EMJ EUROPEAN MEDICAL JOURNAL

RESPIRATORY

October 2013 • emjreviews.com

INSIDE

Review of the

ERS Annual Congress 2013

Barcelona, Spain

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Welcome

It gives me great pleasure to welcome you to the debut edition of *EMJ – Respiratory*. This journal is our biggest to date, and includes the EMJ comprehensive and impartial review of the most prominent meeting of respiratory specialists in Europe, the European Respiratory Society Annual Congress 2013, held in Barcelona, Spain. A range of compelling articles featuring research reports, literature reviews and treatment analyses are also included, forming a well-rounded report of the progress within the respiratory field throughout 2013, and guidance for the future.

In continuation of the hot topics featured at the ERS Congress 2013, *EMJ – Respiratory* focuses on respiratory diseases, namely COPD and asthma. Significant aspects of these diseases are discussed such as: development of new strategies for treatment, improvement of current treatment administration technique and adherence in patients, and research into detection of disease and connections with other health conditions. Also featured are updates on existing respiratory threats such as incense, pollution and harmful chemicals in deodorants, as well as information of new breakthroughs in body-oxygenating microparticles which could keep a patient breathing, even after lung failure.

BBC reporter Vivienne Parry, speaking at the Congress in Barcelona, emphasised the growing impetus within the field of respiratory, and the reflection of this within the media. Parry observed: "The whole area of respiratory medicine is now buzzing in the same way [as the field of Oncology]. A vibrant pipeline, huge excitement, and actually, recognition of lung disease and its importance...In journalism in the past we've been guilty of writing about things that our editors most liked: asthma, TB, things that were 'sexy' and ignoring the huge burden of lung disease. I think that's now changing and has changed already," Parry continued, highlighting the increased recognition and understanding of lung disease, and its implications.

The issue of adherence to treatment was addressed by many new methods, with clear recommendation that both patients and healthcare providers are equally responsible for the improvement of drug efficacy. For example, presentations at the Congress suggested that patients could be virtually monitored via mobile phone technology taking their medication, and for clinicians, a music video has been created to aid them in memorising guidelines for the treatment of asthma patients.

Looking to the future, patient involvement is the critical topic which requires development, particularly when aiming to reach full potential of treatment and improve healthcare policy efficacy. Monica Fletcher, Chair of the European Lung Foundation, explained: "Patient involvement is not clearly understood by either patients or practitioners and often means different things to different people, and that's one of the major issues we are facing."

Ultimately, patients should be at the centre of development, and deserve to be part of the decision-making and treatment process, but more than this, they could actually improve outcomes of others via feedback, as they have relative experience of treatment as a user. Speaking with regards to health policy, guideline development and patient-centred care, Fletcher commented: "Patients who are citizens, have a moral and ethical right to be involved on decisions around healthcare policy," adding the poignant statement: "Care, we ignore that word at our peril."

Kelly-Ann Lazarus

Editor, European Medical Journal

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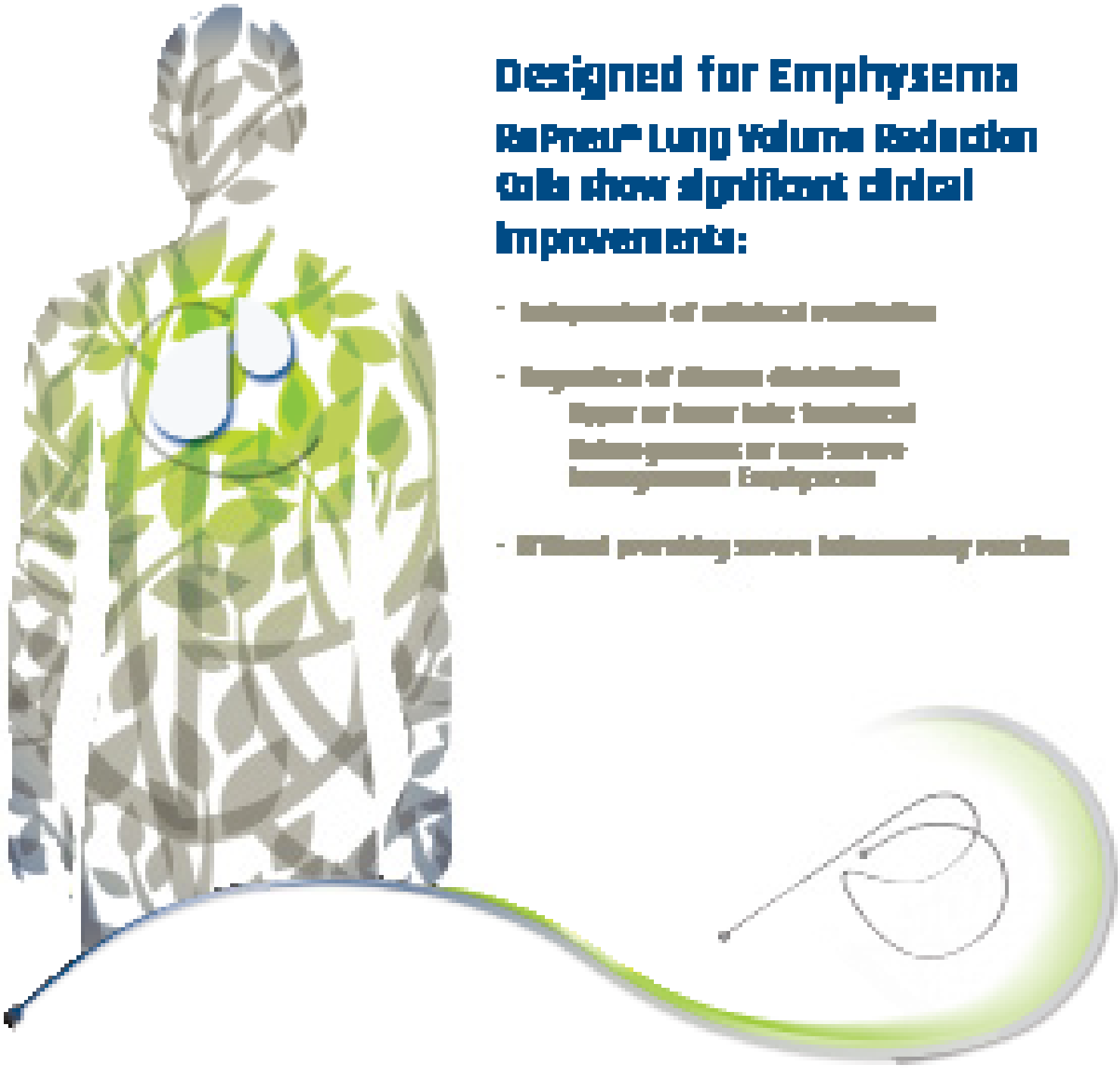

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Foreword

Professor Nicholas Hill

Professor of Medicine,
Tufts University, Massachusetts, USA

Dear Colleagues,

I am honoured to introduce the inaugural volume of *EMJ - Respiratory*. This journal aims to focus on recent advances in the respiratory field, presenting balanced and comprehensive reviews, original articles, practice guidelines, and case reports. The *European Medical Journal* provide articles by leading authorities in the field, written with busy physicians, clinicians and industrial professionals in mind, who are able to keep themselves updated and can access comprehensive bibliographies if they seek additional information. This inaugural volume presents a range of articles representing the journal's scope, including airway disease, interstitial lung disease, infectious lung disease, and rare conditions like pulmonary alveolar proteinosis.

At a time when there are multiple journals already publishing similar materials, others now entering the respiratory field, which is supported by well-known respiratory societies, prominent journals and a plethora of open access online respiratory journals, one might ask what purpose an additional respiratory journal serves? My answer is that multiple interests are served. Competition between journals is healthy, making them more creative with the features they offer and forcing them to strive for the best quality submissions. They also seek to improve the quality of the manuscripts they accept, employing biostatisticians and science writers to work with submitting authors. Perhaps of most value, authors and investigators in the field have more options for publishing, something especially important for younger, lesser-established authors.

On the other hand, one can't help worrying that a saturation point will be reached, where there are not enough high-quality articles to go around and the quality of journals will drop due to dilution. This is unlikely to impact well-established journals with high impact factors, which will continue to attract the best submissions, but it is a concern for lesser-known, newer entries. Furthermore, there is not a limitless supply of experienced, expert reviewers who can help to assure the quality of journal publications. I am not concerned about *EMJ - Respiratory* in this regard, however. The journal is supported by a large, well-experienced publishing company with a large, successful panel of other sub-specialty journals. The staff are experienced and skilled and the editorial board of *EMJ - Respiratory*, consisting of internationally-renowned experts in respiratory medicine, will offer expert guidance. This introductory volume exemplifies the quality of published articles we can expect from this new journal. So please join me in congratulating the editors and staff on this inaugural issue and wish them every success, which will be a boon for the field of respiratory medicine.

Professor Nicholas Hill

Past President of the American Thoracic Society (ATS); Professor of Medicine, Tufts University, and Chief of the Pulmonary, Critical Care and Sleep Division, Tufts Medical Center, Boston, Massachusetts, USA. Professor Hill holds a Distinguished Scholar Award from the American College of Chest Physicians. His clinical specialties include: pulmonary hypertension, mechanical ventilation, noninvasive ventilation, and general pulmonology.

ERS ANNUAL CONGRESS 2013

CONVENTION CENTRE DE GRAN VIA, BARCELONA, SPAIN

7TH - 11TH SEPTEMBER 2013

Welcome to the *European Medical Journal* review of the European Respiratory Society Annual Congress 2013

EMJ EUROPEAN MEDICAL JOURNAL

ERS ANNUAL CONGRESS 2013

CONVENTION CENTRE DE GRAN VIA, BARCELONA, SPAIN

7TH - 11TH SEPTEMBER 2013

Welcome to the *European Medical Journal* review of the European Respiratory Society Annual Congress 2013

THE 23rd Annual Congress of the European Respiratory Society (ERS), which took place from the 7th-11th September 2013, was an exciting event, hosting the largest congregation of respiratory specialists in the world. Held in the vibrant and diverse city of Barcelona, with its backdrop the perfect combination of modern and historical features, artistic, cultural and industrial activities, it was the idyllic setting for hosting this auspicious event.

The Congress attracted approximately 21,000 delegates, 470 invited speakers, 177 exhibitors, and 20 patient organisations, in a united front to strengthen the aims of the ERS and the therapeutic area which they seek to continuously develop. Intriguing topics covered by the Congress included the branch of respiratory medicine combined with large portions of a patient's everyday life, including collaboration with a football team to aid smoking cessation, the launch of a music video to help doctors remember asthma guidelines, and informative sessions on lung diseases ranging from childhood to adulthood.

This year's Presidential Summit was a monumental one, with Vice President Professor Peter Barnes being appointed to the role of President of the ERS. The platform was shared by the former ERS President, Professor Francesco Blasi, and focused primarily on updates from the Dublin session, highlighting research gaps, patients' health requirements, and innovative research for the future.

In an open letter to the members of ERS, posted on the society's official website following the Congress, Professor Barnes stated: "Despite the

"News about the ERS has already generated over 800 news cuttings in both the medical and popular press."

Professor Peter Barnes, President, European Respiratory Society

Congress being over for another year, we are still making headlines across the world. The week began with news about the ERS appearing on the international BBC News website, Svenska Dagbladet in Sweden and on the Agenzia Giornalistica Italia website in Italy.

"News about the ERS has already generated over 800 news cuttings in both the medical and popular press. I would like to thank all of you who joined us in Barcelona and those who contributed to the success of the Congress. We look forward to seeing you again in Munich next year."

Another focal point of the Congress was the ERS-funded award into the research of chronic pulmonary disease (COPD), which was awarded to Dr Martijn A. Spruit whose research observed the effects of pulmonary rehabilitation of patients with COPD. Also, results from TIOSPIR™, one of the largest-ever clinical trials on COPD by Boehringer Ingelheim, were also announced. The use of HandiHaler® or Respimat® in the delivery of tiotropium had varying effects in reducing COPD exacerbation. Furthermore, a new discovery into the link between obstructive lung disease and cognitive impairment, was also discussed.

Asthma was a key topic discussed at the Congress, with substantial results announced from a clinical trial testing the Boston Scientific Alair® Bronchial Thermoplasty (BT) system's effectiveness in treating severe asthma. Furthermore, the association of asthma with argan powder, an ingredient found in cosmetics, was also presented at the Congress, coupled with the possibility of severe asthma sufferers being less responsive to treatment in comparison to mild asthma sufferers.

Welcome to the *European Medical Journal* review of the European Respiratory Society Annual Congress 2013

The launch of the second edition of the 'European Lung White Book' was a highlight of the Congress. The publication has up-to-date information regarding lung health across Europe, including data on epidemiology, prevention, clinical care, education, costs, and research. The book can be used as a full reference for healthcare professionals, policymakers, patient advocates and the media, with recommended changes on the eradication of respiratory illness. Its corresponding website, http://www.erswhitebook.org/, was also launched at the ERS Congress, allowing open access to the book and all crucial information within.

Organised in conjunction with FC Barcelona and following the European Commission's 'Ex-Smokers are unstoppable' campaign, which raised awareness about lung health, spirometry testing and smoking cessation, attendees saw the 'Quit Smoking with Barça' initiative awarded the 2013 European Lung Foundation (ELF) award during the opening ceremony. Targeted at the 25-34-year-old age group, smokers attempting to kick the habit make use of the iCoach tool, which provides motivational assistance from Barça players, coaches, and staff in their specialised field. This campaign hopes to provide information to the many football fans across Europe, in hope of promoting and maintaining smoking cessation.

This was not even the full extent of organisation collaboration during the event, as ERS, along with ELF, hosted an event for free lung testing. Approximately, 1,500 individuals participated in having their lungs tested in Barcelona's city centre, with set targets expected to be surpassed at the next Congress in Munich 2014.

In keeping with the theme of outside events, air pollution was a key subject examined during the week. One of the sessions that focused on the effects of outdoor and indoor air pollution was presented by Professor Nino Kuenzli, highlighting the importance of clean air in respect to the healthy wellbeing of the population. He mentioned that one of the main causes of air pollution is due to traffic, and people – particularly children – living near to roads are susceptible to asthma. He also mentioned that there should be policies in place for the monitoring of air quality.

Even after a remarkably successful Congress, the ERS Board holds even higher aspirations for both the conference and the society as a whole, with newly-elected President Professor Barnes finalising his statement by speaking on his future vision: "Over the next year we have ambitious plans for the ERS and will be exploring several new opportunities in our strategic plan for the next 5 years. I will keep you informed as these new initiatives are developed and the ERS goes from strength to strength."

ERS relaunches White Book

'THE EUROPEAN Lung White Book' publication from the ERS, which sheds light on the extent of lung conditions throughout Europe, was relaunched ahead of the 2013 Congress.

Information regarding the burden, cost and risk factor for a range of respiratory diseases was compiled from the latest available research and statistics, to produce an all-inclusive publication to be used as a reference for healthcare professionals, politicians, and the public.

The four dominating respiratory diseases are lung cancer, chronic obstructive pulmonary disease (COPD), lower respiratory tract infections (including pneumonia), and tuberculosis. It has been documented that lung conditions are responsible for 1 in 10 deaths across Europe, particularly due to lung cancer and COPD, which are predicted to rise in the near future.

Among the wealthy countries in Central and Western Europe, Belgium and Denmark have the highest mortality from respiratory diseases, with 117 deaths per 100,000 population, closely followed by Ireland (114) and the UK (112). Though statistics show the smoking rates in these countries have fallen dramatically since the 1970s, the long-term consequences may continue to manifest in cases of lung cancer and COPD today. In contrast, Finland has the lowest death rate from respiratory conditions (54 per 100,000); this can be attributed to its highly-active programme which targets respiratory illness.

Interms of economic burden, the total annu al average and societal cost per case of lung cancer is €364,213, while for tuberculosis it is €86,217 (this includes multi-drug resistant and extensively drug-resistantforms). The cost of the more common respiratory issues, COPD (€6,147) and asthma (€7,443), represent an even greater socioeconomic burden.

> "By 2030, the WHO estimates that the four major potentially fatal respiratory diseases (pneumonia, tuberculosis, lung cancer and CODP) will account for about one in five deaths worldwide."
>
> *Professor Francesco Blasi, Past President, European Respiratory Society*

'The European Lung White Book' concludes that the proportion of deaths are likely to remain constant in the next two decades, with variations in different diseases balancing out the overall effect of mortality. The equilibrium effect may occur, where a decrease in deaths from lung infections will be accompanied by a rise in lung cancer and COPD mortality.

Professor Francesco Blasi, Past President of the ERS, said: "By 2030, the WHO estimates that the four major potentially fatal respiratory diseases (pneumonia, tuberculosis, lung cancer and CODP) will account for about one in five deaths worldwide, compared to one-sixth of all deaths globally in 2008. However, trends in conditions such as asthma are more difficult to predict."

Smoking is considered the most obvious preventable cause and the root of most respiratory diseases. In 2005, the World Health Organization (WHO) launched the Framework Convention for Tobacco Control (FCTC), a legally binding treaty now signed by 168 countries, in response to the globalisation of the tobacco epidemic. A minority of European counties have not fully implemented this legislation due to commercial, fiscal, and other pressures. Another contributing factor to respiratory disease is poor air quality, with standards recommended by WHO not being enforced by some countries.

Experts state that key measures such as immunisation programmes for those that have vaccines, monitoring of antibiotics and a Europe-wide monitoring of resistance patterns to antibiotics used in treatment of tuberculosis, pneumonia and other respiratory infections, should be maintained so that data are available to address health concerns and decrease the death rates across Europe.

'The European Lung White Book' is available, both online and in print, at http://www.erswhitebook.org/.

FC Barcelona joins with European Commission in bid to boost smoking cessation in Europe

ANNOUNCED at the opening ceremony of ERS Annual Congress, Barcelona, the 2013 ELF (European Lung Foundation) award was presented to the 'Quit Smoking with Barça' programme.

The programme, launched in December 2012, follows on from a previous campaign named 'Ex-Smokers are Unstoppable' (www.exsmokers.eu), and features an initiative which brings together a major public institution, the European Commission (EC), and a world-famous football club, FC Barcelona.

> "What impressed us most about this campaign was its unique approach to reach people who have previously not been successful at quitting smoking, or hadn't even tried to quit."
>
> *Monica Fletcher OBE, Chair, European Lung Foundation*

'Quit Smoking with Barça' is the product of a joint endeavour to help the 28 million smokers of Europe, within the 25-34-year-old age group, to quit smoking and kick the habit for good. Aiming to reach millions of football fans, the programme makes use of a tool dubbed iCoach. The new FCB iCoach features additional motivation directly from Barça players, coaches and staff, each from their own specific area of expertise: health, physical activity, food, stress management, relaxation, and others. Other initiatives, including a cash savings calculator, email tips and

motivational videos, are all designed to support fans to reach their goal of becoming an ex-smoker.

Monica Fletcher OBE, Chair of ELF, commented: "What impressed us most about this campaign was its unique approach to reach people who have previously not been successful at quitting smoking or hadn't even tried to quit. The campaign has helped these people quit for good, making a significant impact in our shared quest to highlight the importance of good lung health."

The programme starts with a questionnaire to assess smoking behaviour. Knowing how much, when, and where you smoke, makes it possible to provide specific advice, adapted to each personal situation.

"We are honoured to be here alongside FC Barcelona to receive this award for the 'Quit Smoking with Barça' campaign. I am pleased that the positive impact we've made through this partnership has been recognised by the wider health community," Paola Testori Coggi, Director General for Health and Consumers at the EC, said.

"70,000 Europeans have signed up to 'Quit Smoking with Barça' and nearly 400,000 are trying to quit with the wider 'Ex-Smokers are Unstoppable' campaign's smoking cessation tool, iCoach. We hope to continue to inspire even more people to reap the benefits of a smoke-free life."

iCoach incorporates techniques from cognitive behaviour therapy and aims to increase smokers knowledge, motivation and self-efficacy gradually over time, to change users' behaviour for the better. It is low-threshold, non-lecturing, and above all, interesting, using interactive elements such as diaries, regular feedback reports, graphic overviews, trigger emails, and so on. Most importantly, iCoach uses a positive approach, emphasising the gains associated with smoking cessation.

"We hope to continue to inspire even more people to reap the benefits of a smoke-free life."

Paola Testori Coggi, Director General, European Commission

The app provides support at every stage of smoking cessation, and even aids ex-smokers in maintaining abstinence by still enabling access to any of the programme's features and restarting the motivational email tips at any time.

Dr Jordi Monés, Head of FC Barcelona's medical area, commented: "We are delighted to have been able to collaborate with the European Commission on such an important public health issue. We are proud to stand alongside the EC, especially here in our city of Barcelona, to collect this ELF award."

Barça became the first football club in Spain to introduce a smoke-free stadium policy at their home ground, under the banner "Camp Nou Sense Fum" (A smoke-free Camp Nou). "It shows that thankfully our message is being noticed and that our combined efforts are making a positive difference," Dr Monés added.

"At Barça we firmly believe in our motto, 'more than a club', and we promote values of respect, health and social commitment. The "Quit smoking with Barça" programme allows us to live that motto."

Visit www.quitsmokingwithBarça.eu for more information on the award-winning programme.

Military weapon fire endangers soldiers' lungs

> "These lung function changes are comparable to the effects caused by other occupational risk factors, such as organic dust in farming and cotton workers."
>
> *Dr Anne-Katrine Boraonder, Oslo University Hospital, Norway*

SOLDIERS are risking their lung health through long-term exposure to firing military weapons, according to research presented at the ERS Annual Congress on Saturday 9th September.

