptor activation in cancer cells. Upon stimulation with EGF molecules, they were able to detect mass redistribution inside living cells in real time, which was attributed to the molecular rearrangements in the cell during the signalling cascade; however, because EFG

stimulation is known to lead to cell rounding and detachment, it is also conceivable that the biosensor was monitoring cell detachment and re-adhesion to the surface. The same approach was employed by Kurucz et al.[34] to monitor the response of human B cells upon stimulation and inhibition of immune receptors. In this case, non-adherent B cells were immobilised on the surface via electrostatic interactions and the dynamic mass redistribution was measured when cells were stimulated with B-cell receptor antibodies, as well as stimulation inhibitors.

Finally, compared to cell activity analysis, capturing and isolating specific immune cells from complex samples is far more challenging in optical label-free biosensors. In order to selectively capture tumour-reactive immune cells, it is necessary to employ specific tumour receptors as biorecognition elements. Unfortunately, the binding strength between T cells and tumour receptors is rather weak, compared to, for instance, antibody-antigen interactions (Figure 3C). In this regard, Soler et al.[35] developed a biomimetic SPR biosensor in a recent study for the detection of T cells through interaction with tumour-specific receptors (Figure 3). By monitoring the interaction in real time, they were able to extract kinetic and affinity parameters and identify tumour-reactive T cells that could be further employed for immunotherapy production.

CONCLUSIONS AND FUTURE PERSPECTIVES

Cell immunotherapies based on adoptive cell transfer approaches have developed huge expectations as novel solutions for efficient cancer treatment and eradication. There are however still considerable challenges to be addressed in order to guarantee their widespread administration safely, timely, and at an acceptable cost. Among others, the manufacturing process of cell immunotherapies needs to be updated, incorporating modern cell analysis techniques that allow for a higher automation in the production process and enhancement of the accuracy and reliability of cell biology studies. Optical label-free biosensors are now being profiled as a unique alternative to traditional cell analysis techniques. These biosensor nanotechnologies have demonstrated capabilities for the real-time identification and monitoring of live cell activity without amplification or staining procedures, enabling the evaluation of cell immune responses in a few hours and only using a few cells. Such features could therefore eliminate several cell culture and expansion steps in the manufacturing process, reducing the costs and time delay from months to days. Further investigation might focus on developing new lab-on-a-chip platforms that combine the recent advances in nanophotonics, microfluidics, and bioengineering to eventually deliver more robust, simple, and reliable devices for cell analysis. The upcoming synergy between nanotechnology and biomedical research may be the key for groundbreaking progress and promotion of cell immunotherapies, holding a bright potential for effective and personalised cancer medicine.

References

1. Wang RF. A special issue on cancer immunotherapy. Cell Res. 2017;27(1):1-2.
2. Kyi C, Postow MA. Checkpoint blocking antibodies in cancer immunotherapy. FEBS Lett. 2014;588(2):368-76.
3. Banchereau J, Palucka K. Cancer vaccines on the move. Nat Rev Clin Oncol. 2018;15(1):9-10.
4. Rosenberg SA et al. Adoptive cell transfer: A clinical path to effective cancer immunotherapy. Nat Rev Cancer. 2008;8(4):299-308.
5. Fesnak AD et al. Engineered T cells: The promise and challenges of cancer immunotherapy. Nat Rev Cancer. 2016;16(9):566-81.
6. Littman D, Hexner E. Cancer immunotherapy with chimeric antigen receptor (CAR) T cells. J Onco-Nephrology. 2017;1(3):151-5.
7. Yee C. Adoptive T cell therapy: Points to consider. Curr Opin Immunol. 2018;51:197-203.
8. Hong E, Dobrovolskaia MA. Addressing barriers to effective cancer immunotherapy with nanotechnology: Achievements, challenges, and roadmap to the next generation of nanoimmunotherapeutics. Adv Drug

Deliv Rev. 2019;141:3-22.