A total of 55 non-smoking men from the Norwegian Armed Forces were tested by researchers from Oslo University Hospital and the Norwegian Defence Research Establishment. Any exposure to fumes from three different types of assault rifle ammunition, one leaded, and two lead-free, was examined.

The study investigated the effects of various weapons, and which components in the emissions were causing health issues, after Norwegian armed forces personnel begun to report ill health effects following live firing sessions over the past 5 years.

Though the type of ammunition used was shown to have no difference in effect, all groups experienced a decline in their lung function. A spirometry test – which measures FEV_1, the maximal amount of air forcefully exhaled in a second – revealed the mean average of lung function was reduced by 5% and 7%, at 1-2 hours and 24 hours after shooting, respectively.

Oslo University Hospital's Dr Anne-Katrine Boraonder, the lead author of the study, said: "The findings from our small sample show that fumes from military arms are causing a decline in lung function shortly after firing practice.

"These lung function changes are comparable to the effects caused by other occupational risk factors, such as organic dust in farming and cotton workers. Although we noticed this decline for all types of ammunition, further research can now be undertaken to look at specific exposure components to help design better ammunition, and to continue implementation of other measures for avoiding these effects."

New electronic tool helping to reduce pneumonia deaths

AN ELECTRONIC decision support tool, which helps to reduce deaths from pneumonia, was presented at the ERS Annual Congress on Wednesday 11th September. Based on initial findings, this new prospect could lead to improvements in pneumonia care and better outcomes for patients.

> "We are encouraged by the impact that our tool has had on death rates, and feel that it is most likely due to more accurate severity assessment and antibiotic decisions being made in accordance with the guidelines."
>
> *Dr Barbara Jones, University of Utah, USA*

In emergency settings, the full implementation of pneumonia guidelines can be difficult. In light of this, researchers have developed an electronic tool linked to patients' medical records, which automatically extracts data that predict the severity of pneumonia. The tool also provides information regarding where the patient should be admitted and recommendations of diagnostic tests and appropriate antibiotics.

The effectiveness of the tool was tested on two groups of pneumonia patients in seven emergency departments, by researchers from Intermountain Healthcare and the University of Utah, USA. The first group of 2,308 patients was analysed before the electronic tool was used, and the second group of 2,450 patients was assessed when four of the seven emergency departments used the tool.

Hospital admission rates, length of hospital stay, deaths, secondary hospital stay, secondary hospitalisation rates, and the adherence to guidelines, were all noted in both groups.

There was a significant reduction in death rates in the emergency departments where the tool was used. Inpatient mortality fell from 5.3% to 3.5% and, after adjusting severity, there was a 25% reduction in risk of death.

Dr Barbara Jones, the leading author of the study, said: "We are encouraged by the impact that our tool has had on death rates, and feel that it is most likely due to more accurate severity assessment and antibiotic decisions being made in accordance with the guidelines. While we are encouraged by the results, we plan to collect more data to explore how the tool is making this impact."

Dr Nathan Dean, a senior author of the study, said: "Although doctors are free to choose at any time whether to follow the recommendations, we think that a tool that is individualised and integrated into the electronic health record is a more efficient way of supporting decision-making and making treatment guidelines quickly accessible during an emergency situation."

Increasing adherence to TB medication through virtual monitoring

MOBILE phone video technology has been proposed to improve patients' adherence to their prescribed TB medication and was presented at the ERS Annual Congress on Sunday 8th September.

This new approach is an alternate version to the directly observed treatment (DOT) recommended by the WHO, an older method deemed time-consuming for the patient and resource-intensive for outreach projects.

The virtually observed treatment (VOT), requires patients to submit a short video of themselves taking their medication to their healthcare provider using a mobile phone. This is usually done after the initial visit to the clinic and continued remotely until a follow-up appointment.

This method was given a trial run at the Royal Free London NHS Foundation Trust to test its effectiveness. There were nine participants who started the programme, two of whom did not submit any video clips and reverted back to the clinical-based approach. Six of the seven remaining patients fully complied with the treatment and sent in their video clips for a minimum of 95% of all treatments.

There was an observed 86% of scheduled doses with compliance to the treatment. Due to this finding, it was concluded that this is a practical method for monitoring TB treatment.

Lead author, Dr Sara Hemming, said: "These preliminary findings suggest that telemedicine can help us overcome the difficulties we've seen in directly monitoring patients taking their medicine. Some people are unable or unwilling to visit clinics for a variety of reasons, but by enabling healthcare professionals to virtually monitor patients, we can still ensure effective medication use without the need for a one-to-one session.

"This has the potential to not only reach people who are otherwise unengaged, but also reduce costs and resources. A larger trial comparing the two techniques is needed to ensure the safety and reliability of this technique, and also determine who benefits most from this approach."

Past President of ERS, Professor Francesco Blasi, said: "The European Lung White Book, which is launched this week, calls on countries with high rates of TB, to set up strategies to manage the large numbers of people with drug-susceptible and multidrug-resistant-TB (MDR-TB).

"This study shows one way that could improve the effectiveness of treatments for people who are particularly hard-to-reach. The outcomes of this pilot study are positive and I look forward to seeing the results of a larger trial."

"This has the potential to not only reach people who are otherwise unengaged, but also reduce costs and resources."

Dr Sara Hemming,
Royal Free London NHS Foundation Trust

Kicking the habit: e-cigarettes versus nicotine patches

THE COMPARISON of e-cigarettes with nicotine patches was carried out in a pioneering trial and was presented at ERS 2013. Both nicotine replacement therapy methods resulted in similar degrees of success in terms of smoking cessation for a period of 6 months after the 13-week course of the study.

This trial was led by Associate Professor Chris Bullen, Director of the National Institute for Health Innovation at the University of Auckland New Zealand; his team of researchers enlisted 657 smokers looking to quit smoking. The volunteers received a 13-week supply of the test material, which is commercially available. They were divided into three groups: 292 received e-cigarettes (containing 16 mg of nicotine), 292 received nicotine patches, and the remaining 73 received placebo e-cigarettes that contained no nicotine.

Professor Bullen said: "Our study establishes a critical benchmark for e-cigarette performance compared to nicotine patches and placebo e-cigarettes, but there is still so much that is unknown about the effectiveness and long-term effects of e-cigarettes. Given the increasing popularity of these devices in many countries, and the accompanying regulatory uncertainty and inconsistency, larger, longer-term trials are urgently needed to establish whether these devices might be able to fulfil their potential as effective and popular smoking cessation aids."

After the 13-week duration and a further 3-month follow-up, the participants underwent testing to determine if they were able to abstain from cigarettes. Participants in the e-cigarettes group had the highest rate of success in terms of completely quitting smoking, with 7.3% compared with 5.8% of those in the nicotine group and 4.1% in the placebo group.

> "Our position is clear: we need more research on the positive or negative effects of these products."
>
> *Professor Franceso Blasi, Past President, European Respiratory Society*

Interestingly, approximately 90% of participants in both of the e-cigarette groups would be inclined to recommend their product to family and friends, while in the patches group, just over half (56%) would do so.

"Our position is clear: we need more research on the positive or negative effects of these products," Past President of the ERS, Professor Franceso Blasi, said.

"This study has taken us one step closer to understanding the effectiveness of these devices as a quitting aid, but we still need long-term independent clinical trials and behavioural studies."

Road pollution kills

LIVING closer to busy roads can mean a higher risk of death for people suffering from bronchiectasis, according to data presented at ERS on the 8th September.

The study observed the association between the number of deaths in a group of 189 people with non-cystic fibrosis, between June 2006 and October 2012, and their residential distance to a main road. Bronchiectasis, often caused by cystic fibrosis (CF), is a condition where the lungs' airways become abnormally widened, resulting in a build-up of excess mucus.

Results found a hazard ratio of 0.36 for every ten-fold increase in the distance to a major road, the conclusions adding to the growing body of evidence demonstrating the potentially damaging effects of road-side pollution.

Lead author, Dr Pieter Goeminne, said: "Our results are the first to link air pollution with the risk of death in people with bronchiectasis and adds to a number of other studies showing the dangers of living close to a busy road.

"The findings of this study should encourage policymakers to make air quality a key focus of transport policies and consider the proximity of main roads to residential areas."

Then-ERS President, Professor Francesco Blasi, said: "This study has added crucial evidence to our understanding of how living close to a busy road can affect people with poor lung health.

"The European Lung White Book provides several key recommendations to help policymakers address this issue, and I would call on EU member states to make air quality an integral part of their transport policies."

EFA: Outrage at exorbitant oxygen prices for passengers

THE EUROPEAN Federation of Allergy and Airways Diseases Patients Associations (EFA) hit out at certain airlines charging exorbitant prices for oxygen therapy to people with severe respiratory diseases at the ERS Congress on Monday 9th September.

The EFA believes that these practices are discriminatory and so have collaborated with the European Lung Foundation (ELF) in the production of a new booklet. 'Enabling Air Travel with Oxygen in Europe' aims to provide information to patients, their carers, the general public and air crew.

EFA President Breda Flood said: "As EFA President, it is my pleasure to present this booklet as a cornerstone for a movement to call out the discriminatory policies employed by certain airlines and call upon policymakers

to improve the situation for patients with chronic respiratory diseases." These rules and regulations can vary between airlines, making it very confusing and costly to certain travellers. Airlines such as British Airways and TAROM have provided oxygen completely free of charge to their customers, while others continue to charge flat fees for both European and long-haul flights, potentially resulting in an increase in airfares of up to seven-times the normal amount.

Both the EFA and ELF state within the booklet that patients with respiratory illness and a dependence for oxygen during travel should have it available at all times and free of charge, either in the form of airlines' oxygen containers, or their own portable oxygen concentrators (POCs), checked and approved by the European Aviation Safety Agency.

Citing wheelchair accessibility on airlines as an example, the EFA believes that the introduction of oxygen therapy policies is possible across Europe, if backed by a European Commission mandate.

Monica Fletcher OBE, Chair of ELF said: "We believe it is completely unacceptable to discriminate against patients with lung diseases. The ELF Air Travel database, providing oxygen policies for major airlines, can help people select the most economical options, however our goal would be for clearer guidelines and for oxygen to be free and available on all airlines."

Innovative music video created to aid clinicians

A MUSIC video created to help doctors become more aware and remember guidelines used to treat asthmatic patients has been created. Dr Tapas Mukherjee from Glenfield Hospital in the UK, found there was a lack of knowledge in acute severe asthma management. Study results revealed that only 45% of healthcare professionals had used the hospital guidelines, while 62% of doctors were unaware of them.

It was this gap in knowledge which inspired Dr Mukherjee to create a music video, transforming the guidelines into memorable lyrics and posting the video on social media websites.

"As doctors are often working in busy environments, we have to think of creative ways of reaching them with important clinical information," said Dr Mukherjee.

The music video had a formidable impact, as the follow-up study revealed that 100% of healthcare professionals involved were now aware of the guidelines.

These results did not only improve all aspects of asthma management and knowledge, showing significant results for chest radiograph indication and target oxygen saturation, but it also displayed the power of music and social media websites.

Dr Mukherjee suggested: "The method could be adapted to different scenarios and the possibilities are not limited by resources of money, but only by imagination."

Dr Mukherjee's achievements were recognised as he won both the British Thoracic Society Innovation in Education Award 2012, and the NHS Expo/Network Casebook II Innovation Award 2013.

Global trial TIOSPIR™ is breath of fresh air

A LANDMARK trial reinforced the importance, safety, and efficacy of the two available SPIRIVA® formulations, it was revealed at ERS 2013 on Tuesday 10th September.

Including over 17,000 patients, TIOSPIR™ was one of the largest global chronic obstructive pulmonary disease (COPD) trials, designed to test both the unique Respimat® Soft Mist Inhaler and the dry powder inhaler HandiHaler® delivery devices.

> "The critical message from this large-scale TIOSPIR™ trial is that physicians can be confident that they can prescribe this proven maintenance therapy across the severity spectrum of COPD patients."
>
> *Prof Antonio Anzueto, University of Texas, USA*

Published in the *New England Journal of Medicine*, the 3-year trial demonstrated that both formulations had a significant impact on patients' lives.

Professor Antonio Anzueto, Professor of Medicine at the University of Texas Health Science Center, USA, said: "Importantly, this large and rigorous clinical trial provides evidence that tritropium is safe in a broad population of COPD patients, including those with a history of cardiac disease.

"The critical message from this large-scale TIOSPIR™ trial is that physicians can be confident that they can prescribe this proven maintenance therapy across the severity spectrum of COPD patients."

Participants were recruited and randomised between May 2010 and April 2011, in more than 1,200 investigator sites in 50 countries. Results showed that the median time to COPD exacerbation was over 2 years for both formulations. Respimat®, distributing 2.5 μg in two puffs once daily, and HandiHaler® distributing 1.8 μg, were found to be fully-effective at 756 days and 719 days respectively.

New possibilities for sleep apnoea and melanoma

THE SEVERITY of sleep apnoea can indicate how aggressive malignant skin melanoma will be, research presented at ERS 2013 has shown.

While previous studies have been conducted in mice to demonstrate the response of reduced oxygen levels in the blood on tumour growth, this is the first trial which has studied the effects on humans, which has linked skin melanoma specifically with sleep apnoea.

The research, carried out by Dr Francisco Campos-Rodriguez, of the Hospital de Valme, Spain, discovered that of the 56 patients that were studied, 14.3% had severe sleep apnoea. Melanoma in these patients was more aggressive, compared to the 60.7% of patients in which sleep apnoea was not as severe.

Moreover, the severity of sleep apnoea was linked with both tumour growth rate, and the depth of invasion.

Lead author Dr Francisco Campos-Rodriguez, from the Hospital Universitario de Valme in Seville, Spain, has emphasised that although this is a small trial, it is the first one which has been conducted in humans. Also, if these results are mirrored in his larger trial, involving 450 patients, the clinical implications for treating sleep apnoea could be very important, potentially paving the way for new possibilities for treating patients with both conditions.

Antibiotics prescribing method can reduce patient harm

A NEW way of prescribing antibiotics has proven to reduce patient harm and will also combat the rise in antibiotic resistance, according to data shown at ERS 2013.

The results from the study indicated that there was a 20% reduction in antibiotic use, as well as a 40% reduction in antibiotic-related side-effects. Dr Matthew Lloyd, the lead author from the University of Dundee, UK, said: "The threat from growing resistance to antibiotics is increasing, which is in part attributable to inappropriately lengthy courses of antibiotics. Our study aimed to implement a simple system for preventing patients taking antibiotics for longer than they should."

Throughout the 12-month trial, 500 patients with lower respiratory tract infections were observed. The researchers monitored the side-effects of the antibiotics, including symptoms, length of hospital stay, and death rates.

The new prescription strategy ensured there were automatic stop dates, and pharmacists ensured that these stop dates were clearly visible for patients. There were also time limits on prescriptions, which depended on the severity of the infection.

Dr Lloyd found the results promising, saying: "Through prescribing automatic stop dates and working with our multidisciplinary colleagues, we can help prevent this problem and reduce patient harm."

These new protocols could prove to be highly valuable in reducing both patient harm, and also combatting antibiotic resistance.

> "The threat from growing resistance to antibiotics is increasing, which is in part attributable to inappropriately lengthy courses of antibiotics."
>
> *Dr Matthew Lloyd, University of Dundee, UK*

Project kick-started to categorise asthma subgroups

AN EU-FUNDED project, U-BIOPRED (Unbiased BIOmarkers in PREDiction of respiratory disease outcomes), hopes to kick-start the process of categorising asthma disease and allow researchers to develop specialised treatment for each subtype.

Initial research, presented at ERS, has found common characteristics among children and adults with severe asthma. 55% of adults that are prescribed oral corticosteroids show greater airway obstruction than mild/moderate cohort. Also, despite taking high doses of corticosteroids, severe asthma sufferers still experience exacerbations and other unpleasant symptoms. In children, the level of airway obstruction in severe and mild/moderate asthma was similar. The severe asthma group had higher fraction exhaled nitric oxide (FeNO) levels, which is used to diagnosed asthma.

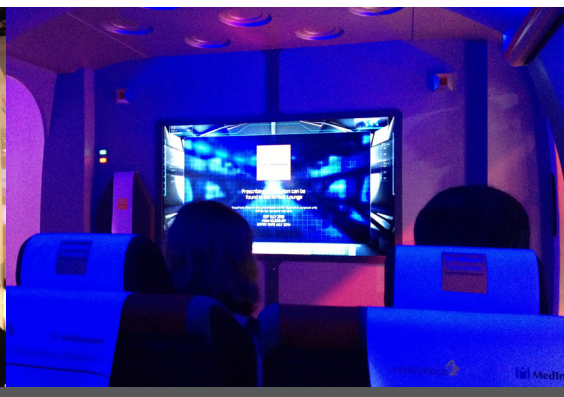
This is the first analysis of a wide range of patients from intensive research projects that will collect over 3 million samples from 300 children and 700 adults with severe and non-severe asthma, and without asthma.

Professor Peter Sterk, the project leader for U-BIOPRED, said: "In order for us to help improve the lives of these people, we need to make a full biological and clinical 'fingerprint' of each patient, by embarking on a huge analysis of data including a wide-range of samples from CT scans, to sputum samples, analysis of a person's genetics and results from bronchoscopies. The U-BIOPRED project is doing that."

In the UK, 5.4 million people are currently receiving treatment for asthma. Asthma in adults is more common in women than men, and it is becoming increasingly known that there are different types of the condition. It is not understood why some people suffer a more severe form of the disease, hence need for categorisation of subtypes.

"We would like to understand why people with more severe asthma are less responsive to the effects of corticosteroids," Dr David Gibeon, lead author of this study from Imperial College, London, said.

"This initial analysis will provide an overview of the groups which exist within asthma, which will help us develop a more personalised approach to treating the individual patient with asthma."



Cancer breathalyser: Diagnosing lung cancer via patients' breath

A COLLECTION of exhaled breath from people that are deemed at high risk of lung cancer can be used to diagnose the disease, according to initial findings presented at ERS 2013.

The current tests used to diagnose lung cancer are blood and urine tests, followed by CT scans and chest radiographs. This new method merely involves analysing the breath of the patient to quickly assess their symptoms.

The new research is based on the fact that animals are capable of detecting diseases through breath tests, with scientists harnessing this concept by attempting to replicate an "electronic nose" technology. This works by detecting different profiles of volatile organic compounds (VOCs) in breath samples. Scientists have not clearly identified the key VOCs which linked to different diseases, but this study suggests that it is possible to differentiate lung cancer from different lung conditions and healthy people.

Researchers from the University of Latvia collected exhaled breath samples from 252 lung cancer patients, 223 patients with different lung diseases or healthy volunteers, 265 non-smokers, and 210 smokers.

The electronic nose correctly identified 128 non-smokers as having lung cancer, and misdiagnosed 5 people who did not. There were 114 people in the smokers group who were correctly identified as having lung cancer, with 5 being misdiagnosed.

Lead author, Dr Maris Bukovskis, from the University of Latvia, said: "We have shown that it is possible to use breath tests to correctly identify lung cancer with a high rate. The results of our study take us one step further to understanding this important new technology.

"The major problem with electronic nose technology is that it is individual, and each piece of equipment must be trained to distinguish between odours. This causes a problem of standardising the practice between different centres. The next step will be to test the practice between different centres to help us think about how we can ensure consistency between all the results."

Revolution in testing for TB

NEW tests which could diagnose drug resistance in tuberculosis earlier have been developed by US researchers, according to data revealed at ERS. In the past, traditional methods of drug-susceptibility took between 21 days and 3 months. These new tests only take 15 days.

Professor Antonino Catanzaro, from the University of California, USA, said: "Our findings suggest these three tests could provide a quicker way to identify patients who need alternative treatment regimes. This is very important and could potentially save lives as well as help to curb the rise of drug-resistant TB."

The researchers trialed these three new tests: pyrosequencing, a DNA-sequencing technique, the HAIN line probe test, which detects genetic mutations in the bacteria, and the microscopic observation drug susceptibility (MODS) test, in over 1,000 patients in India, Moldova, and South Africa, and compared them to standard drug susceptibility tests.

The findings showed a 95-98% accuracy rate, and can be used as an alternative to standard testing, increasing the possibilities open to clinicians. Furthermore, the efficiency of these tests will provide a quicker cure time and will prevent the development of even more drug-resistant TB.

Professor Catanzaro pointed out however, that although the tests have advantages and disadvantages, a range of options are crucial to ensure that all TB treatment programmes worldwide have access to a method which is tailored to them, one which takes into consideration financial restrictions.

Argan powder linked with occupational asthma

THE FIRST evidence of respiratory issues associated with the use of argan powder in the production of cosmetics has been presented at ERS.

Nine workers from a cosmetic factory in France, who were exposed to the product in its three forms – crude granules, powder, and liquid – were participants in the study. Each individual completed a medical history questionnaire and underwent lung function tests, allergy tests, and an inhalation challenge test. The latter test examined the airways' specific reaction to argan.

The results show that four out of the nine participants had asthma or rhinitis symptoms and blocked nose when handling argan powder, three of whom had occupational asthma caused by argan powder evidenced by specific challenge tests. Out of the proportion of participants, two had a positive test for skin prick-test to argan powder.

Dr Emmanuelle Penen, lead author of the study, said: "Occupational asthma can be a debilitating condition if it prevents a person from working. This study is very preliminary but does suggest an association between argan powder and occupational asthma. Our initial findings warrant further research to understand any health risks associated with the compound."

Obstructive lung disease patients experience cognitive functioning decline

NEW information was released indicating the link between obstructive lung diseases (OLDs) and weakened cognitive functioning at ERS 2013 on Monday 9th September.

This research suggests that those with OLD, including chronic obstructive pulmonary disease (COPD), regularly experience a decline in cognitive functioning. This new study however, determines which particular cognitive functions are impaired.

Dr Fiona Cleutjens worked with the Netherlands' Center of Expertise in Chronic Organ Failure (CIRO), and concentrated on people with OLD, investigating which domain-specific cognitive functions were influenced. Researchers used the UK Biobank Resource, a reserve of over 500,000 Scottish, English and Welsh people aged between 40 and 70, focusing on genetics, environmental exposures, and lifestyle.

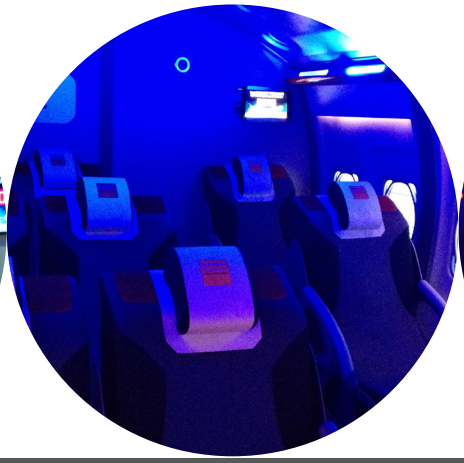
43,039 people were involved in this research; of this, 5,764 had OLD. They were required to engage in various cognitive tests to examine cognitive functioning. The 13.4% with the condition had poorer test results compared to those without the disease, with the exception of the fluid intelligence test.

The results of the prospective memory test indicated that those without OLD performed better, including pairs-matching tests and a reaction time test.

Researchers have thus concluded that people with OLD are highly prone to experiencing cognitive impairment, most notably memory and information processing. Dr Cleutjens stated: "We know that OLD can often exist alongside other conditions and our new study has found evidence that OLD is linked with problems with memory and information processing. This can be very debilitating, especially for someone who is already dealing with the symptoms of OLD.

"Our findings suggest that healthcare professionals need to be aware of the possible impact of cognitive impairment in the self-management, clinical management and pulmonary rehabilitation of OLD patients."

The researchers could not determine the causality of this association, although Dr Cleutjens suggested hypoxic injury to the brain as a possible mechanism.



Annual COPD award announced

AN ERS-FUNDED annual award for research in chronic obstructive pulmonary disease (COPD) was officially opened to applicants at the ERS Congress in Barcelona.

The 'ERS COPD Research Award', supported by Boehringer Ingelheim, is offered as an achievement award to young researchers who have made an outstanding scientific or research contribution to any sector of COPD.

The award will be presented at the 2014 ERS Annual Congress in Munich, and the winning candidate will be requested to make a short presentation of the outcome of their project within 1 to 2 years.

This year's award went to Dr Martijn A. Spruit, from Horn, the Netherlands, for his research on the effects of pulmonary rehabilitation in individuals with such complex chronic lung diseases as COPD.

Although one award is granted annually for €50,000, in 2014 the award will be split by the ERS Scientific Committee into two. In addition to the main awards, a travel grant of €1,000 will be given to each of the 15 best abstracts on COPD.

Available only for members of ERS aged under 45, the online application form is available at www.ersnet.org/funding, with the deadline set at February 28, 2014.

POOR ASTHMA CONTROL, DEVICE HANDLING AND PHENOTYPE

Summary of Presentations Given at the Teva Symposium, ERS Annual Congress 2013, Barcelona, Spain

Chair: Roberto Rodiguez-Roisin[1]
J. Christian Virchow,[2] Cynthia Rand,[3] Richard Dekhuijzen,[4] Michael Wechsler[5]

1. Senior Consultant Physician, Service of Respiratory Medicine, Hospital Clinic, Barcelona, Spain
2. Head, Department of Intervnal Medicine, Pulmonary Division, University of Rostock, Germany
3. Pulmonary and Critical Care Medicine, Johns Hopkins University School of Medicine, Maryland, USA
4. Radboud University Medical Center, Nijmegen, the Netherlands
5. Professor of Medicine, Director of Asthma Programme, National Jewish Health, Colorado, USA

Disclosure: The speakers received honorarium from Teva for participating in this symposium.
Acknowledgements: Writing assistance provided by Trilogy Writing and Consulting Ltd.
Support: The publication of this article was funded by Teva and Teva received a Medical Accuracy review.
Citation: EMJ Respir. 2013;1:30-52.

INTRODUCTION

The aim of this symposium is to discuss the impact of poor treatment and poor inhaler technique on asthma outcomes. In addition, the symposium will explore the aetiology of asthma and the mechanistic role of IL-5 in severe asthma.

Developing the 'Ideal Inhaler'

J. Christian Virchow

Generally in asthma management the hypothesis is that poor device techniques in asthma patients affect outcome; it is assumed that if the patient does not or cannot inhale correctly there will be no change in outcome (the outcome would not be worse, but there would be no effect because no inhalation has taken place). Secondly, it is predicted that the poorer the inhaler technique the poorer the outcome for the patient will be. Thirdly, it is likely that improvements in technique or device will improve compliance and possibly outcome.

The reasons for poor asthma control are: underestimation of disease severity by both the patient and the physician; delay in diagnosis or possibly the wrong diagnosis; under treatment including a delay in inhaled corticosteroid (ICS) therapy; poor compliance; incorrect inhaler choice; incorrect inhaler technique and insufficient instructions to the patient. In addition, guidelines are not always implemented correctly,[1] all of these reasons contribute to poor asthma control.

It is often thought that patients referred for uncontrolled asthma should receive more therapy however, this is not always accurate. Bush et al.[2] stated in a recent editorial that *'...at least half of those referred to specialists with so-called therapy resistant asthma, in fact need to get the basics right rather than indulge in expensive biologicals'*.

It is difficult to adequately define what constitutes errors that occur with inhalation devices. Therefore, it is important to establish a hierarchy of errors: important errors and less important errors. Important errors are probably those where no drug is delivered at all and less important errors are when a partial amount of the drug released is actually inhaled.

Many studies that have looked at the efficacy of inhalation technique contain a patient population

that are 'inhalation experts' due to patients who were unable to inhale being excluded. Consequently, the efficacy of inhalation therapy shown in controlled clinical studies has always been evaluated in patients who can inhale. However, in clinical practice many patients fail to inhale correctly.

The requirements of a device for optimal inhalation are that it tolerates errors and does not allow any crucial errors to occur that result in failure of the drug being delivered and consequently treatment failure. The choice of inhalation device is complex and the guidelines do not advise on the choice of device. It is considered that the correct choice of inhalation device is the cornerstone in the effective management of asthma and chronic obstructive pulmonary disease (COPD). The reality is that inhalation devices are often chosen on an empirical basis, e.g. some like red more than pink, others like powder more than pressurised metered-dose inhalers (pMDI). Device selection is rarely based on evidence based awareness, and a considerable number of physicians caring for asthmatics have poor knowledge on the appropriate selection and use of inhaler devices.[3,4]

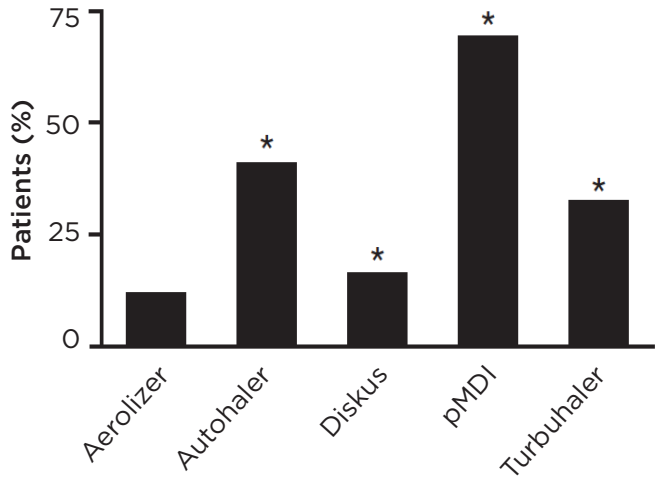


Figure 1. Percentage of 3,811 patients with at least one device-dependent error.
*p<0.05 versus best results adjusted by age and gender.
Blaiss MS.[6]

Poor inhaler technique is highly prevalent as shown in a study by Giraud et al.[5] The study of 3,955 patients classified patients into good users (29%) and misusers with at least one error or omission (71%). In addition, patients were sub classified into misusers with poor coordination who were unable to coordinate inhalation and actuation of the device (39%) and misusers with good coordination but made an error with the inhalation (32%). In a study of 3,811 patients a significant number of patients with poor inhaler technique had at least one device-dependent error ($p<0.05$ versus best results adjusted by age and gender).[6] The results showed a considerable difference between the types of device being used (Figure 1). pMDI were shown to be the most problematic inhalation device concerning device dependent errors.

Understanding and teaching inhaler technique is very important. A study from the United States[7] evaluated 56 medical interns (who were at the height of their theoretical medical knowledge) and assessed the process of correct pMDI administration this was:

1. Remove cap
2. Shake inhaler
3. Hold inhaler upright
4. Tilt head back or keep at level
5. Exhale to functional residual capacity or residual volume
6. Insert or keep mouthpiece 2–4 cm away from mouth
7. Begin breathing then actuate canister once
8. Continue slow, deep inspiration
9. Hold breath for 5–10 seconds
10. Exhale, wait 20–30 seconds before second dose
11. Shake again before a second actuation

56% of the group had not received any training with the devices; on the first attempt 5% of the group got the process of administration correct. After a large group lecture only 13% achieved the correct process of administration. It required one-on-one training to achieve the correct process of administration in 73% of the group. This is an important consideration when educating patients in the correct use of inhalers. When each specific step in the process was analysed, only 82% of the group removed the cap,

after one-on-one instruction this increased to 100%. In the most important part of the inhalation process in terms of lung deposition (begin breathing then actuate canister once), less than 40% of the group were correct, after group training there was a negligible increase in using the correct technique. It was only after one-on-one instruction that the number that used the correct technique increased to 79%. This study shows that correct pMDI administration was a challenge to healthy medical interns and should be considered in light of patients who have severe breathing difficulties.

Problems with the correct administration of pMDIs are extremely prevalent and teaching inhaler techniques has variable results, for example, in a study of 1,200 patients 86% used incorrect inhaler technique.[8] Following instruction and using a device that monitors correct inhalation 76% of patients used incorrect inhaler technique however, on the third attempt this reduced to 61% of the patients using the incorrect inhaler technique.

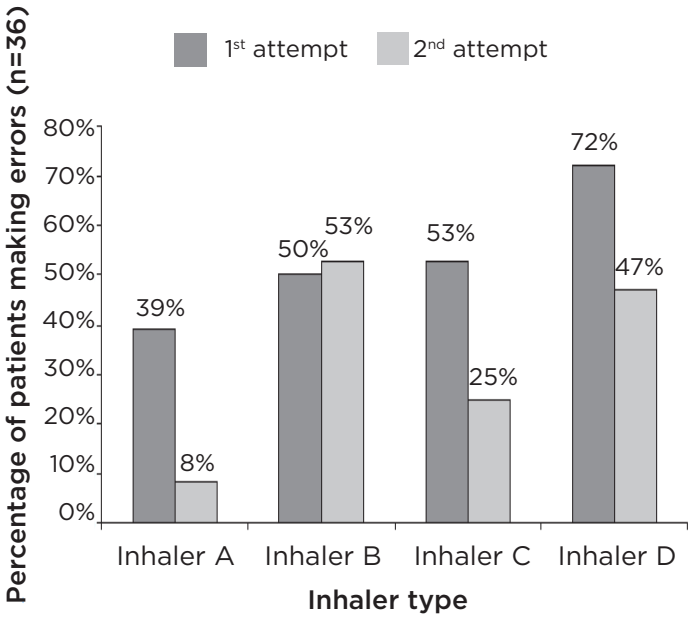


Figure 2. Critical errors with four DPIs, after reading the instructions and after personal instruction.
Schulte M, et al.[9]

Dry powder inhalers (DPIs) show different results; Schulte et al.[9] evaluated the use of DPIs after reading the instructions and after a personal instruction (Figure 2). The results showed that 72% of patients using inhaler 'D' made critical errors after reading the instructions, following personal instruction this reduced to 47%. Using inhaler 'B' 50% of patients made critical errors after reading the instructions and this number increased to 53% after personal instruction, indicating that personal instruction was detrimental to correct inhaler technique in this instance.

A study in the United States evaluated five visits (at 0, 1, 2, 3 and 6 months) to the pharmacist.[10] 97 patients received an average of 2.5 minutes of individual coaching on inhaler technique per visit. At entry 7% used the Turbuhaler correctly and 13% used the Diskus correctly. After 3 months this significantly improved to 85% and 96% respectively, however at the 6 month evaluation correct inhaler use reduced to 50% in the Turbuhaler group and 79% in the Diskus group, indicating that between the 3 and 6 month visits a large proportion had forgotten what they had been taught by the pharmacist. Even though there was a reduction in correct inhaler use at six months, the results showed that there was improvement in peak expiratory flow (PEF) and asthma quality of life (AQoL) at both 3 and 6 months.

It has been shown that switching inhalers is problematic and having more than one inhaler type can be confusing for the patient. There is an increased level of misuse if patients have different types of inhaler or their inhalers are switched.[11] In addition, patients can get confused over the appropriate inhaler technique for different devices.[12] Van der Palen et al.[13] compared the use of Diskhaler, Rotahaler and Turbuhaler and found that when used in combination with each other the percentage of patients who use the correct technique is low. For example, when using the Diskhaler and Turbuhaler only 35% of patients used the correct technique and attained 100% scores when using the two devices. The number of patients using the correct technique was even less with a DPI and pMDI (<35%). In practice many patients are on DPI fixed dose combinations because there is a lack of availability of short-acting beta2-agonists in the same device.

The consequence of switching inhalers was illustrated in a retrospective matched cohort study.[11] The study used the UK General Practice Research Database to identify patients who changed inhaler without consultation with their General Practitioner. The results showed

successful treatment in 19.7% of patients in the switched cohort compared with 34.3% in patients maintaining treatment with the same inhaler. Unsuccessful treatment was 50.7% and 37.9% respectively, indicating that if consultation had been sought it would possibly have reduced the number of patients who experienced unsuccessful treatment.

Improving inhalation technique can improve asthma control. In a study that evaluated patients with their own pMDI and inhalation technique compared with the use of a breath-actuated pressured inhaler,[14] the measurement of bronchodilation showed that if the patients used a breath-actuated pressured inhaler or were instructed on the use of their own pMDI, the effect was much better compared with the patient's own pMDI inhalation technique. This indicates that improved inhaler technique leads to improved pulmonary deposition and therefore improved asthma control. In addition, improved inhalation technique affects patient outcome, this was shown in a study of outpatient management by a paediatrician or asthma nurse in children with severe asthma.[15] The results showed an improvement of correct inhalation technique from 65% to 95% and this resulted in a lower corticosteroid dose and improved asthma control. Improving inhaler technique was the main factor in showing that it was possible to have improved asthma control with less corticosteroid.

Inhaler misuse is associated with decreased asthma stability. Giraud et al.[5] assessed whether the improper use of pMDIs was associated with decreased asthma control. The study of 3,709 patients were given an asthma instability score (AIS), a score of 0 indicated stable asthma and a score of 9 indicated totally unstable asthma. The results showed that asthma was less stable in pMDI misusers than in good users (AIS: 3.93 versus 2.86), and among misusers asthma was less stable in poor coordinators (AIS: 4.38 versus 3.56 in good coordinators). These results indicate that misuse of pMDIs, which is more frequent in poor coordinators, is associated with poor asthma control.

Medical visits for an exacerbated asthma condition or emergency visits have been shown to be more frequent in misusers with poor coordination, than in misusers with good coordination or in good users ($p<0.00001$).[5] Even if the device is used incorrectly but with good coordination, asthma stability is better than when the device

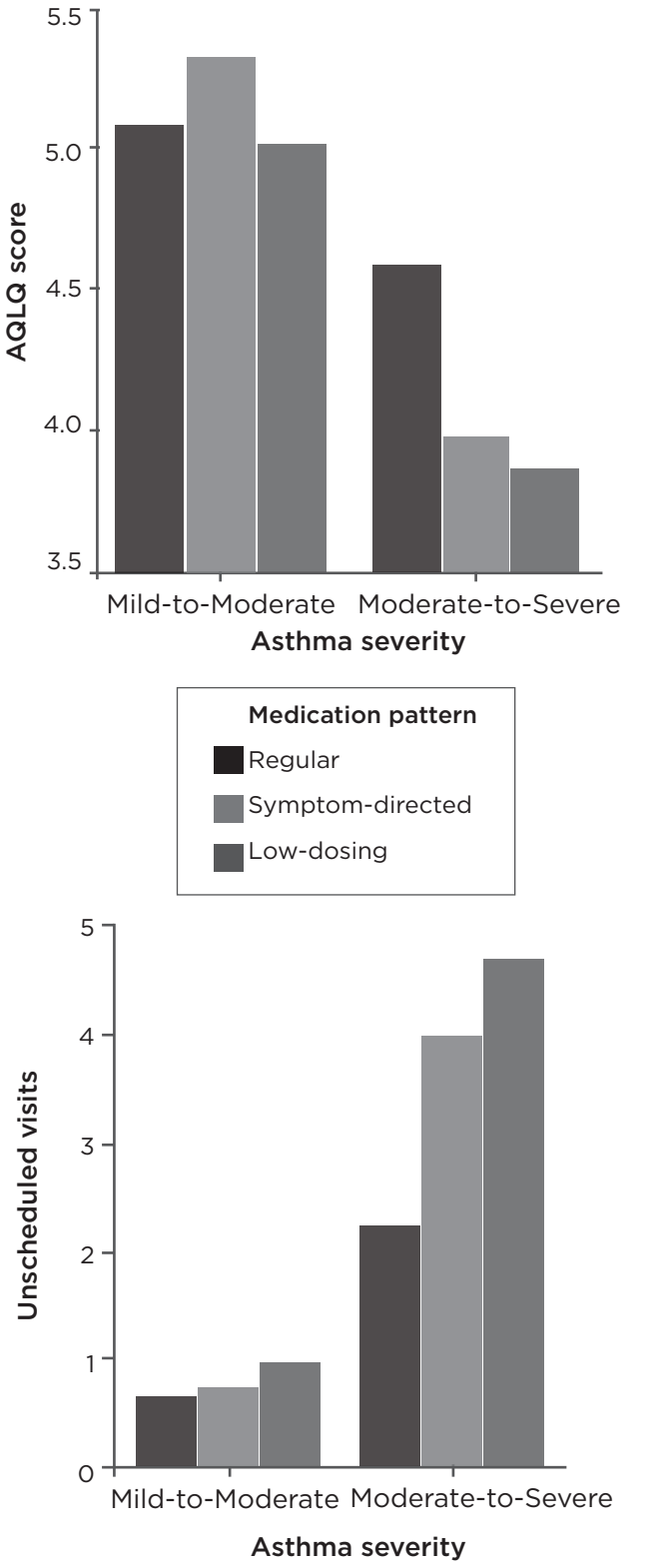


Figure 3. Non-adherence can be effective in milder asthma.
Greaves CJ, et al.[16]

is used correctly with bad coordination. This indicates rational non-compliance, which means that the device is more relevant for treatment success than the delivered drug, i.e. a patient may decide that the amount prescribed for them

may be more than they need for their personal feeling of control. This is shown in another study that assessed AQoL and unscheduled visits in mild-to-moderate asthmatics compared with moderate-to-severe asthmatics.[16] The results showed that mild-to-moderate asthmatics that take their medication regularly have a good AQoL and few unscheduled visits. In addition, in this group of patients, symptom directed medication use showed an increased AQoL and only a small increase in unscheduled visits. Patients receiving a low-dose experienced more unscheduled visits compared with patients that had a regular medication pattern or symptom directed medication (Figure 3). In moderate-to-severe asthmatics, an improvement in AQoL and a reduction of unscheduled visits was seen only if medication was taken regularly. In the mild-to-moderate asthma group, regular versus symptom directed inhaler use showed equally good asthma control.

Whether the compliance and the effect of treatment and inhaler competence are negatively correlated is an unresolved hypothesis. Patients need to take an increased number of inhalations per day if they have poor inhaler competence in order to compensate for the insufficient inhalation. If patients are competent at inhaling and are receiving the optimum dose from each inhalation, they may reduce their compliance because they feel they are getting enough relief and do not require anymore. Inhaler competence is the prerequisite for rational non-compliance but asthma management should not be controlled by patients who cannot inhale. There are economic advantages of correct inhaler use. Correct inhaler use results in improved asthma control, no stepping up, reduced medication needs, reduced medication costs, reduced need for additional prescriptions such as fixed dose combinations and programs to improve inhaler competence, compliance and adherence, all of which are likely to be cost saving.[12]

The recommendations for prescribing aerosol therapy are that all medications should come from the same device and only chlorofluorocarbon-free aerosol devices should be used. The decision for prescribing an inhaler should be based on the patient's ability to perform an inspiratory vital capacity manoeuvre. Inability to perform an inspiratory vital capacity manoeuvre requires inhalation from tidal volume e.g. a nebulizer or a hydrofluoroalkane (HFA) pMDI with a spacer and a valve. If the patient can perform an inspiratory vital capacity manoeuvre, the patient requires a single breath inhalation with a dry powder inhaler or HFA-pMDI (plus or minus a spacer).[1]

Poor device technique evidently affects outcome. This appears to be due to the fact that there is a large spectrum of handling errors that increase with the number of inhalers that individual patients have. Secondly, healthcare professionals' knowledge about inhaler technique is inadequate. These factors can be addressed by teaching patients to take their medication correctly; it has been shown that improved inhaler technique improves outcome. In addition, there are device-specific differences; DPIs appear to be more effective than pMDIs in terms of patient handling, therefore, checking a patient's inhaler technique prior to new prescriptions is essential; therapy should be individualised according to patient preferences and ability. The device should be simple and self-explanatory with no crucial errors and personal instructions should be repeated at every visit.[1,17]

The question is which is more important: the inhaler or the drug?