9. Zanchetta G et al. Emerging applications of label-free optical biosensors. Nanophotonics. 2017;6(4):627-45.
10. Soler M et al. Label-free plasmonic biosensors for point-of-care diagnostics: A review. Expert Rev Mol Diagn. 2019;19(1):71-81.
11. Nguyen H et al. Surface plasmon resonance: A versatile technique for biosensor applications. Sensors. 2015;15(5):10481-510.
12. Masson JF. Surface Plasmon resonance clinical biosensors for medical diagnostics. ACS Sensors. 2017;2(1):16-30.
13. Drescher DG et al. Analysis of protein interactions by surface plasmon resonance. Adv Protein Chem Struct Biol. 2018;110:1-30.
14. Šakanovič A et al. Surface plasmon resonance for measuring interactions of proteins with lipids and lipid membranes. Methods Mol Biol. 2019:2003;53-70.
15. Lopez GA et al. Recent advances in nanoplasmonic biosensors: Applications and lab-on-a-chip integration. Nanophotonics. 2017;6(1):123-36.
16. Escobedo C. On-chip nanohole array based sensing: A review. Lab Chip. 2013;13(13):2445-63.
17. Daghestani HN, Day BW. Theory and applications of surface plasmon resonance, resonant mirror, resonant waveguide grating, and dual polarization interferometry biosensors. Sensors. 2010;10(11):9630-46.
18. Quaranta G et al. Recent advances in resonant waveguide gratings. Laser Photon Rev. 2018;12(9):1800017.
19. Fernández Gavela A et al. Last advances in silicon-based optical biosensors. Sensors. 2016;16(3):285.
20. Prieto F et al. An integrated optical interferometric nanodevice based on silicon technology for biosensor applications. Nanotechnology. 2003;14(8):907-12.
21. Zinoviev KE et al. Integrated bimodal waveguide interferometric biosensor for label-free analysis. J Light Technol. 2011;29(13):1926-30.
22. Patko D et al. Single beam grating coupled interferometry: High resolution miniaturized label-free sensor for plate based parallel screening. Opt Express. 2012;20(21):23162-73.
23. Ciminelli C et al. Label-free optical resonant sensors for biochemical applications. Prog Quantum Electron. 2013;37(2):51-107.
24. Zhou Q et al. Biosensors for Cell Analysis. Annu Rev Biomed Eng. 2015;17(1):165-90.
25. Méjard R et al. Optical biosensing for label-free cellular studies. TrAC Trends Anal Chem. 2014;53:178-86.
26. Liu C et al. Real-time monitoring biomarker expression of carcinoma cells by surface plasmon resonance biosensors. Chem Commun (Camb). 2012;48(84):10389-91.
27. Li X et al. Plasmonic nanohole array biosensor for label-free and real-time analysis of live cell secretion. Lab Chip. 2017;17(13):2208-17.
28. Oh BR et al. Integrated nanoplasmonic sensing for cellular functional immunoanalysis using human blood. ACS Nano. 2014;8(3):2667-76.
29. Oh BR et al. Multiplexed nanoplasmonic temporal profiling of T-cell response under immunomodulatory agent exposure. ACS Sensors. 2016;1(7):941-8.
30. Raphael MP et al. Quantitative imaging of protein secretions from single cells in real time. Biophys J. 2013;105(3):602-8.
31. Li X et al. Label-free optofluidic nanobiosensor enables real-time analysis of single-cell cytokine secretion. Small. 2018;14(26):e1800698.
32. Ye Fang et al. Characteristics of dynamic mass redistribution of epidermal growth factor receptor signaling in living cells measured with label-free optical biosensors. 2005;77(17):5720-5.
33. Fang Y et al. Resonant waveguide grating biosensor for living cell sensing. Biophys J. 2006;91(5):1925-40.
34. Kurucz I et al. Label-free optical biosensor for on-line monitoring the integrated response of human B cells upon the engagement of stimulatory and inhibitory immune receptors. Sensors Actuators B Chem. 2017;240:528-35.
35. Soler M et al. Two-dimensional label-free affinity analysis of tumor-specific cd8 t cells with a biomimetic plasmonic sensor. ACS Sens. 2018;3(11):2286-95.

What's New

New Device for Monitoring Multiple Myeloma Developed

MULTIPLE myeloma, the second most common type of haematological cancer, is a disorder of plasma cells for which diagnoses often require invasive means such as bone marrow aspiration, to varying efficacy. Circulating clonal plasma cells (cCPC) are thought to potentially have significant prognostic value in multiple myeloma patients; upon leaving the bone marrow and entering the circulatory system, an increased mortality risk may present of which awareness of would clearly be beneficial to the treating clinician. A problem exists however in the fact that, especially at low levels, cCPC are difficult to detect. Now, a team of researchers from the University of Toronto, Toronto, Canada, have developed a new device they claim can capture these cells and help assess relapse risk in remissive patients.

The researchers initially adopted a computer model to design a microfluidic device harbouring tiny, diamond-shaped pillars capable of binding cCPC from small blood samples. This is made possible through the fact that the pillar organisation permits transfer of normal blood cells through a filter whilst retaining the morphologically distinct and more rigid cCPC. This 'label-free' system, one in which antibodies are not incorporated, provides a clear advantage in allowing the safe extraction of cCPC following capture for subsequent analysis.