'...an old but well-known drug in a new, more reliable inhaler is probably more useful than a new drug in an old (flawed) inhaler'.[1]

How Important is Inhaler Adherence to Asthma Outcomes?

Cynthia Rand

The discussion surrounding poor asthma control continues. In the United States there is a significant problem with poor asthma control among African-Americans and higher rates of morbidity are seen in this group of patients. The asthma hospital discharge rate by race in the United States, 1980 to 2006,[18] illustrates a fundamental issue in asthma treatment. 30 years ago the armamentarium for managing asthma was very limited because there were only a few drugs available. Over a period of 25 years this has increased enormously. Now there are an increasing number of therapies that have been shown to be effective in controlled clinical trials and yet there does not appear to have been dramatic changes in asthma control, this is reflected by the continued

number of asthma hospitalisations and emergency room visits. There are many potential reasons for this and there is no definitive solution or perfect drug for treatment.

Efficacious drugs require patient adherence in order to achieve effective treatment outcomes. Patient adherence is how patients use their asthma medications and the relationship between that behaviour (the fundamental link) and asthma control. The European Community Respiratory Health Surveys (ECRHS)[19] that were conducted across multiple continents in asthma patients asked 'if you have been prescribed medicine for your breathing do you normally take all the medicine?' Consistently across all patient populations, patients reported that they did not take all the medication prescribed, clinicians are aware that this is common practice in asthma patients.

In the United States a four-state survey that looked at paediatric asthma asked parents how they gave preventive medication to their child with persistent asthma.[20] The participants were subdivided into white children (n=822), black children (n=294) and Latino children (n=369), the overall results suggested that the overall rate of adherence was less than 50%. Self-reported use of any preventative medicine in the last 3 months was 44% in the white subgroup, 30% in the black subgroup and 30% in the Latino subgroup (p <0.001). Looking at utilization, the suggestion was that the highest rate of morbidity, as reflected by emergency department use (≥1 emergency department visit in the last 12 months), was seen in those children who reported the lowest use of preventer medication; 39% in the black subgroup and 24% in the Latino subgroup, compared with 18% in the white subgroup (p<0.001). These results are surprising because one would think that parental concern in controlling their children's health would play a significant role in how parents used the medication prescribed for their children.

The Childhood Asthma Management Program (CAMP) is the largest study ever conducted in the United States. In an ancillary study[21] conducted in three of the eight CAMP Clinical Centres, adherence was assessed by using self-reported

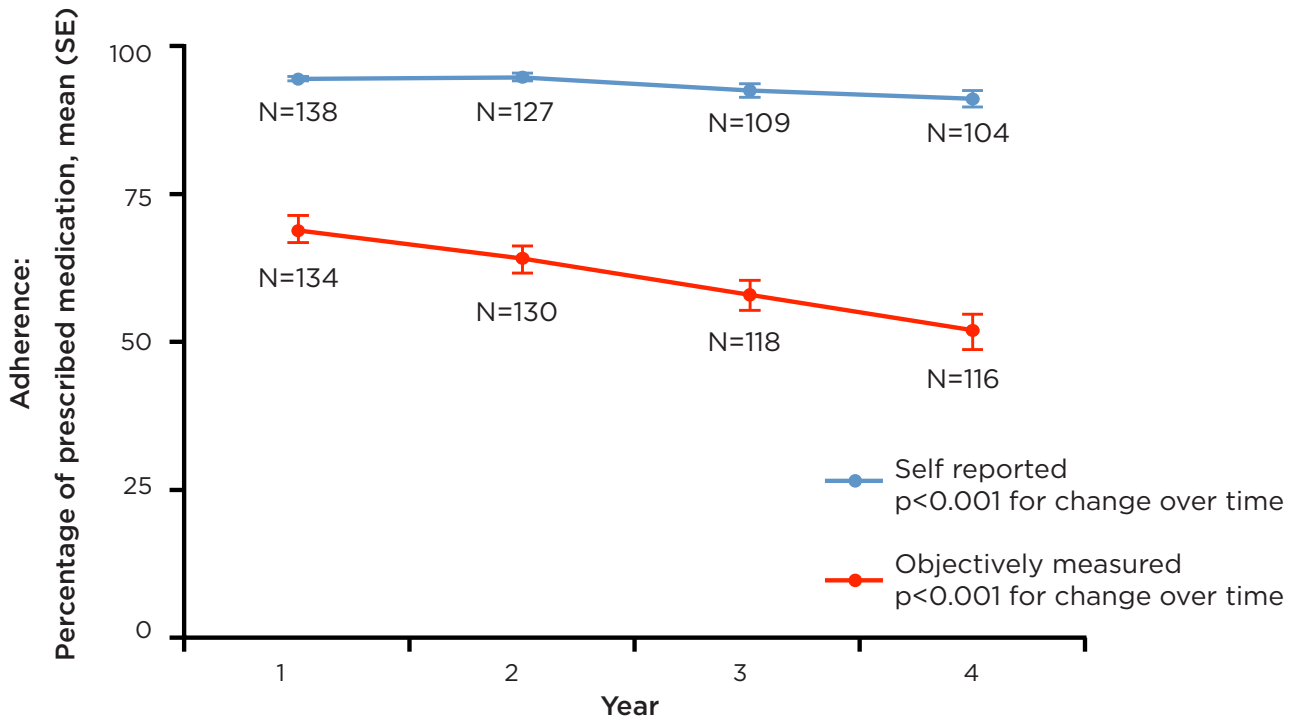


Figure 4. 4-year asthma diary and counter measures of adherence in the CAMP study.
CAMP: Childhood Asthma Management Program; SE: standard error.
Krishnan JA, et al.[21]

and objective data in 5-to 12-year-old children with mild or moderate asthma, who were randomly assigned to 200 mg of inhaled budesonide twice a day (n=84) or placebo (n=56) for 4 years. The Kappa statistic was used to evaluate agreement between self-reported adherence (daily diary cards), and objectively measured adherence (number of doses left in study inhalers) (Figure 4). It has been shown that, when asked to complete asthma diaries, patients consistently report a high use of therapy when asked to present the information in this form.

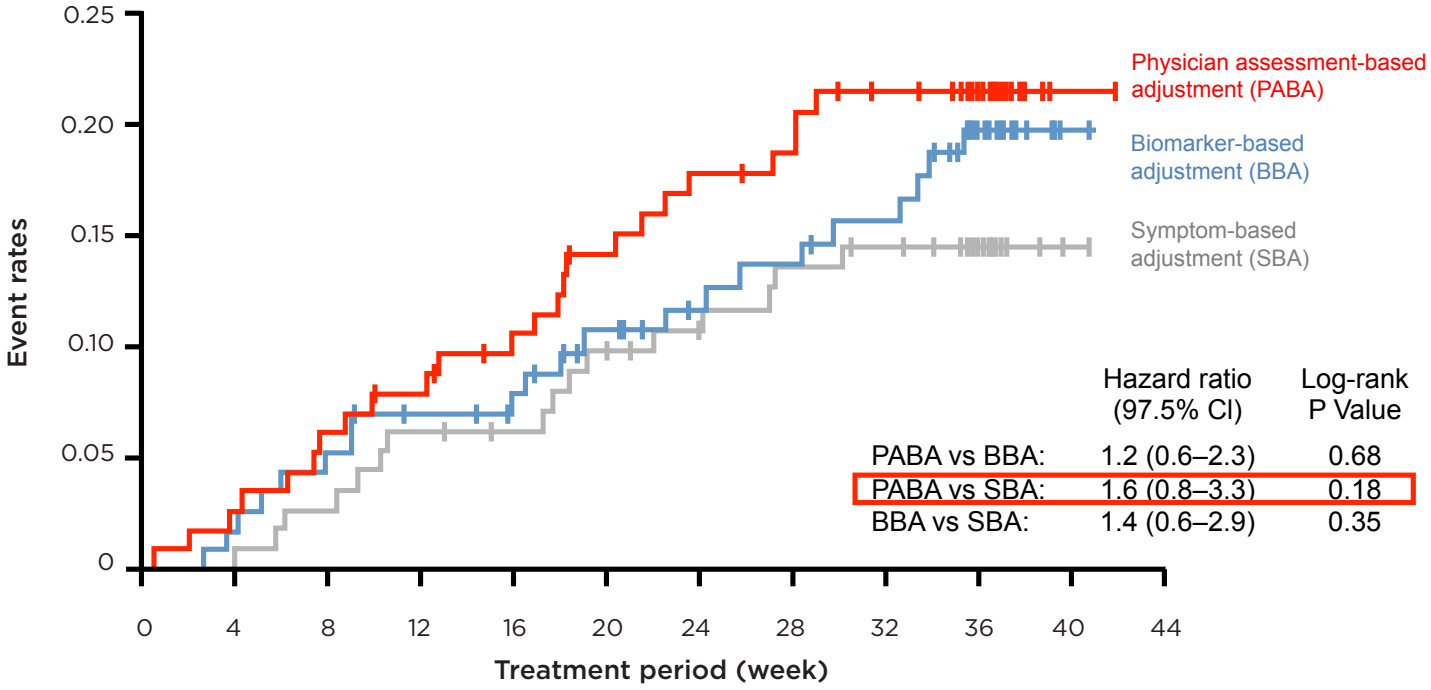
In this study self-reported asthma diary data suggested there was 85%-90% adherence. However, the data that were collected using objective measures showed that adherence was less than 75% at the beginning of the study, dropping to 50% by the end of Year four. The results revealed that overall adherence was low even among children who were in a carefully controlled trial with careful monitoring, nurse instruction at every visit and pre-selection of who was enrolled in the study; this shows that even under optimal conditions adherence was low.

The prevalence of no-adherence in severe brittle asthma (which is difficult to control) was examined in a retrospective, cross-sectional study of 182 patients attending the Northern Ireland Regional Difficult Asthma Service.[22] All 182 patients reported that they were taking their medication as prescribed. They all had 'difficult' asthma, which was defined as persistent symptoms despite treatment, and according to the Global Initiative for Asthma (GINA) guidelines,[23] were step 4 (reliever medication plus two or more controllers) or step 5 (reliever medication plus additional controller options). All of the patients were in specialist care consultation. The study evaluated the medication refill rates in the pharmacy records and found that 53% of the patients in this difficult to control asthma clinic had less than 75% adherence. Another study that evaluated the rate of adherence in patients receiving five different therapy types in a difficult to control asthma clinic, found that the majority of patients (74.8%) were non-adherent with their asthma medication.[24] This shows that, in the subgroup of patients with severe asthma, non-adherence is the missing link between effective therapies and effective control. In addition, Krishnan et al.[25] assessed medication adherence in patients who were hospitalised for asthma, 20% of whom had been intubated for asthma. Each patient's medication was electronically monitored following discharge from hospital. A monitoring device was used on the patient's inhaler and one on their medication bottle. This allowed data to be collected for inhaled steroids and oral corticosteroid adherence. The results showed that during the first 2 weeks following discharge from hospital, adherence dropped to less than 50%. This indicates that behaviour plays a fundamental role in adherence, even in life-threatening asthma.

Evidence suggests that inhaler adherence is associated with improved asthma outcomes. This was shown in a prospective study that evaluated patterns of steroid use among a range of patients with severe and mild-to-moderate asthma.[26] Adherence was assessed 6 months before a period in which exacerbation was monitored. A significant association with adherence of inhaled steroids and future events in this population was observed. Approximately 24% of asthma exacerbations were attributable to ICS non-adherence, and in this patient population, the benefit was only evident if the patients took 75% of their medication.

How much asthma morbidity could be averted by increased medication adherence? This question has been considered hypothetically using an algorithm, which was a very sophisticated modeling strategy. The algorithm looked at what is known about adherence and what is known about the association between adherence and outcomes.[27] The results suggested that in the United States alone, if patients were 100% adherent per year it would reduce unscheduled asthma visits by 3,700,000 visits (30%), reduce emergency department visits by 1,000,000 (20%), and would reduce hospitalisations by 300,000 (20%). Therefore, the potential benefit if adherence were improved is substantial.

It has been shown that the impact of adherence on AQoL and unscheduled visits in patients with mild-to-moderate asthma that are using asthma therapy on a symptom-directed-as-needed-basis, or lower dosing, does not appear to make a difference in terms of QoL or exacerbation. However, in patients with more severe asthma, the association between inhaler use and improved AQoL is clearly important.[16] This indicates that for patients with mild to moderate asthma, under-adherence may be acceptable. Similarly, in the BASALT trial[28] the outcomes of patients using symptom driven therapy versus physician



No. at risk											
PABA	114	111	107	104	99	94	90	88	82	60	1
BBA	115	112	108	104	101	94	89	87	83	58	1
SBA	113	113	110	106	104	100	95	90	87	68	1

Figure 5. Asthma treatment failure with symptom-based use of ICS versus physician or biomarker-based use of ICS: The BASALT trial.
BASALT: Best Adjustment Strategy for Asthma in the Long Term.
Calhoun WJ, et al.[28]

driven therapy showed that patients with mild-to-moderate asthma had improved outcomes compared with those using biomarker-based or physician-led therapy (Figure 5). The authors concluded that patients with mild-to-moderate asthma that under-adhere do quite well, this is consistent with physicians' observations in clinical practice. Whereas, the results show that the consequences of non-adherence to therapy are clearly more critical in patients with severe asthma.

The impact of adherence to therapy was evaluated by baseline forced expiratory volume in one second (FEV_1) in patients with mild-to-moderate asthma receiving prescribed fluticasone or montelukast asthma treatment.[29] Therapy was electronically monitored with the objective of identifying a dose response relationship between the amount of therapy patients took and the number of symptom free days. The only group that showed a dose response relationship was the group containing those patients who had slightly more severe disease (Figure 6), with baseline FEV_1 ≤86% of predicted. This supports the notion that adherence is fundamentally important in patients with greater underlying disease and less critical in patients with milder disease.

There are hidden barriers to adherence and asthma self-management; these include doctor-patient communication, depression and negative medication beliefs (patients do not like taking medications, are asymptomatic or fear side effects). Effective communication with patients is essential; in a study that used audio tapes of the physicians communicating with their patients about taking new drugs, it was found that 55% of the physicians only gave explicit instructions on the number of doses to take.[30] In addition, only 34% of the physicians discussed how long to take the medication for, and overall full medication directions were conveyed to less than 60% of

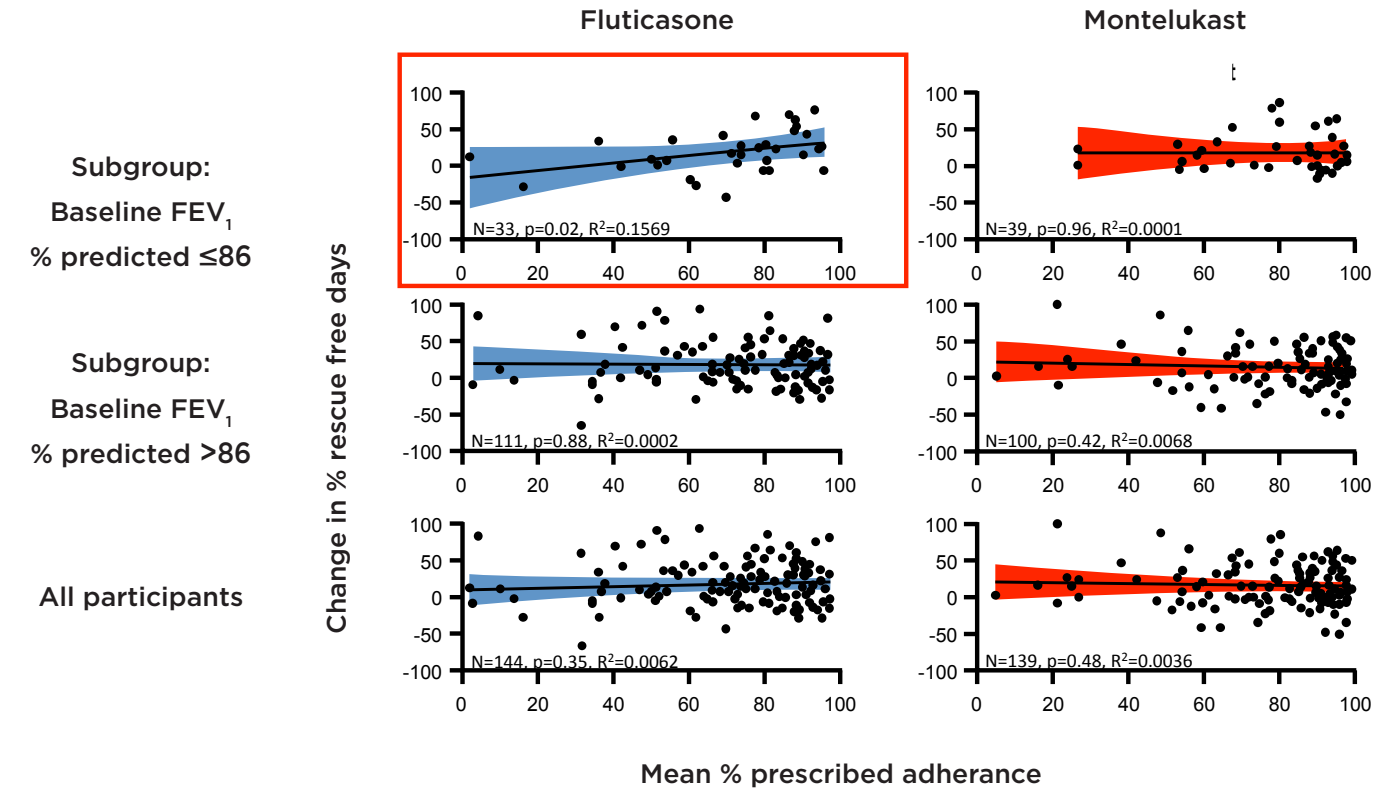


Figure 6. The impact of adherence with therapy on symptoms by baseline FEV_1. Change in percentage of rescue-free days by mean percent prescribed adherence.
Rand C, et al.[29]

patients. This highlights the fact that physicians should be explicit on how to use medication.

Beliefs about asthma medications are related to adherence with therapy. This was shown in a study in which parents of asthmatic children were asked their beliefs about asthma medication.[31] The study evaluated the association between what parents thought about therapy and whether the medication was actually taken. The results showed that when parents endorsed the statement 'my child does not need to take asthma medication every day', the child did not take the medication every day. When the parents endorsed the statement 'my child doesn't need as much medication as the doctor prescribed', the parents did not give full medication to their child. Overall, the study showed that when patients have high concerns about taking medication every day they are far more likely to be non-adherent. Therefore, ascertaining the medication beliefs of patients allows physicians to identify who is at risk from non-adherence.

Depression has been identified across every chronic illness as a substantial risk factor for non-adherence. Compared with non-depressed patients, depressed patients are three times more likely to be non-adherent with medical treatment recommendations.[32] In all racial and ethnic populations, patients with low economic status are at increased risk for both depression and poor adherence.[33] This was shown in a study in the United States that evaluated elderly asthma patients with depression.[34] The results showed that elderly patients with depression were substantially more likely to have poor medication adherence with their controller therapy and were substantially more likely to be hospitalised for asthma. This supports the fact that establishing whether depression is present may indicate whether patients are taking their medication as prescribed.

Adherence should be a component of personalised therapy, which can be accomplished by: 1) creating a paradigm shift and reframing adherence from good patient/bad patient to critical information or valuable clinical data that allow improved patient management; 2) routinely collecting refill data and self-reported adherence data, including behaviour, beliefs and concerns;

3) using patient adherence levels and patterns to identify patient-specific responses to treatment and personal risks of non-adherence; 4) personalising and tailoring asthma therapy not only for the drug, the dosing and the device, but for the adherence expectations in order to best fit each patient's risk, needs and preferences.

Non-adherence with inhaler therapy is common even in severe disease. Across all populations there is clear evidence that adherence is associated with better outcomes. In addition, there is evidence that patients with mild-to-moderate persistent asthma may tolerate lower levels of adherence with minimal impact. Clinicians should work together with their patients to personalise therapy by determining the optimal level of dosing and adherence necessary to effectively control asthma.

The Various Types of Uncontrolled Asthma

Richard Dekhuijzen

The primary goal of asthma management is to achieve overall asthma control. Asthma management is aimed at the level of current control and the level of future risk. Current control is defined by symptoms, activity, reliever use, and lung function. Future risk is defined by instability or worsening of the disease, exacerbations and the severity of exacerbations, loss of lung function, and the adverse effects of long-term medication.[23]

The algorithm for assessing the level of asthma control is to check daytime symptoms, limitations of activities, nocturnal symptoms/awakening, need for rescue/reliever treatment, and lung function (PEF, FEV_1). Levels of control are defined as uncontrolled, partially controlled or controlled. The prevalence of uncontrolled asthma was evaluated in a large telephone survey of asthma patients;[35] the survey found that 95% of patients were uncontrolled. A later study evaluated patients who had a physician's diagnosis of asthma and a prescription for inhaled steroids.[36] The results showed that 51% of these patients were uncontrolled and 21% were partially controlled. Uncontrolled asthma is a huge problem; O'Byrne et al.[37] identified that the areas where problems occur are: learning abilities in younger children, focused attention, exercise limitation and reduced cardiovascular fitness, increased risk of severe exacerbations, accelerated lung function decline, increases in medical consumption, mortality, and costs.

There are three main categories of uncontrolled asthma. The first category is untreated asthma due to poor availability of diagnostic procedures and/or medication; this is an immense problem in low income countries. The second category is uncontrolled asthma due to inappropriate recognition and/or handling of modifiable factors and comorbidities. The third category is severe asthma with poor asthma control and/or frequent exacerbations, despite high dose ICS and a second controller such as oral corticosteroids (OCS).[38] It is important to know the specific cause or causes of uncontrolled asthma in an individual patient as this will initiate individualised non-pharmacological interventions, prevent over-prescription and/or overuse of medication, and initiate specific pharmacotherapy.

Several approaches have been suggested to find the cause of uncontrolled asthma, for example Bel et al.39 suggested an algorithm for uncontrolled/severe asthma (Figure 7). If a patient with asthma is uncontrolled despite 500 mcg ICS a day, with or without a long acting beta2-agonist (LABA) it is a concern, and a practical approach to treatment is required. The ABCDE(F) scheme[40] algorithm in uncontrolled asthma can help in the treatment of such patients:

A Is it really (and only) **A**sthma?

B Are all **B**ronchial triggers known?

C Is **C**ompliance optimal?

D Can the patient handle the **D**evice

E Is **E**very small airway reached?

F Is a specific **Ph**enotype present?

A Is it really (and only) **A**sthma?

There are a lot of diseases that will mimic or overlap asthma, e.g. a viral wheeze in children, emphysema, bronchiectasis, COPD, and chronic cough.[41] In addition, there are many suspected alternative or additional diagnoses in adults which are sometimes difficult to distinguish from asthma, e.g. vocal cord dysfunction, recurrent pulmonary embolism, bronchiolitis, Churg-Strauss

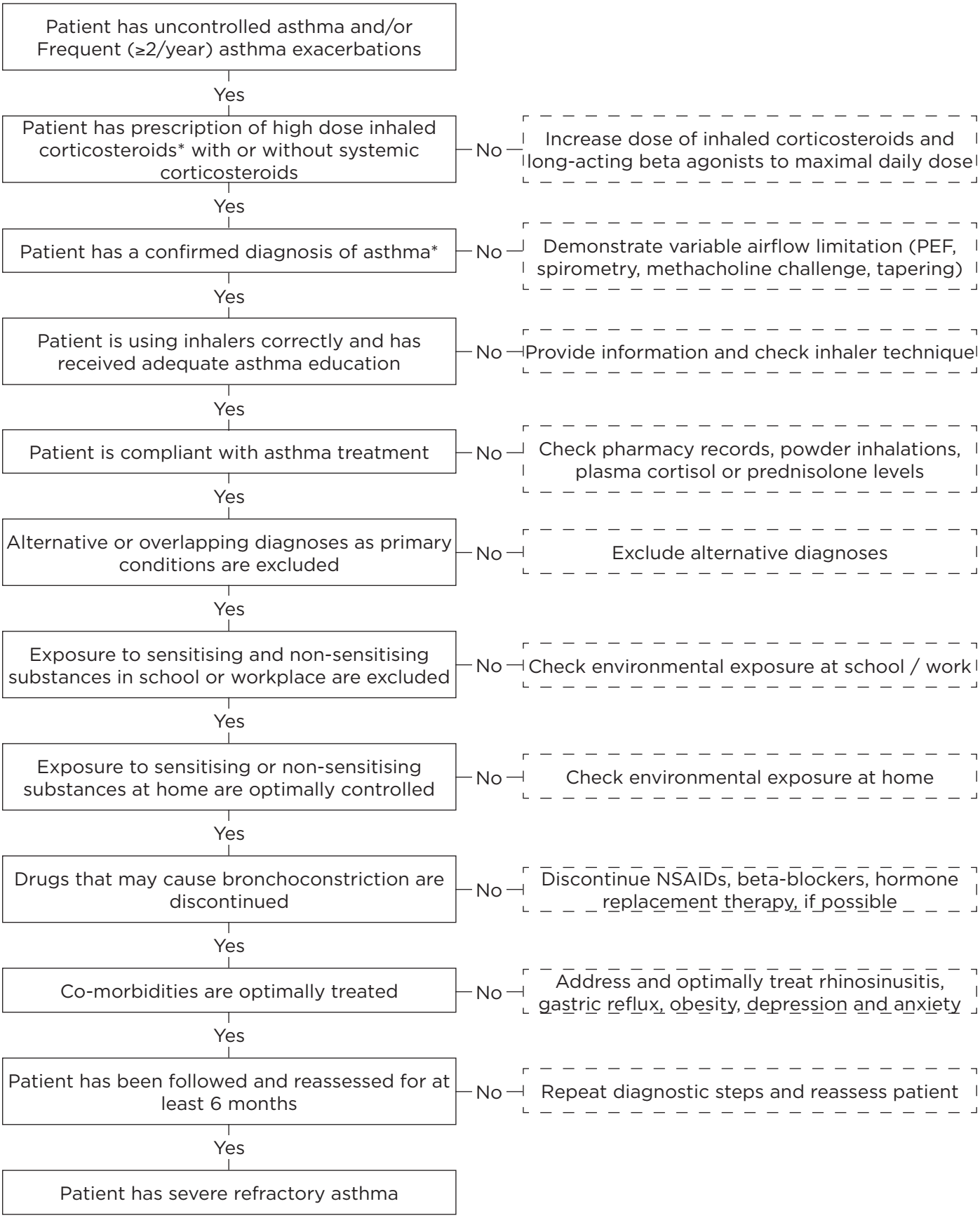


Figure 7. An example of an algorithm in case of uncontrolled/severe asthma.
NSAID: non-steroidal anti-inflammatory drug.
Bel EH, et al.[39]

syndrome etc.[39] Another consideration is that the asthma patient may have multiple comorbidities. In a study of risk factors of frequent exacerbations in difficult-to-treat asthma patients the frequency distribution of comorbid factors was significantly associated with frequent severe exacerbations.[42] In all patients with frequent exacerbations (n=39), more than one factor (severe sinus disease, gastric reflux, recurrent respiratory infections, psychopathology, or obstructive sleep apnoea) could be detected. In addition to asthma, 12% of the patient population had one comorbid factor, 36% had two comorbid factors and 40% had three comorbid factors. Comorbidities have an impact on outcome and the study showed that patients with >3 exacerbations per year had significantly more prevalent psychosocial dysfunction, severe sinus disease, gastric reflux, or recurrent respiratory infections compared with patients with only one severe exacerbation ($p<0.05$). This highlights the importance of establishing whether the patient really (and only) has asthma.

B Are all **B**ronchial triggers known?

There are many trigger factors which include: outdoor and indoor allergens, environmental pollutants, toxic fumes, occupational agents, and medication.[39] 10-15% of adults with asthma have work-related complaints; rhinitis is often the presenting symptom. Over 200 compounds have been associated with work-related asthma; a thorough history is required to enable the possibility of identifying the causative agent.[43] Some triggers are very well known for example smoking; patients with asthma who are current smokers have less asthma control compared with those who have never smoked or those who are ex-smokers.[44]

C Is **C**ompliance optimal?

In chronic asthma patients, inhaled medication is in the cluster of the lowest adherence; only 20% to 30% still use their asthma medication as prescribed after 2 years.[45] The consequence of low adherence is less asthma control.[44]

D Can the patient handle the **D**evice?

There are three important issues that need to be considered in order to make an optimal match between the patient and the device: 1) is the patient able to inhale consciously? The elderly, cognitively impaired, and children should be considered; 2) is the patient able to generate a sufficient inspiratory flow rate? 3) Is the patient able to coordinate well?[46] A patient who demonstrates that conscious inhalation is possible, and has sufficient inspiratory flow and good coordination, can be prescribed almost any device (Figure 8).

E Is **e**very small airway reached?

More attention is being paid to pathology in the small airways in patients with asthma. In the bronchioles, the patency of the small airways is significantly reduced in patients with asthma.[47] Closing volume (CV) and closing capacity (CC) are parameters for airway closure and air trapping, and thus measure small airway patency. Severe asthmatic patients with recurrent exacerbations (unstable asthma) have increased CV and CC compared with equally severe but stable asthmatic control patients, even in well-controlled episodes. Patients with recurrent exacerbations are prone to earlier airway closure and are at risk for excessive airway narrowing.[48] This suggests that airway closure at relatively high lung volumes but clinically stable conditions might be a risk for severe exacerbations in asthmatic patients.

Patients with less severe asthma (step 2, 3 and 4) have abnormal values of peripheral airway resistance. A study that assessed small-airways disease using alveolar nitric oxide (NO) and impulse oscillometry in asthma and COPD showed that 64-70% of step 2, 3 and 4 asthmatic patients were shown to have abnormal patency of the small airways.[49] This indicates that even in relatively mild disease, there is small airway involvement and this should be considered in terms of its contribution to the severity and lack of control in these patients. In patients with mild asthma, bronchial NO is not correlated with asthma control. However, asthma control and alveolar NO demonstrate a statistically significant relationship.[50] This suggests that inflammation in the periphery of the lung may contribute to less control of asthma. The involvement of the small airways is difficult to assess in clinical practice. If an assessment of the history of the patient's asthma is made, bronchial triggers excluded, compliance confirmed, and the device is satisfactory but there is still uncontrolled asthma, it may indicate small airway involvement. A suggested treatment would be to give an ICS

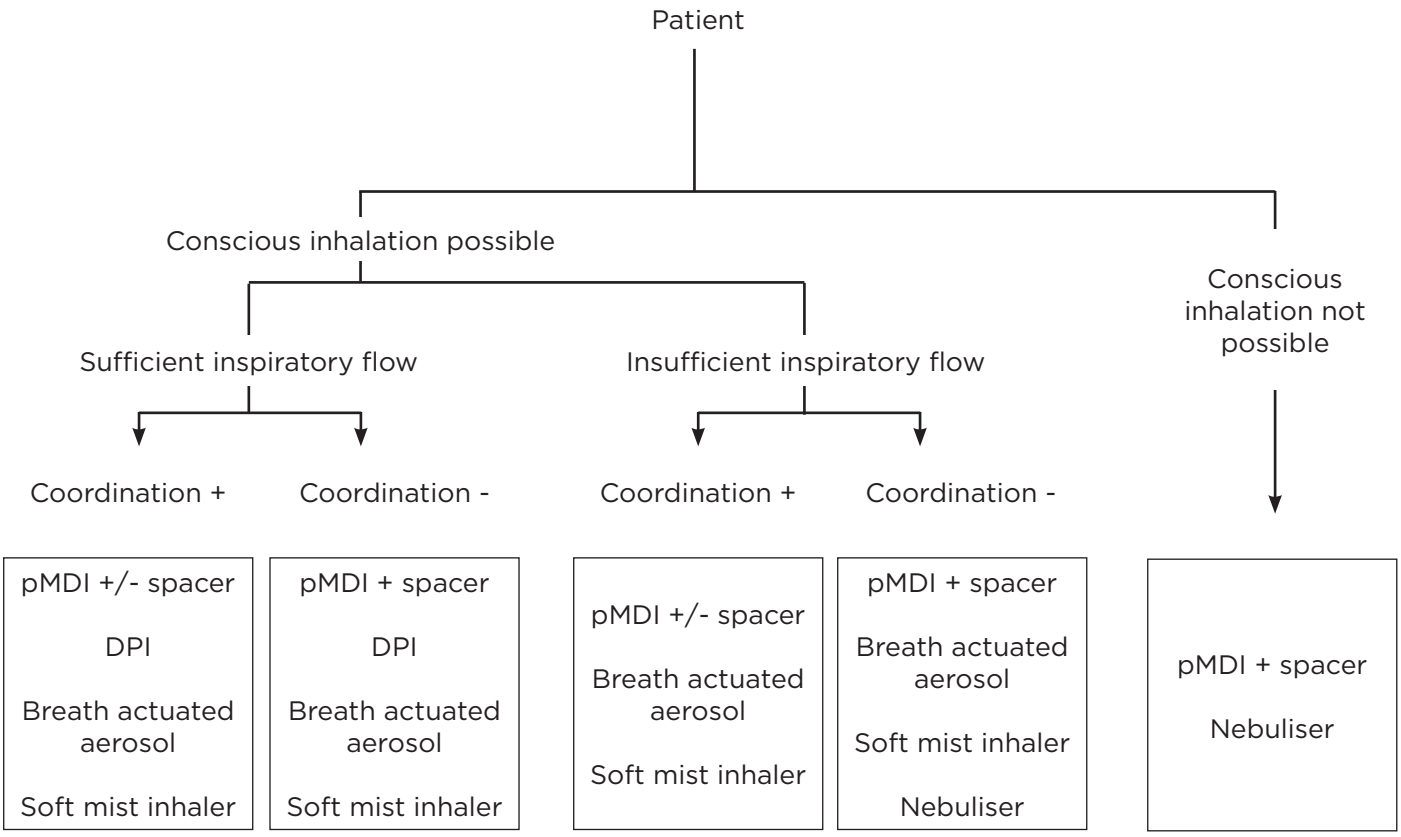


Figure 8. Inhaler therapy for adults with asthma: can the patient handle the device?
pMDI: pressurised metered-dose inhaler; DPI: dry powder inhaler.
Dekhuijzen, PN.[46]

with asmall particle size for 3 to 6 months, if the treatment is successful, the patient will indicate an improvement signified by fewer complaints, more exercise capacity and fewer exacerbations.

F Is a specific **Ph**enotype present?

Asthma is driven by T helper 2 (T_H2) (in children this includes allergic asthma, exercise-induced asthma and aspirin-exacerbated respiratory disease) and non-T_H2 phenotypes (in adults this includes very late-onset asthma in women, obesity-associated asthma, smoking-associated neutrophilic asthma, smooth muscle mediated paucigranulocytic asthma).[51] The phenotype may have therapeutic consequences. There is a difference between phenotypes and their response to therapy, for example the phenotype 'early-onset allergic' is corticosteroid responsive which is T_H2 targeted and is relatively easy to treat. Conversely, the treatment of adult onset obesity-related asthma is more difficult because there is a lack of T_H2 and the target for therapy is less clear. This type of asthma is responsive to weight loss, antioxidants and possibly hormonal therapy.

In summary, uncontrolled asthma occurs frequently and is a huge problem for the patients. There is a wide spectrum of causes of uncontrolled asthma and it is of great importance that the cause of uncontrolled asthma is found in individual patients. Several of the causes of uncontrolled asthma can be handled by non-pharmacological interventions. There are specific phenotypes where specific pharmacological interventions are indicated.

Severe Asthma: The Role of IL-5

Michael Wechsler

There is a huge proportion of the asthma population that remains poorly controlled. This is mainly due to poor inhaler technique and poor adherence. New options are required and many different therapies are being developed which will become available within the next decade. The main goal in the treatment of asthma patients is to optimise their asthma management. It is clear that adherence and inhaler technique need to

be addressed, but additional new therapies need to be developed to help in the management of these patients.

The national Asthma Education and Prevention Program (NAEPP) 2007 guidelines[52] for the management of asthma recommend escalation of therapy in patients who are poorly controlled. This means that as patients become increasingly inadequately controlled, doses of corticosteroids are increased and long acting beta-agonists, leukotriene modifiers and anti-immunoglobulin E (IgE) are added to the treatment regimen. However, despite these recommended measures and the use of the current therapies for asthma (short-acting beta-agonists, LABA, ICS, leukotriene modifiers, anti-IgE, systemic steroids, immunotherapy, anticholinergics [short-acting], and ipratropium), asthma control remains poor. The question is 'what to do next?'

The underlying pathophysiology of asthma denotes the specific cellular elements that need to be targeted; these are the mast cell, basophil, eosinophil, neutrophil, macrophage, dendritic cell, lymphocyte, and fibroblast, all of which are involved in asthma pathogenesis. One of the key cells involved in asthma pathogenesis is the eosinophil. Eosinophilic cytokines contribute to the chronic inflammatory process, in addition they have an interrelationship with other cells (epithelial, basophil, smooth muscle, mast, endothelial and neutrophil). Eosinophilic cytokines contribute to the activity, dysregulation and protonation of all the other cells that contribute to asthma pathogenesis. A number of cytokines released by eosinophils have autocrine growth-factor activities. These cytokines, Regulated on Activation, Normal T cell Expressed and Secreted (RANTES), IL-3, IL-5, and granulocyte-macrophage colony stimulating factor (GM-CSF), create a feedback loop for eosinophil expansion and activation. IL-5 is especially important in asthma and facilitates the maturation, activation and degranulation of eosinophils. In addition, IL-5 enhances the longevity of eosinophils by inhibiting apoptosis.[53] Other cytokines produced by human eosinophils that may have activities in acute and chronic inflammatory responses include IL-1, IL-6, IL-8, Tumour necrosis factor alpha (TNF-α), and both transforming growth factors (TGF), TGF-α and TGF-β.[54]

The effector functions of eosinophil-derived cytokines are tissue repair and remodelling, innate immune cell interactions with mast cells, modulation of adaptive immunity, autocrine regulation, effects on nerve cells, and angiogenesis.[55] Eosinophils and their cytokines play a large number of roles in many of these different features of asthma.

There is a substantial amount of evidence linking eosinophils to asthma. It has been shown that there are increased numbers of eosinophils in symptomatic allergic asthma patients,[56] whether it is in the blood, the sputum or the tissue. Patients with airway hyper-responsiveness and airway limitation are associated with increased numbers of eosinophils. Understanding the role of eosinophils in the management of asthma is essential because treatments that decrease eosinophil numbers, whether it is systemic steroids, inhaled steroids, leukotriene modifiers, or IL-5 targeted therapy, result in improvement in asthma control.

Castro et al.[57] studied bronchial biopsies taken before and after treatment in 25 subjects with moderate persistent asthma. The subjects were treated for 30 days with inhaled fluticasone propionate (1760 μg/day) followed by a withdrawal period that lasted until peak expiratory airflow decreased by 25% and FEV_1 by 15%, or 6 weeks elapsed. The results showed that the number of eosinophils in the bronchial biopsies was increased by glucocorticoid withdrawal in both groups. This suggests that eosinophils play an important role in the inflammatory pathway. Similarly, allergen challenges result in increased eosinophils, this was demonstrated in a study of patients who underwent an allergen challenge.[58] The results showed that patients were shown to have a significant increase in sputum eosinophils after the allergen challenge compared with before the allergen challenge.

It has been suggested that asthma can be classified phenotypically as eosinophilic or non-eosinophilic.[59] It is estimated that 40-60% of asthma is in the eosinophilic subset,[60] and this number would probably increase significantly if corticosteroids were withdrawn. It has been shown that the severity of symptoms is increased in patients with eosinophilic asthma[61] and in those that have persistent eosinophilia despite the use of corticosteroids. Exacerbations are associated with sputum eosinophilia; Jatakanon et al.[62] studied the effect of changes in airway eosinophils in 15 patients with stable asthma. Mild exacerbations were induced in

the patients with stable asthma controlled with medium to high-dose ICS. The only significant difference between these two groups at baseline was a higher baseline sputum eosinophil count in subjects with subsequent exacerbations ($p<0.05$). Eosinophilia correlated with decreased lung function (PEF and FEV_1) and an increase in NO in these patients.

Further studies have shown the role of eosinophilia in poorly controlled asthma patients, Green et al.[63] studied 74 patients with moderate to severe asthma allocated randomly to management either by standard British Thoracic Society asthma guidelines (BTS management group) or by normalisation of the induced sputum eosinophil count and reduction of symptoms (sputum management group). The sputum eosinophil count was 63% (95% CI 24–100) lower over 12 months in the sputum management group than in the BTS management group (p=0.002). Patients in the sputum management group had significantly fewer severe asthma exacerbations than patients in the BTS management group (35 vs 109; p=0.01) and significantly fewer patients were admitted to hospital with asthma (1 vs 6, p=0.047). There were no differences between the groups in the average daily dose of inhaled or OCS. A treatment strategy directed at normalisation of the induced sputum eosinophil count as an inflammatory surrogate count reduces asthma exacerbations and admissions without the need for additional anti-inflammatory treatment. A similar study analysed data obtained from 164 subjects with mild to moderate asthma compared the effects of continued ICS use with the effects of a switch to salmeterol or placebo.[64] The study demonstrated that sputum eosinophils guided treatment strategy resulted in a 48% reduction in ICS therapy.

There are several potential eosinophil selective targets: chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2), mucin-like hormone receptor 1 (EMR-1), Siglec-8, chemokine receptor type 3 (CCR3), and IL-5 (Figure 9).[65] There is also the group of corticoid receptors, all of which are potential targets that could reduce the number of eosinophils in the airway.