Following the device's manufacture, it was successfully tested *in vitro* using a myeloma cell line supplemented into blood samples from healthy donors. Both a high enrichment ratio (>500) and sufficient capture efficiency (40–55%) for cCPC were demonstrated using the particle. In a separate analysis, the group also discovered that the number of cCPC captured from relapsed disease patients was significantly higher than those who were still in remission or healthy volunteers.

Co-senior author Dr Lidan You was optimistic in proclaiming that the device "shows great potential as a noninvasive method for either early detection or monitoring of multiple myeloma disease progression." Despite this initial success, further work is needed utilising a larger sample size to evaluate the relationships between cCPC number and types, stages, and recurrence of multiple myeloma.

"shows great potential as a noninvasive method for either early detection or monitoring of multiple myeloma disease progression."

What's New

Rapamycin: A Novel Anti-Ageing Cream?

RAPAMYCIN, a commonly used immunosuppressor used to prevent organ rejection, has recently been found to decrease the rate of ageing in human skin. The researchers of this trial believed that the skin, a complex organism with immune cells, nerve cells, and stem cells, would be ideal to investigate the ageing process and test the biology of a drug. By doing so, they discovered that topical delivery of rapamycin decreased wrinkles, reduced sagging, and improved skin tone.

Researchers from Drexel University College of Medicine, Philadelphia, Pennsylvania, USA have observed that regular application of rapamycin to the backs of the hands reduces wrinkles and sagging and improves skin tone. The study recruited 13 volunteers >40 years old who applied rapamycin cream to the back of one hand and a placebo cream on the back of the other hand every 1 or 2 days before going to bed. For 8 months, the participants attended evaluation visits every 2 months in which investigators took photographs of their hands to evaluate skin wrinkles and general appearance. At the 6-month visit, blood samples were taken from the participants, showing that rapamycin was not absorbed into the participants' bloodstream, and at the 8-month visit they underwent a skin biopsy on both hands.

After 8 months, results indicated that the majority of the rapamycin-treated hands displayed increased collagen protein and statistically significant lower p16 levels, and immunohistochemical analysis revealed improvement in the histological appearance of skin tissue. Elevated levels of p16, a key marker of skin cell aging,

may cause dermal atrophy, slow healing after cuts, and increased risk of infection or complications after an injury. p16 acts as a stress response that human cells undergo when damaged and as a way of preventing cancer. This response prevents mutation of cells that would lead to a tumour by slowing down the cell cycle process; therefore, instead of creating a tumour, p16 contributes to the ageing process. Rapamycin interacts with cells by blocking mammalian target of rapamycin, a mediator in cell metabolism, growth, and ageing, and thus reduces p16 levels resulting in fewer senescent cells.

Associate Prof Christian Sell, Drexel University College of Medicine, stated that: "If you ramp the pathway down you get a smaller phenotype. When you slow growth, you seem to extend lifespan and help the body repair itself." As this is early research with a small sample size, future studies will investigate how to apply the drug in a clinical setting and explore potential applications in other diseases.

"When you slow growth, you seem to extend lifespan and help the body repair itself."

Home Testing Available for Urinary Tract Infections with New App

SMARTPHONE healthcare apps are part of an expanding industry and are allowing for the advent of more personalised medicine, as well as improved doctor and patient communication. Scanwell Health are in the process of launching an app that will allow patients to carry out urinary tract infection testing at home, with a portal to converse with a doctor and receive a prescription, if necessary.

Cases of urinary tract infections are the cause of almost 10 million hospital visits every year in the USA, and a reliance on patient-reported symptoms has led to inappropriate antibiotic prescriptions in 30–50% of cases. In this age of increasing antibiotic resistance and the impending consequences accompanying this, new approaches are critical to ensure patient safety.

The remote testing involves a urinalysis test strip, which is able to give a result in around 2 minutes. By assessing the composition of the urine and any excess proteins present, the diagnostic test results are shown via a colour change which can be detected by a smartphone camera. Once the results are in, a patient is able to contact a virtual network of healthcare practitioners and a remote diagnosis can be obtained.

A national launch in the USA will now see the app, which was recently approved by the U.S. Food and Drug Administration (FDA), rolled out to pharmacies in all 50 states. Scanwell's founder and CEO Stephen Chen is excited about the future of this technology, stating: "We have a number of additional diagnostic tests in the pipeline that have the potential to change the way we diagnose and treat infections and monitor chronic diseases."

Working alongside the Chronic Renal Insufficiency Cohort Study, it is Chen's hope to "work with additional partners to bring these tests to people across the country." The next aim is for remote chronic kidney disease testing and monitoring, and additional chronic diseases tests are already being designed.

"We have a number of additional diagnostic tests in the pipeline that have the potential to change the way we diagnose and treat infections and monitor chronic diseases."