IL-5 binds to a heterodimeric cell surface receptor comprising an alpha chain specific for IL-5 (IL-5Rα), and a beta chain that is shared with the IL-3 and GM-CSF receptors (βc). IL-5Rα is expressed on eosinophils and their precursors, basophils,

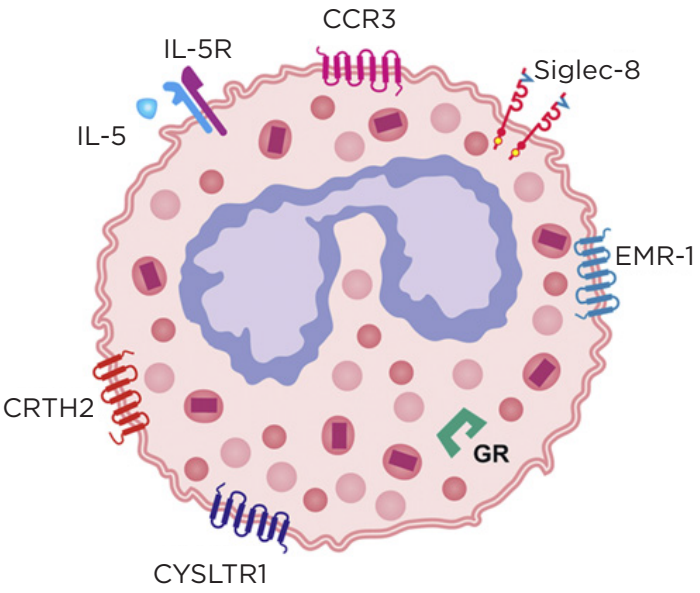


Figure 9. Potential eosinophil selective targets. CCR3: C-C chemokine receptor type 3; EMR-1: mucin-like hormone receptor 1; CRT: chemoattractant homologous receptor; GR: glucocorticoid receptor. *Wechsler ME, et al.*[65]

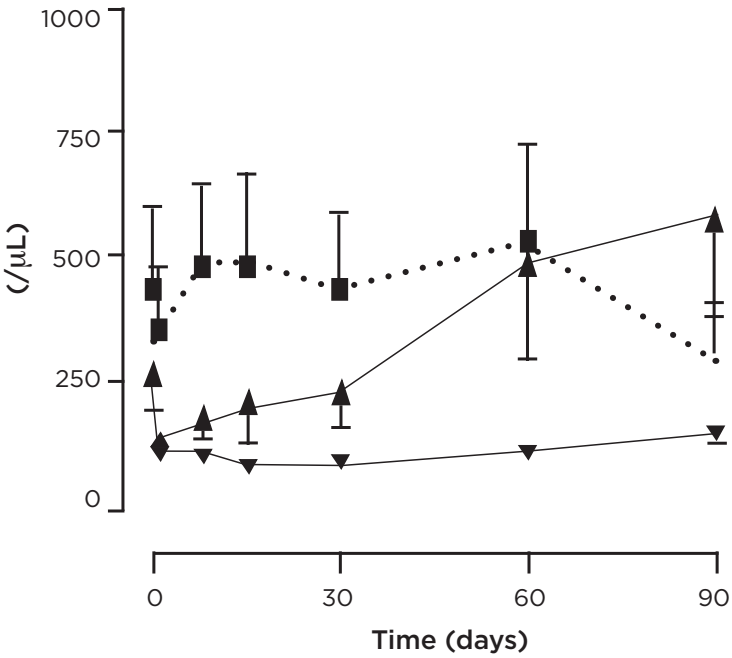
bronchial smooth muscle cells and murine B1 lymphocytes.[66] IL-5 promotes the intramedullary eosinophilopoietic development of eosinophil precursors to mature eosinophils and releases stored mature eosinophils from within the marrow.

IL-5/eosinophils may have a major role in asthma-associated remodelling. IL-5 is a major regulator of eosinophil proliferation and maturation. Cho et al.[67] sensitised wild-type (WT) and IL-5–deficient (IL-5 knock out [KO]) mice to ovalbumin (OVA), and challenged by repetitive administration of OVA for 3 months. WT mice had a significant increase in the number of peribronchial cells staining positive for major basic protein and TGF-β. In contrast, IL-5–deficient mice had a significant reduction in thenumber of peribronchial cells staining positive for major basic protein, which was paralleled by a similar reduction in the number of cells staining positive for TGF-β, suggesting that eosinophils are a significant source of TGF-β in the remodeled airway. OVA challenge induced significantly higher levels of airway epithelial Vβ6 integrin expression, as well as significantly higher levels of bioactive lung TGF-β in WT compared with IL-5–deficient mice. Increased airway epithelial expression of Vβ6

integrin may contribute to the increased activation of latent TGF-β. The IL-5 KO mice had significantly less peribronchial fibrosis (total lung collagen content, peribronchial collagens III and V) and significantly less peribronchial smooth muscle (thickness of peribronchial smooth muscle layer, smooth muscle actin immunostaining) compared with WT mice challenged with OVA. This indicates that IL-5, eosinophils and TGF-β appear to have an important role in airway remodeling.

Blocking IL-5 may significantly contribute to the management of asthma. There are at least three anti–IL-5 drugs in development: mepolizumab, reslizumab[68] and benralizumab.[65] These therapies target IL-5 (benralizumab targets the IL-5 receptor specifically); their mode of action is that they neutralise IL-5 and or have cytotoxic effects in the cells. The effects of the therapies are mainly a decrease in eosinophil counts and a decrease in eosinophil activation in the tissue, their aim is to improve asthma control.

In pre-clinical studies, reslizumab was administered to allergic mice resulting in pulmonary eosinophilia inhibition, and an anti-inflammatory effect was observed.[69] The anti-inflammatory activity is additive with oral prednisolone. In ovalbumin-sensitised guinea pigs reslizumab was administered before OVA challenge and resulted in the decrease of pulmonary eosinophilia and hyper-reactivity.[69] In addition, it significantly inhibited bronchoconstriction.[70]



Blood eosinophil counts after IV administration of placebo (N=8) (squares) or reslizumab at 0.3 mg/kg (N=6) (triangles) or 1.0 mg/kg (N=12) (inverted triangles)

Figure 10. The effects of reslizumab on blood eosinophil counts.
Kips JC, et al.[73]

In humans, Leckie et al.[71] showed that anti–IL-5 therapy resulted in a reduction of blood eosinophil count. 2-5 mg/kg and 10 mg/kg doses of anti–IL-5 caused a reduction in eosinophils that was sustained for up to 16 weeks, whereas placebo did not result in the reduction of eosinophils. It was also demonstrated that anti–IL-5 therapy resulted in a reduction of sputum eosinophil counts; significant reductions were seen in the 10 mg/kg anti–IL-5 group compared with placebo at 9 days and 30 days. The problem was that anti–IL-5 did not improve lung function in this study.[72] These were broad studies that looked at all asthmatics and did not stratify them according to baseline eosinophilia. Although there was a reduction in blood and sputum eosinophils there was no change in airway late phase reactivity to allergens, no change in lung function and no change in asthma symptoms.

It is becoming increasingly recognised that a wide group of asthmatics is not the most ideal study population. This is because asthmatics are heterogeneous, which indicates that different endotypes of asthma should be studied. This suggests that IL-5 therapy needs to be targeted at identified potential responders, and these should be stratified accordingly.

Kips et al.[73] studied anti–IL-5 in severe persistent asthma. Four different doses (0.03 mg/kg; 0.3 mg/kg; 0.1mg/kg; 1.0 mg/kg) of reslizumab versus placebo were given to 32 patients. The results showed there was a short-lived decrease in blood eosinophil count after the 0.3 mg/kg dose (52.5% reduction at 48 hours). There was a more pronounced response to reslizumab 1.0 mg/kg, remaining significant to 30 days post-treatment (p=0.05 versus placebo) (Figure 10). There was a substantial variability in sputum eosinophil counts, but no consistent changes over time were observed in any of the treatment groups. A trend towards increased FEV_1 was observed, with significant improvement at 24 hours with the 0.3 mg/kg dose (p=0.019 versus placebo). No significant changes in

FEV_1/FVC (forced vital capacity) ratio, peak flow, symptom score, or physician-evaluated overall condition were seen. Although there were no changes in peak flow or symptom score, it was thought that if stratified grouping was improved in larger patient populations, and as dosing regimens advance, it is possible that improvements in peak flow and symptom score would be seen.

In a further study, reslizumab was evaluated in poorly controlled eosinophilic asthma patients.[74] This Phase II, placebo-controlled, double-blind study randomised 106 patients with eosinophilic asthma who had ≥3% eosinophils at screening. Reslizumab at a 3 mg/kg dose was compared with placebo (IV dosing at weeks 0, 4, 8, and 12). The mean change in the asthma control questionnaire (ACQ) showed an improvement in the reslizumab group of -0.7 versus -0.3 in the placebo group (p=0.0541) (Figure 11). In addition, there was an improvement of ≥0.5 in ACQ scores in 59% of patients receiving reslizumab, versus 40% in the placebo group (odds ratio 2.06; p=0.0973), and a greater change from baseline in patients with nasal polyps of -1.0 in the reslizumab group compared with –0.1 in the placebo group (p=0.012). The mean change in FEV_1 was -0.08 in the placebo group compared with +0.18 in the reslizumab group (p=0.0023). The sputum eosinophil count was reduced by 95.4% in the reslizumab group compared with 38.7% in the placebo group (p=0.0068), and the reduction from baseline in blood eosinophil count was significantly greater in the reslizumab group (p<0.0001). Asthma exacerbations were reported in 8% of patients receiving reslizumab compared with 19% of those receiving placebo (p=0.0833), showing over a 50% reduction in asthma exacerbations in the reslizumab group.

A randomised trial of mepolizumab versus placebo in 20 patients with sputum eosinophilia and asthma symptoms despite prednisone, resulted in a significant reduction in exacerbations in the mepolizumab group compared with placebo (p=0.002).[75] In addition, there was a significant reduction in steroid dose (84% reduction in the mepolizumab group compared with 48% in the placebo group), there was a sustained benefit for 8 weeks of reduced eosinophils, and there were no serious adverse events. Another study evaluated mepolizumab and exacerbations of refractory eosinophilic

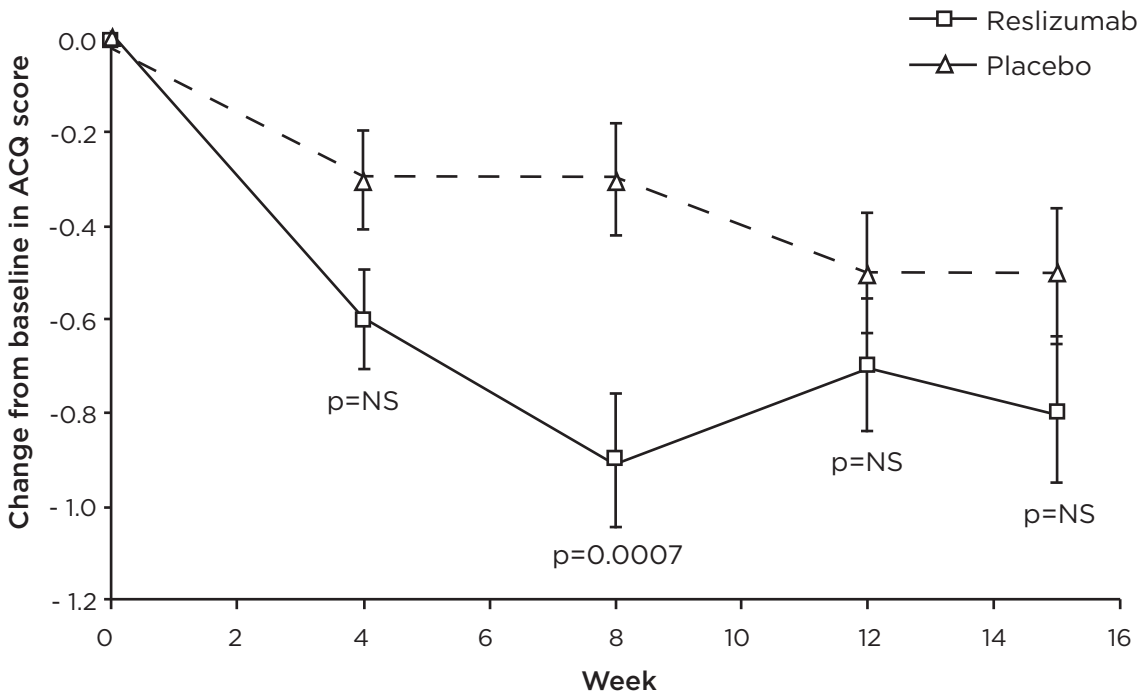


Figure 11. The efficacy of reslizumab on ACQ score.
Castro M, et al.[74]

asthma in 61 patients.[76] The patients were treated with mepolizumab or placebo for 12 months. The results showed that there was a significant reduction in exacerbations in the mepolizumab group compared with placebo; the cumulative number of exacerbations at month 12 was 57 versus 109, respectively. No effect on lung function, symptom scores or NO was seen. The results of this study are supported by another study, which found the total number of exacerbations over time was reduced with mepolizumab compared with placebo.[77] Patients with sputum eosinophils >3%, or fractional exhaled NO >50, or blood eosinophils >300, received 75,250 or 750 mg of mepolizumab or placebo. The results showed a significant reduction in asthma exacerbations in all three of the doses given (Figure 12). There were no effects on FEV_1, ACQ or in the AQoL. However, the reduction of the number of exacerbations equated to 50% per patient per year and this demonstrates a clinically important outcome.

Based on the studies that have been published to date, it has been shown that anti-IL-5 therapy is more likely to benefit patients with eosinophilic asthma. This group of patients not only shows a decrease in sputum eosinophilia but also an improvement in FEV_1 as well as an improvement in asthma control, and more importantly a decrease in exacerbations. Anti-IL-5 has been shown to be well tolerated with a similar percentage of patients with adverse events as placebo; the most commonly reported adverse events are headache, fatigue and nasopharyngitis.[74]

In the majority of the studies that have been done, eosinophilic asthma has been defined as persistent sputum eosinophils ≥2.5–3%.[78] Sputum cell count has been relied on because it is a valid, repeatable and responsive metric that is specific and comprehensive. Sputum cell counts have been independently evaluated in 18 different research laboratories across four continents. The

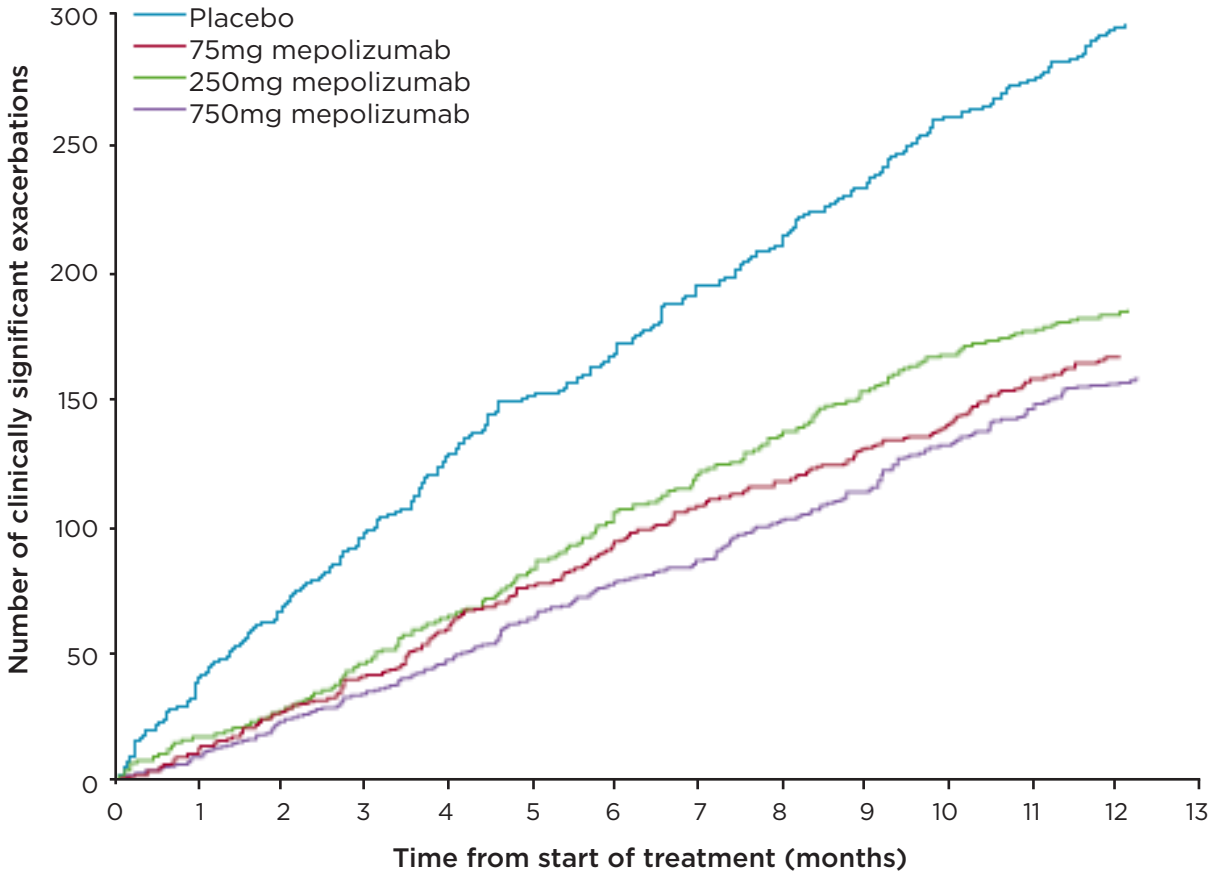


Figure 12. Total exacerbations over time are reduced with mepolizumab versus placebo.
Pavord I, et al.[77]

process for measuring sputum cell counts requires some training but produces excellent results.

In the persistent eosinophil phenotype, key metrics have shown that if patients are selected based on screening of eosinophils, good results are achieved. This has been illustrated in two studies that did not screen eosinophils in patients; in one study only 5% of the patient population had >3% eosinophils[73] and in the other, only 30% of the patient population had >3% eosinophils;[73] consequently, these studies did not demonstrate success with anti-IL-5 therapy. However, in studies with patients who have >3% eosinophils,[74-76] success has been demonstrated with this therapy.

The current gold standard in the diagnosis of eosinophilic asthma is sputum eosinophilia. The utilisation of blood eosinophilia is being considered as a marker and a metric because it is widely available and is a Food and Drug Administration standard. In the future it is hoped that other sputum biomarkers can be used to help identify responders and non-responders to anti-IL-5 therapy.

Anti-IL-5 is effective in reducing eosinophilia in blood and sputum. This has been established by improvements in lung function being apparent in patients with eosinophilic asthma. In addition, trends towards improved asthma control have been observed, with more pronounced effects being seen in patients with eosinophilic asthma and nasal polyps. Anti-IL-5 is well tolerated with a similar adverse event profile to placebo. Despite the fact that there are many other therapies available, newer therapies are required. Problems with adherence and inhaler technique require solving, and other ways to manage patients need to be identified. Accordingly, new therapies are required, and anti-IL-5 is a new therapy that appears to be effective, it targets a variety of different cells and makes biological sense. The future development of anti-IL-5 therapy provides an exciting option for the management of asthma patients.

Panel discussion

Question: Why were sputum tests used when there are much easier and cheaper alternatives?

Michael Wechsler: The reason that sputum eosinophils were selected is because 1) eosinophils in sputum are in the compartments of interest. 2) There hasn't been a correlation between blood eosinophils and a response to therapy. It has been investigated, and, while in some patients' blood eosinophils there is a good response, not everyone with high blood eosinophils is necessarily a responder. Patients with low blood eosinophils do have higher sputum eosinophils because sputum is in the compartment of interest. I do think it is important to try and develop a simple assay; sputum is more difficult than the blood test. However, the more we do these kinds of studies the easier they will get, and certainly some people would argue that sputum is, in some ways, less invasive; you just spit into your pallet, and this maybe another reason for this approach. But we require a measure that predicts responsiveness whether it be in blood, sputum or urine.

Question: Which are better: leukotriene modifiers or an anti-IL-5?

Michael Wechsler: Leukotriene modifiers are effective therapies in patients with mild-to-moderate asthma and they do have some mild anti-eosinophilic properties. But in general you cannot use eosinophilia as a predictor of response to leukotriene modifiers, and the degree of benefit that has been seen with leukotriene modifiers compared with that seen with anti-IL-5 in patients, who are already on ICS and a LABA, is not to the same degree. So while leukotriene modifiers have beneficial properties and do have some anti-eosinophil effects they are not to the same degree as anti-IL-5.

Question: What do the other speakers think about anti-IL-5 therapy?

Cynthia Rand: So why would somebody who looks at adherence comment on this? Well I will make two comments and listen for a response. The first is that this is directly observed therapy, treatment where you confirm having received the treatment has been shown in other therapies to have a dramatic effect on outcome. So a study has not yet been done that actually teases out the difference between directly-observed therapy in this population, where you have matched and observed doses of inhaled steroids versus one of the biologics. So it raises a question for me as to what extent that is a contributor to outcome. And the second is the limitation we know we have in people who are non-adherent, the eosinophil count also goes up, so the question is to what extent in the studies that have been done have they sufficiently, that is really sufficiently, screened out non-adherence as a contributing cause for the increased eosinophils? So I think it is a really intriguing and promising area and clearly I absolutely agree it has had some impact on some populations, but what is a little less clear to me is whether or not it has actually provided a different way of delivering therapy to a population that was under-adherent in the first place.

J. Christian Virchow: From a clinical perspective, if you see patients who have eosinophilia, these are the ones that are easy to treat, and I would imagine that the number of patients with asthma who are difficult to treat usually lie in the high eosinophil range. These patients are not huge in numbers, but I do see a very clear need for these therapies in more complicated cases, such as allergic bronchopulmonary aspergillosis, where these patients have loads of eosinophils and need high doses of systemic corticosteroids. Secondly, there is a big need in patients who have asthma and eosinophilia; their problem is not so much the lower airways which can be treated, but the upper airways severe polyposis, and we know from these studies that if you give reslizumab to patients with nasal polyposis there is a reduction in the spores, whereas high doses, even toxic doses, of corticosteroids are required to get the disease in check. I certainly see a big need in these patients for anti-IL-5 therapy. I do see some patients with high steroid dosing-requiring asthma where you would go to a balance therapy with anti-IL-5 but also a dose of corticosteroids. I think that we need to keep in mind that our patients are well controlled on high doses of corticosteroids but only controlled according to GINA; they come to you and say I still feel miserable even though I take most of my inhalers but I hate taking this red stuff because it is not normal.

Michael Wechsler: Very recently, Sally Wenzel published a paper in *The New England Journal of Medicine* that examined patients with eosinophilia, who were poorly controlled on ICS and beta agonists; all patients were screened and there were still over 20% of patients who had persistent eosinophilia. So it is important to recognise that yes we can work on inhalers, we can work on compliance, but there are still a large proportion of patients that, no matter what you do, the patients take their medications, they take them properly and they are still symptomatic, and this is what this kind of therapy targets. We do need to work on those issues and other conditions need to be excluded.

Question: Can disease management programmes improve adherence?

J. Christian Virchow: Well I guess they could but the evidence for that is not from very well-controlled studies. Recently I saw a comprehensive care programme for asthma, very similar to a disease management programme in COPD, and saw higher mortality in the programme compared with those not in the programme. I think from what we have heard, anything that educates patients has at least the potential to increase compliance. Based on what we know I would say yes.

Cynthia Rand: There are lots of different flavours of disease management programmes and it depends on what they consist of. I think what the evidence suggests is that those which address more complicated issues have better outcomes. Clearly I think the theme that runs through all of our talks is that there is tremendous variability in asthma patients and, the extent to which you understand their

unique barriers and the phenotype underlying the severity, the better you can match the right treatment to help control their illness. Two comments that were raised by the audience, which I think are very important to raise: one that I failed to mention (as did others) is the cost of medication and the extent to which that can put up a barrier for a patient. I think it was highlighted before that many of our patients, and I speak from the US perspective where we do have some substantial cost issues, patients are not just treating one illness; they are having to cover costs for multiple different chronic illnesses, and indeed that has been shown to be a significant barrier, and that reducing that cost would improve adherence. And the second point that was made was that instead of searching for biomarkers for specialised therapies, perhaps we need behavioural studies in patients to increase adherence – well what am I going to say, I am going to say yes of course – but I truly do think we need to partner on these issues. There is no one solution for our patients, and behavioural strategies are not at the route of problems with asthma, but to manage any other chronic illness and how we can help patients better follow therapies.

Question: Coming back to the area of anti-IL-5 is there any experience in childhood asthma?

Michael Wechsler: There are some studies that are on-going in paediatric patients but nothing has been published to date.

Question: Another important question, which is particularly relevant to countries like many in Europe where we have consultation for free and access to medicine. How are you going to persuade the health authorities to fund this treatment how expensive will it be?

Michael Wechsler: The specific pricing for these therapies has not been developed as of yet; we have some experience based on anti-IgE therapy. The rationale for prescribing these expensive therapies is to prevent asthma exacerbations, and I showed you at least three different studies that demonstrate that anti-IL-5 reduced exacerbations. Exacerbations are very costly; they include hospitalisations, emergency room visits, and it is estimated in the United States that we spend at least 15 to 20 billion dollars a year treating patients with asthma, and at least two thirds to three quarters of that expenditure is in the management of asthma exacerbations and in hospitalisations, and so if we can reduce asthma exacerbations by 50%, as was shown in these studies, then this can result in significant savings, so that is the rationale we argue.

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LONG-TERM NON-INVASIVE VENTILATION (NIV) FOR COPD PATIENTS WITH CHRONIC RESPIRATORY FAILURE

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Disclosure: SN has received travel grants from Weinman, speaking fees from Respironics and ResMed, a research grant from Starmed, and free loans of equipment from Respironics, ResMed and Siare. BE has no conflicts of interest to declare.
Received: 20.08.13 **Accepted:** 16.10.13
Citation: EMJ Respir. 2013;1:54-62.

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a major health issue worldwide, with disease burden, healthcare costs, and mortality rates all significant and increasing. Non-invasive ventilation (NIV) is used to manage acute exacerbations of COPD associated with mild-to-moderate acidosis. Continuing NIV after discharge can reduce the risk of re-exacerbation, and decrease hospitalisation rates and healthcare resource use. A number of positive effects have been documented during NIV treatment in patients with stable hypercapnic COPD. These include reductions in hypercapnia and hypoxaemia, improvements in quality of life (QoL) and neuropsychological function, reduced hospital admissions and costs, and improved benefit from pulmonary rehabilitation. The effect of NIV targeting carbon dioxide reduction on long-term survival remains to be clearly determined, but is the subject of ongoing research. Overall, accumulating evidence suggests that NIV also has a role in the long-term management of stable hypercapnic COPD. It is expected that long-term NIV will be most useful for the subgroup of patients with frequent exacerbations of disease. Co-morbidities such as obesity, heart failure, or sleep-disordered breathing could further support the use of long-term NIV in the setting of stable hypercapnic COPD.

Keywords: Non-invasive ventilation, chronic obstructive pulmonary disease, randomised controlled trials, survival rate, hypercapnia.

INTRODUCTION

The term chronic obstructive pulmonary disease (COPD) encompasses the diagnoses of chronic bronchitis and emphysema. COPD is characterised by persistent, non-reversible airflow limitation. The main underlying feature is loss of elastic recoil of the lungs as a result of parenchymal destruction, along with airway inflammation.[1] The World Health Organization (WHO) estimates that 65 million people have moderate to severe COPD worldwide,[2] and COPD is a significant cause of mortality, ranking as the fifth leading cause of death in 2002.[2] The prevalence of COPD is rising; mortality from COPD is expected to increase to the third leading cause of death worldwide by 2030.[2]

Death from COPD is an important issue, however, the chronic nature of the disease and its significant impact on quality of life (QoL) (for patients and their caregivers) means that the burden of disease extends well beyond mortality. The European COPD Coalition estimates that the life experience of one in ten adults is limited by COPD.[3] Severe COPD has a symptom burden that is comparable to that of cancer,[4] and has a greater negative impact on health status than self-reported cardiovascular disease or diabetes.[5]

COPD also places a significant demand on healthcare resources. The annual direct cost of COPD in the US has been estimated to be more than $32 billion (USD),[6] and the mean in-hospital treatment cost for one patient with an acute exacerbation of COPD has been reported as $9,545 (USD).[7] In the European Union, total direct costs for respiratory disease account for around 6% of the total healthcare budget; in turn, COPD consumes 56% of the spend on respiratory disease (€38.6 billion).[8]

COPD is a chronic, progressive disease, and many patients will eventually develop chronic respiratory failure. This can be either hypoxic or hypercapnic in nature. Type 1 respiratory failure is characterised by hypoxaemia secondary to impaired gas exchange in the lungs, and can be treated by with long-term oxygen therapy. Hypercapnia is the main feature of type 2 respiratory failure and occurs as a result of impaired ventilation.[9] Type 2 respiratory failure is often associated with severe exacerbations of COPD,[10] and effective management strategies are essential.[11] Maintenance of appropriate gas exchange and sufficient ventilation has been highlighted as one of the key therapy objectives.[11] Clinical trial data show that mortality is much higher in COPD patients admitted to an intensive care unit (ICU) because of acute respiratory failure and who were discharged with non-reversible hypercapnia (defined as $PaCO_2$ ≥50 mmHg), than in those who were normocapnic at admission or discharge (reversible hypercapnia).[12] Higher mortality rates have also been documented in a subgroup of COPD patients who had a rise in $PaCO_2$ of ≥5 mmHg/year compared with those who had stable $PaCO_2$ values.[13]

NON-INVASIVE VENTILATION

Acute non-invasive ventilation (NIV) has been shown to be a very effective treatment approach in patients with an acute exacerbation of COPD with mild-to-moderate respiratory acidosis, reducing mortality, need for endotracheal intubation, and length of hospital stay.[14-21] For patients who are treated acutely, continuation of NIV at home after discharge from hospital may be associated with a lower risk of recurrent severe COPD exacerbation.[22] In patients with chronic, stable COPD, the use of NIV in the home appears to decrease the need for physician care and hospitalisation.[23-27] In COPD patients at risk of recurrent admission, the use of home-based NIV has the potential to reduce hospital admissions and healthcare resource use.[28]

Long-Term NIV in Chronic Hypercapnic COPD: The Rationale

Physiologic studies have shown that mechanical ventilation can improve alveolar ventilation while reducing inspiratory effort in patients with stable chronic hypercapnic respiratory failure.[29] The possibility that reductions in CO_2 could be maintained underlies the rationale for using NIV in patients with chronic hypercapnic respiratory failure. The concept that hypercapnia in stable COPD patients, and thus hypoventilation, may be caused by chronic muscle fatigue, is why it was suggested to rest the respiratory muscles. Diaphragmatic contractile dysfunction has also been documented, even in the early stages of COPD.[30] However, this has not been confirmed in autopsy studies looking at the remodelling of the diaphragm, although fatigue-resistant fibres were seen more frequently in COPD patients compared with controls.[31]

Patients with COPD are likely to develop nocturnal hypoventilation, especially during rapid eye movement (REM) sleep when upper airway tone and accessory muscle activity is impaired. Nearly half (42%) of the COPD patients in one study had a >10 mmHg increase in $PaCO_2$ at night, resulting in progressive resetting of respiratory control to higher $PaCO_2$ values.[32,33] A low central respiratory drive may also contribute to the development of hypercapnia in COPD.

A number of potential mechanisms have been proposed to explain the beneficial effects of NIV in hypercapnic patients with COPD. One study reported an increase in the central responsiveness to CO_2, which was associated with improved day time blood gases, however, no change in inspiratory muscle pressure was found.[34] Other possibilities include changes in lung mechanics, improvement in ventilation/perfusion (V/Q) matching, recruitment of non-ventilated or poorly-ventilated alveolar units, and decreased pulmonary hypertension. However, this remains a controversial area of research and has been for more than 20 years, with all the mechanisms proposed not being mutually exclusive and having the potential to contribute to beneficial effects to differing extents.[35]

*See page 58 for abbreviations

Table 1. NIV in chronic hypercapnic COPD: RCTs with duration ≥3months.

Author, Date Study type Pt numbers	Treatment arms (median/mean $PaCO_2$, mmHg)	Mean EPAP/IPAP (cmH_2O)	Mean NIV use	Effects of NIV
Strumpf, 1991[50] RCT, crossover, 7 pts completed	Nocturnal NIV+SC (49.0) SC (49.0)	2/15	6.7 h/night	•Improved neuropsychological function •No changes in gas exchange, lung function, respiratory muscle strength, exercise endurance, sleep quality, and dyspnea ratings
Meecham Jones, 1995[47] RCT, crossover 14 pts completed	Nocturnal NIV+LTOT (55.8) LTOT (55.8)	2/18	6.9 h/night	•Improved daytime and overnight hypoxaemia and hypercapnia, sleep time, sleep efficiency, and QoL •No effect on lung function or 6MWD
Gay, 1996[44] RCT, parallel 4 pts completed NIV 6 pts control	Nocturnal NIV (54.7) Sham control with lowest expiratory pressure (48.5)	2/10	5.1 h/night	•No effect on gas exchange, lung function, exercise capacity, and sleep, but proportion of REM sleep decreased
Garrod, 2000[43] RCT, parallel 17 pts (NIV) 20 pts (exercise)	NIV+exercise training (44.2) Exercise training (46.1)	4/16	2.1 h/day >4 h in 29% of pts	•Improved oxygenation, exercise capacity and QoL (CRDQ total and fatigue scores)
Casanova, 2000[23] RCT, parallel 20 pts (NIV +SC) 24 pts (SC)	Nocturnal NIV+SC (50.7) SC (53.2)	4/12	6.2 h/day (1st half of study) 5.9 h/day (2nd half of study) <3 h/day in 11% of pts	•Improved dyspnoea (Borg scale) and psychomotor coordination •No effect on gas exchange, pulmonary function, cardiac function, exacerbations (number and severity), hospitalisations, intubations, and survival •Hospitalisation rate decreased in the first 3 m but was similar in the treatment groups at 6 m
Clini, 2002[24] RCT, parallel 43 pts (NIV + LTOT) 47 pts (LTOT)	NIV+LTOT (54.0) LTOT (55.5)	2/14	9.2 h/day (study protocol criteria was use for ≥5 h/night)	•Improved hypercapnia, resting dyspnoea and HRQoL (MRF-28) •No effect on lung function, inspiratory muscle strength, exercise tolerance, sleep quality, hospital/ICU admissions and length of stay, or mortality
Duiverman, 2008[40] RCT, parallel 24 pts (NIV + rehab) 32 pts (rehab)	Nocturnal NIV+rehabilitation (51.7) Rehabilitation (51.1)	6/20	7.7 h/day	•Improved hypercapnia, minute ventilation in quiet breathing, HRQoL (CRDQ fatique domain, MRF-28 total score and cognition domain) and daily step count •No effect on hypoxaemia, lung function, exercise tolerance

McEvoy, 2009[46] RCT-parallel 72 pts (NIV + LTOT) 72 pts (LTOT)	Nocturnal NIV+LTOT (52.6) LTOT (54.4)	5/13	4.5 h/day >4 h/day in 60% of pts	•Improved survival, sleep-related hypercapnia and sleep architecture •No effect on gas exchange and pulmonary function (assessed in first 12 m in a subgroup of pts with available data) •Worsening QoL (assessed in first 12 m; SF-36 general health and mental health) and mood state (vigour and confusion-bewilderment variables)
Cheung, 2010[22] RCT, parallel 23 pts (NIV) 24 pts (CPAP)	NIV (76.5) CPAP with 5 cmH_2O (80.3)	5/15	7-9 h/night (average)	•Reduced risk of recurrent severe COPD exacerbation •No effect on gas exchange, time to first readmission, adverse events, and survival
Funk, 2011[42] RCT, parallel (after 6 m of NIV treatment) 13 pts continuation 13 pts withdrawal	NIV continuation (57.0) NIV withdrawal (56.0)	5/20	NR	•Higher probability of clinical worsening in the NIV withdrawal group •Improved daytime pH and exercise capacity •No effect on gas exchange, pulmonary function, HRQoL, and the incidences of antibiotic or oral steroid therapy due to COPD exacerbation
De Backer, 2011[36] RCT, parallel 10 pts (NIV) 5 pts (medical therapy)	NIV (55.4) Medical therapy (52.4)	NR	Study protocol criteria was use for >5 h/day	•Improved gas exchange (change in oxygenation correlated with improved ventilation-perfusion match in functional imaging) and exercise tolerance •No effect on lung function
Duiverman, 2011[41] RCT, parallel 24 pts (NIV + rehab) 32 pts (rehab)	Nocturnal NIV +rehabilitation (NR) Rehabilitation (NR)	6/23	6.9 h/day	•Significant improvements in gas exchange, exercise tolerance, dyspnoea, FEV_1 (annual decline was 17 mL in NIV+rehab group versus 83 mL in rehab group), mood and HRQoL (MRF-28, SRI physical function domain). •No change in exacerbation rate
Murphy, 2012[48] RCT, crossover 7 pts completed	High-pressure NIV (64.5) High-intensity NIV (64.5)	5/29	6 h 37 min/night (high pressure) 6 h 33 min/night (high intensity)	•Similar gas exchange, sleep quality and quantity, and patient adherence in both NIV treatment arms •Respiratory component of SRI was lower in the high-intensity NIV group

*6MWD: 6 minute walking distance; COPD: chronic obstructive pulmonary disease; CPAP: continuous positive airway pressure; CRDQ: Chronic Respiratory Disease Questionnaire; EPAP: expiratory positive airway pressure; FEV_1: forced expiratory volume in one second; h: hour; HRQoL: health-related QoL; IPAP: inspiratory positive airway pressure; LTOT: long-term oxygen therapy; m: month; MRF-28: Maugeri Foundation Respiratory Failure questionnaire; NIV: non-invasive ventilation; NR: not reported; $PaCO_2$: partial arterial carbon dioxide tension; pt: patient; QoL,: quality of life; RCT: randomised controlled trial; REM: rapid eye movement; SC: standard care; SRI: Severe Respiratory Insufficiency questionnaire.

Long-Term NIV in Hypercapnic COPD: Clinical Studies

The effects of adding chronic NIV to long-term oxygen therapy have been assessed in a number of studies (Table 1).[22-24,28,36-50] The most commonly studied outcomes can be classified as: physiological (gas exchange, lung function, and respiratory mechanics), health-related (dyspnoea relief, exercise tolerance, and health-related QoL), and disease-related morbidity (exacerbation frequency and characteristics, hospitalisations, intensive care unit admissions, and intubations). To date, no clear effect of NIV on major outcomes such as survival has been documented.[22-24,28,36-43,45-50]

A number of positive effects have been reported with use of NIV in patients with stable hypercapnic COPD. These include: reductions in hypercapnia and hypoxaemia,[22-25,28,35-41,43-46] improvements in QoL[24,47,51] and neuropsychological function,[50] reduced hospital admissions and costs,[28] improved benefit from pulmonary rehabilitation,[40,41,43] and a higher survival rate.[46] Details of the results from randomised, controlled clinical trials with duration of ≥3 months are shown in Table 1, and the findings of non-randomised trials are shown in Table 2.

Critical analysis of the available data is appropriate before any definitive decisions are made about the use of NIV in stable hypercapnic COPD. The difference in results between studies could be due to a number of factors, including heterogeneous patient populations (resulting from differing inclusion/selection criteria), differences in ventilator, ventilator settings and interfaces, and choice of study endpoints (e.g. CO_2 reduction). Another factor to consider is the relatively small number of patients included in each of these studies (Table 1). The conclusions of a recent meta-analysis of data from seven randomised, controlled clinical trials stated that no definitive conclusions can be drawn regarding the effects of NIV because the quality of evidence is only moderate as a result of the small sample sizes.[52] Therefore, there is a need for studies in this area to enrol a larger number of patients to provide more robust data.

One of the most significant unresolved issues in the field of NIV for stable COPD is defining the optimum ventilation strategy. Before concluding that NIV has no beneficial effects, it is important to confirm that effective ventilation has in fact been delivered. Finding an appropriate physiological target to guide the choice of ventilator mode and settings has proven difficult; to date, this has usually been defined as a specified decrease in $PaCO_2$. Studies that have utilised lower inspiratory pressures (10–12 cmH_2O) have not reported any beneficial effects of NIV on gas exchange or pulmonary function.[23,44] Conversely, use of higher inspiratory pressures (16–22 cmH_2O) in one randomised, controlled trial was associated with positive results for NIV,[47] suggesting that higher ventilating pressures might be more effective. Data from a retrospective study using inspiratory pressures of 17–40 cmH_2O showed improvements in hypercapnia and FEV_1 after 2 months, and a 2 year survival rate of 86%. However, there was no control group and patients also received daytime ventilation.[53]

Adherence to NIV is also a factor which could influence the outcome of therapy. The majority of studies encourage patients to use NIV for at least 5 hours per night, and only randomised studies advocating this level of therapy for at least 3 weeks were included in one meta-analysis. However, the overall finding was for a lack of significant effect of NIV on lung function, gas exchange and sleep efficiency, although improved walk distance was noted in some patients.[54] It is interesting to note that studies, which included in their protocol sufficient time for familiarisation with NIV treatment, tended to show greater benefits.[24,53,55]

Although some beneficial effects of NIV on health-related outcomes have been reported, the variety of assessment tools used makes determination of the overall effect of NIV on these parameters very difficult. For example, dyspnoea has been

Table 2. NIV in chronic hypercapnic COPD: non-randomised trials.

Author, Date Study type Pt numbers	Study arms	Mean NIV use	Effects of NIV
Tuggey, 2003[28] 13 pts	Cost analysis in patients with recurrent exacerbations before and after domiciliary NIV	-	•Use of NIV decreased costs by €11,720 per patient per year •Number of hospital admissions decreased from 5 to 2 •Length of hospital stay decreased from 78 to 25 days
Nava, 2001[49] 13 pts (NIV) 8 pts (rehab)	Nocturnal NIV Rehabilitation	6.6 h/night	•Reduced resting $PaCO_2$ (associated with decreased diaphragmatic effort) •Acute reduction in $PaCO_2$ in the first trial of NIV was a strong predictor of the final outcome

COPD: chronic obstructive pulmonary disease; h: hours; NIV: noninvasive ventilation; $PaCO_2$: partial arterial carbon dioxide tension.

assessed using four different scales in different studies. Similar differences have been documented with respect to health-related QoL: studies that have used instruments that are validated and specific for chronic respiratory failure (such as the Maugeri Respiratory Failure-28 (MRF28) and Severe Respiratory Insufficiency questionnaires) have found NIV to have a positive effect on QoL, whereas trials utilising more general tools (Short Form-36 and St George's Respiratory Questionnaire) reported no change or even worsened QoL during NIV.[24,40,46,47,56] It is being increasingly recognised that disease-specific tools are more appropriate and useful in COPD patients with chronic respiratory failure.[55,57,58]

Long-Term NIV in Hypercapnic COPD: the Practicalities

Despite a lack of consensus and large-scale controlled clinical trial data, accumulating evidence for the beneficial effects of long-term NIV in hypercapnic COPD patients is starting to be incorporated into clinical practice guidelines. The UK NICE guidelines[59] state: "Adequately treated patients with chronic hypercapnic respiratory failure who have required assisted ventilation (whether invasive or non-invasive) during an exacerbation or who are hypercapnic or acidotic on LTOT should be referred to a specialist centre for consideration of long-term NIV." This recommendation refers to a very specific subgroup of patients with very severe disease who are at the highest risk for mortality and are likely to benefit from NIV therapy as a last resort.[22,34,42,60] In these patients, NIV might induce relative clinical stability by reducing the likelihood of recurrent exacerbations and hospitalisations. German invasive and non-invasive ventilation guidelines[61] recommend long-term NIV when $PaCO_2$ is >50 mmHg during the day, or >55 mmHg at night.

The Eurovent study showed that decisions about when and how to start NIV in patients with COPD are highly dependent on local guidelines and each physician's current clinical practice.[62] In addition, optimal ventilator settings for NIV in patients with chronic hypercapnic COPD are the subject of debate. It has been shown that the empirical titration of NIV (i.e. based on the clinician's experience and judgment) may result in worse patient/ventilator interaction and sleep compared with physiologic titration (i.e. based on recording of respiratory mechanics, with the goal of reducing inspiratory effort by ≥50% and the load imposed by the presence of intrinsic positive end-expiratory pressure (PEEP) by 80%).[63-65] Recently, high inspiratory pressures (≈30 cmH_2O) and respiratory rates (20 breaths/minute) were used to ventilate chronic hypercapnic COPD patients in order to achieve maximal $PaCO_2$ reduction.[39] This approach, called high-intensity NIV (HI-NIV), has been shown to improve spontaneous diurnal blood gases to a greater extent than the traditional approach using lower pressures.[39] In a physiological study, HI-NIV was

able to significantly improve $PaCO_2$ and work of breathing compared with low intensity NIV.[66] However, marked reductions in cardiac output and stroke volume might limit the application of HI-NIV in patients with pre-existing cardiac disease.[66] A long-term randomised study is ongoing in Germany to determine the effect of NIV targeting CO_2 reduction on long-term survival.[67] There is a practical consensus that patients with COPD who have substantial daytime hypercapnia and superimposed nocturnal hypoventilation are most likely to benefit from NIV.

Ventilator settings are often determined based on gas-exchange parameters recorded during the daytime and on patient-reported tolerance, but NIV is usually applied in order to correct nocturnal hypoventilation. Therefore, nocturnal monitoring and/or alternative monitoring with the ventilator software would be preferable to allow better titration of NIV pressures, taking into account sleep abnormalities.[68]

CONCLUSION

NIV is currently used primarily for the management of acute exacerbations of COPD. However, accumulating evidence suggests that NIV also has a role in the long-term management of patients with stable hypercapnic COPD. Early nocturnal NIV therapy in these patients may reduce hospitalisation rates, improve QoL, and reduce healthcare costs, but further research is needed. It is expected that long-term NIV will be most useful for hypercapnic COPD patients with frequent exacerbations of disease. Co-morbidities such as obesity or obstructive sleep apnoea (OSA) might be further factors that support the use of long-term NIV.

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EFFECTIVENESS OF INHALER DEVICES IN ADULT ASTHMA AND COPD

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Disclosure: Please refer to page 71.
Received: 27.08.2013 **Accepted:** 26.09.2013
Citation: EMJ Respir. 2013;1:64-71.

ABSTRACT

Inhalation therapy remains the cornerstone of treatment for bronchial diseases. Despite being pharmacologically efficacious, currently available inhaled drugs can have decreased real-life effectiveness due to a variety of factors, including poor inhalation technique. Each device type has its own specifications regarding the optimal way to use it, in terms of device handling and characteristics of the inhalation manoeuvre. Poor inhalation technique is associated with decreased treatment effectiveness. Choosing the optimal device, together with proper education, improves inhalation technique, adherence and outcomes or effectiveness, but has to be performed regularly and rigorously, including visual checking of the patient's ability to use the inhaler. Some testing devices are also available, as well as various training materials. All healthcare professionals caring for the patient can be involved provided that they have also been properly trained. To optimise treatment effectiveness, healthcare providers should prescribe inhalation device(s) optimised to the patient, accounting for the specific characteristics of each individual, his/her disease, and involved healthcare professionals.

Keywords: Asthma, COPD, inhaler, metered-dose inhaler, dry-powder inhaler, technique, effectiveness, education.

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are among the most frequently occurring respiratory diseases and represent a major public health burden.[1,2] They are both pulmonary diseases, resulting from interactions between environmental exposures and genetic predispositions. The inhaled route is crucial for their treatment since it allows pharmacological agents (currently, β2-adrenergic and anticholinergic bronchodilators and topical corticosteroids) to be delivered directly to their target receptors while minimising systemic exposure and, thus, side-effects.[3,4]

Several types of portable inhaler devices are now available to administer inhaled drugs, and can be classified in several ways. The first classification differentiates: (1) propellant-driven pressurised metered-dose inhalers (pMDIs), which include standard (i.e. non-breath-actuated) devices, which

can be used with spacers, and breath-actuated pMDIs (BAIs); versus (2) non-propellant-driven dry-powder inhalers (DPIs), which can be multi-dose (with an integrated reservoir) or single-dose (using capsules). An alternative classification differentiates devices that require accurate coordination between triggering and inhalation (i.e. pMDIs without spacers) and those that were developed to alleviate the need for proper coordination, i.e. spacers, BAIs and DPIs.[5] Among pMDIs, devices delivering HFA-propelled extra-fine particles have been developed. These devices increase deposition in small airways and reduce the influence of errors in coordination and inhalation flow.[6,7] Nebulisers are not discussed here since they are considered as second line devices for long-term treatment of asthma and COPD.[1,2]

The important characteristics of the dose emitted include the mass median aerodynamic diameter (MMAD) and the fine particle dose (FPD). The MMAD provides an understanding of the size of the emitted particles, with those <5 μm being the most likely to be deposited into the airways. The FPD is the amount of particles with a size <5 μm.[5]

The effectiveness of inhaled treatments is influenced by several factors, including: (1) their efficacy, i.e. their positive effects when used under optimal conditions, directly resulting from their pharmacological properties; and, (2) the way they are used, i.e. the appropriateness of prescription and patients' adherence and ability to use inhalation devices. A systematic review of efficacy studies (i.e. randomised controlled trials) performed a decade ago did not find significant differences between devices.[8] Usually, patients were highly trained regarding their inhalation technique and those who did not demonstrate correct use post-training were excluded. Thus, the real-life effectiveness of the various available devices is not known and could be markedly influenced by the way they are used. A systematic review, by Brocklebank et al. in 2001, underlined the need for additional studies aimed at determining whether some differences between devices can be identified in terms of effectiveness.[9] Some studies have been conducted thereafter to address this issue.[10,11]

Misuse of inhalers will prevent the pharmacological agent from reaching its target, resulting in a decreased effect.[12] The amount of drug depositing in the lung depends on three factors: the drug formulation, the technical characteristics of the device (such as its external and internal design and, for DPIs, the resistance to inhalation), and the ability of the patient to handle it and inhale properly (Figure 1).

Many studies have investigated the frequency and consequences of inhaler misuse. Their results support the major importance given to inhaler technique as a determinant of treatment success. However, only few guidelines address the issue of inhaler technique in detail.[5,13] As a consequence, several international groups developed a specific interest for research and communication on this topic, such as the Asthma Drug Management Team (ADMIT) (http://www.admit-online.info/en/), and the recently established Respiratory Effectiveness Group (REG), (http://www.effectivenessevaluation.org/).

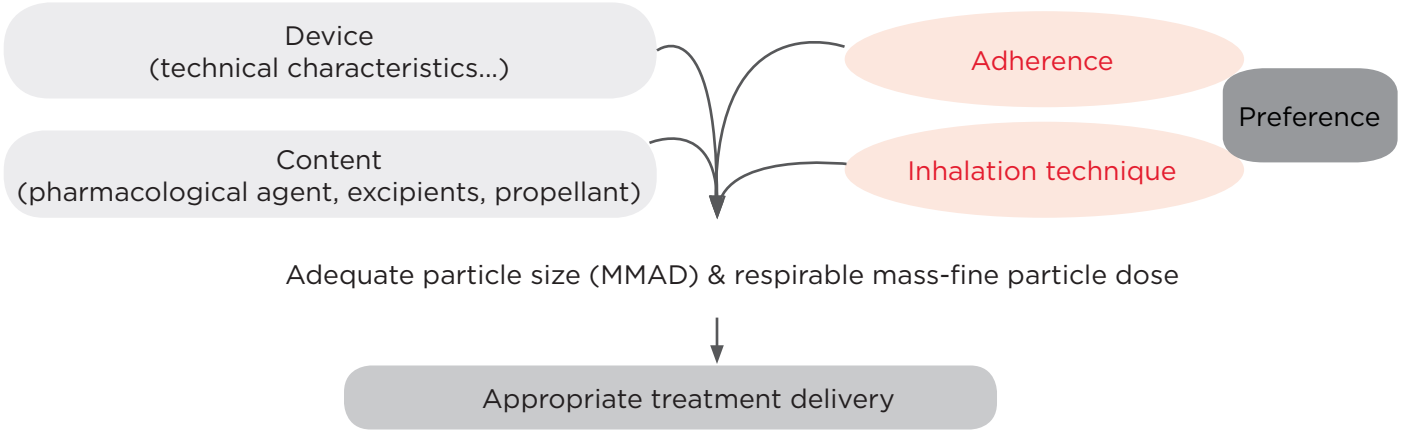

Figure 1. Basic principles in inhalation therapy: factors that influence the effectiveness of inhaled medications. MMAD: mass median aerodynamic diameter. Fine/respirable particles: MMAD <5 μm.

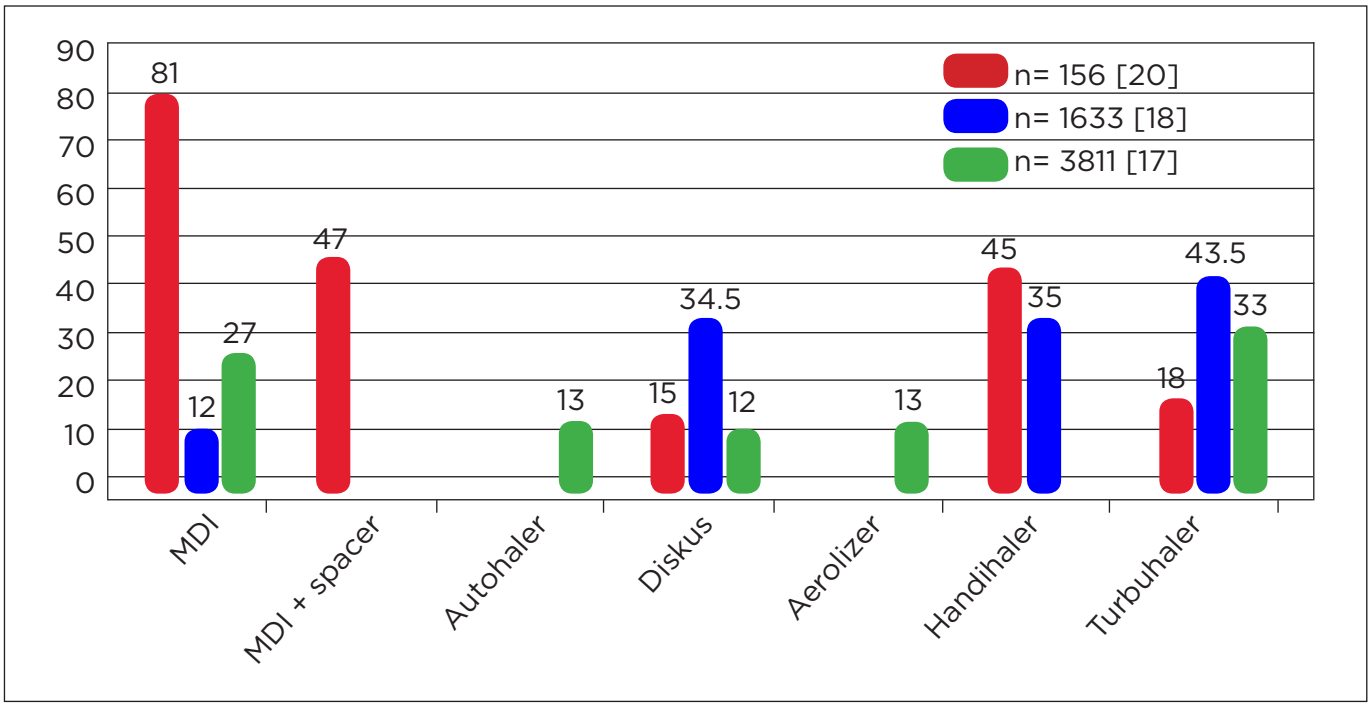

Figure 2. Frequency of inhalation errors by device type. Data from three studies.[19,20,22]

BASIC PRINCIPLES OF INHALER USE

The following steps of inhaler use are common to all devices: (1) device preparation (shaking for some pMDIs, adaptation to the spacer when required, priming for BAIs and reservoir DPIs, capsule insertion for single-dose DPIs); (2) exhalation followed by deep inhalation (coordinated with device triggering for pMDIs); (3) breath-holding (lasting at least 4 seconds); and, finally (4) normal breathing lasting at least 30 seconds before repetition of all these manoeuvres when a second dose is required. With pMDIs and BAIs, the drug formulation is propelled by the pressurised gas contained in the cartridge when the device is actuated, either manually (pMDIs) or automatically when inhalation begins (BAIs). Therefore, the main initial driving force is propulsion by the pMDI device and, as such, the inhalation needs to be slow. In contrast, when using DPIs, the only driving force is inhalation. The formulation of a DPI is broken up (de-aggregated) during the first part of each inhalation into particles that have characteristics suitable for deposition into the lungs (i.e. a diameter of <5 μm). This break-up is caused by a turbulent force that is generated inside the DPI by the interaction between the patient's inhalation and the resistance of the device. Each DPI has its own resistance ranging from low to high.[5] To obtain a set inhalation flow with higher resistance, the patient needs to use a more forceful initial inhalation.[14] Hence, compared to a pMDI, the inhalation manoeuvre from a DPI needs to be forceful, which many translate to 'as fast as you can'. Since the break-up of the dose occurs in the first part of the inhalation, the instruction with a DPI is 'forceful from the start'. Together with the intrinsic aerodynamic properties of drug particles, this explains why inhalation should preferentially be slow with pMDIs (especially to limit oropharyngeal impaction), while it has to be fast from the beginning with DPIs.[15-17] These inhalation manoeuvres are required to provide ideal inhalation flows of 30 L/min for pMDIs and between 30-90 L/min for DPIs. Finally, to ensure good penetration into the lungs, a deep inhalation manoeuvre is required and is usually defined as lasting at least 4-5 seconds.

FREQUENCY AND DETERMINANTS OF INHALER MISUSE

All studies in this area agree on a high proportion of misuse among patients with asthma or COPD, even in those who had been exposed to devices for long periods.[18-22] However, the frequency of misuse for each particular type of device varies between studies, depending on the studied population and

on the criteria used to define proper technique. Figure 2 combines results from some of these studies. Altogether, standard pMDIs are definitely the devices with the highest proportion of errors, but misuse is also frequent with the other types of devices; these have been developed to limit the impact of poor coordination but still need proper preparation, inhalation and breath-holding. Preparation errors relate, for example, to improper device priming manoeuvres including improper device position. Thus, none of the devices currently available can be considered as 'ideal' in that all require some patient skill. As a consequence, training is always required to ensure adequate patient education about specific handling requirements, as well as on the inhalation technique per se.[5]

Some patients appear to be at higher risk of poor inhalation technique.[22,23] Some risk-factors relate directly to the patient, these include: extreme ages (i.e. young children and the elderly), very severe airflow obstruction, cognitive dysfunction, motor handicap of the upper extremities, and co-morbidities such as stroke etc. In addition, patient preference could be associated not only with compliance but also with inhalation technique.[24] Other risk factors relate to the prescription and delivery of the treatment: patients who are simultaneously prescribed different types of devices (e.g. a pMDI and a DPI)[25] tend to use their devices less appropriately than those using a single device type (although some controversial data on this topic have been published in children),[26] and device switching without a face-to-face review is associated with worsening of asthma control.[27] The last group of risk factors relates to measures that should accompany the prescription: patients are to be trained on the optimal way of using their device, and inhalation technique has to be checked regularly. Virtually all healthcare professionals can be involved in patient training and follow-up regarding inhaler technique: primary care physicians, lung specialists, pharmacists, nurses, and physiotherapists. Since healthcare providers' skills and knowledge of inhalers can also be poor, they require training on correct inhaler handling.[28]

IMPACT OF INHALER MISUSE ON TREATMENT EFFECTIVENESS

Clinical consequences of poor inhalation technique have been studied mostly in asthma, where it is easier to explore links between inhaler use and control. Such studies are more difficult in COPD due to the very progressive nature of the disease and relatively small magnitude of observable treatment effects in the short-term.

It has long been known that pMDI misuse decreases the amount of drug deposited in the lungs and that, in patients with poor coordination, deposition can be improved by the use of spacers, BAIs or DPIs.[29] Corresponding studies used mostly scintigraphy with Technetium-radio-labelled particles as a means to provide images showing the level of deposition in the device, throat, lungs, and stomach.[30] Others have measured blood levels of administered drugs or their metabolites, blocking digestive absorption with charcoal so that systemic exposure results from lung absorption only, which directly correlates with lung deposition.[31] It has also been demonstrated that the magnitude of bronchodilation, obtained by β2-agonists, decreases in poor users.[32] Finally, more recent studies managed to show a link between poor inhaler technique and poor asthma control.[18,33-35] In one of these studies, a "dose-dependent" relationship was even found between the number of errors in pMDI use and the level of control (named "instability" at that time).[18] Asthma control was worse in poor pMDI users and, among errors in inhalation technique, poor coordination had the greatest impact on control. More recently, these authors found a triangular relationship between control, adherence to treatment and inhaler technique.[33] Poor inhalation technique and poor compliance were both independently associated with control, the other risk factor for poor control in that study being smoking.

Altogether, these results show that inhalation technique should belong to the systematic check-list when assessing a patient with insufficient asthma control, together with smoking status, other environmental exposures (to allergens or irritants), body mass index, adherence to treatment, associated diseases, or an alternate diagnosis (gastro-oesophageal reflux, COPD, hyperventilation syndrome, vocal cord dysfunction, congestive heart failure, vasculitis, allergic bronchopulmonary aspergillosis).[36,37]

COMBATTING INHALER MISUSE TO IMPROVE TREATMENT EFFECTIVENESS

Three directions can be followed to decrease the frequency and consequences of poor

inhalation technique: research to improve inhalers, training patients and doctors, and individualising inhaler choice.

Developing the 'Ideal Inhaler'

The ideal inhaler would be user-friendly, delivering optimal respirable amounts of drugs irrespective of the prescriber and patient's skills, without any need for external supervision and independently of environmental conditions. More realistically, it should have the characteristics described in Table 1. To date, none of the currently available inhalers can be considered as 'perfect' regarding all these characteristics. All need some training and regular checking of inhalation technique. However, some new inhalers try to simplify required manipulation. It must also be noted that most devices still do not deliver all pharmacological classes of respiratory drugs. Thus, the choice of the prescribed pharmacological agent restricts (and can be restricted by) the number of devices that can be proposed.

Checking and Training

Several observational studies have shown that training patients on inhaler use improves not only inhalation technique but also adherence to treatment and, most importantly, disease control.[15,33,38,39] Most of these studies are purely observational with a 'before-after' design, and include only patients with asthma. Only a few specifically recruited patients with COPD, in whom similar improvements were demonstrated.[39] Interestingly, one observational study found that control improved only in those patients in whom inhaler technique improved following training by pharmacists, suggesting that the effect of training on control is actually determined by its effect on inhaler technique.[33] Similar results were very recently found in COPD patients.[40] However, it has also been shown that the effect of training is inconstant and sometimes transient.[41]

Several tools and strategies can be used to provide training: visits to health care professionals using placebo demonstration devices or the patient's own device, video demonstration of adequate and incorrect technique, tele-counselling,[42] and web-based programs.[43] As mentioned above, all healthcare professionals should be involved in this training process: primary care physicians, lung specialists, nurses, physiotherapists, and pharmacists. The choice of tools and involved professionals often depends on local availability and organisation. It is also of utmost importance to educate professionals properly in the first place[44] and then repeat training regularly.

In all cases, it must be outlined that providing patients with written material (such as brochures and leaflets) is not sufficient. A critical step in education is the regular observation of the patient using his/her inhaler. The other major step is the live demonstration of proper technique when necessary, followed by repeated observation of the patient's ability to correct his/her technique. Simply asking the patient whether he/she has difficulties using his/her inhaler does not provide reliable information.[21]

Table 1. Theoretical characteristics of the ideal inhaler.

Criteria	Device and inhalation technique characteristics
General appearance	User-friendly
Priming	None
Coordination between triggering and inhalation	Not required
Effect of errors in inhalation technique	None
Dose consistency	Perfect, independently of environmental conditions (temperature, humidity etc) and inhalation manoeuvre
Dose counter	Present, based on actual inhalation rather than manipulation
Perception of drug delivery	Clear perception (but not based on unpleasant sensations such as bad taste)
Feedback	Confirmation that a dose has been inhaled, technique used was correct Reminder about adherence

Of note, some testing and training devices have been developed to help healthcare professionals check inhalation technique and train patients.[15,41] They are known to improve inhalation technique, but their cost-effectiveness remains unknown at present. In addition, no study has investigated the effect of training or the most desirable training method/tool in situ over a prolonged period of time.

Personalising Device Choice

Patients with asthma or COPD do not all require the same treatment, and do not all have the same skills and preferences. Therefore, tailoring the treatment to each specific patient is of utmost importance.[3,45-49] Several factors have to be taken into account when selecting a specific inhaler device for each patient. These factors can be categorised in four ways: (1) Patient-related factors, including (i) age and ability to inhale consciously, handle the device and coordinate the use of the device and the inspiratory effort, (ii) patient's preference, (iii) adherence and compliance, (iv) language and literacy, and (v) presence of comorbidities that could be aggravated by some respiratory treatments. (2) Disease-related factors, since (i) severe and/or acute airflow obstruction may compromise the ability to generate an adequate inspiratory flow and (ii) therapeutic strategy and indications are not the same for asthma and COPD. (3) Device-related factors, as the optimal inhalation profile differs between pMDIs (slow inspiration is preferable) and DPIs (forceful high-flow inhalation is not required, with fast acceleration especially for reservoir devices). For example, observational comparative effectiveness studies suggested that BAIs and standard pMDIs (using HFA-propelled extra fine particles with a size of approximately 1 μm) could improve treatment effectiveness as compared to standard metered-dose inhalers,[7,8] due to the more limited influence of errors in coordination/inhalation technique on lung deposition with these devices.[6,7] Finally, (4) Caregiver-related factors, accounting for the availability and knowledge of professionals involved in information and education (general practitioners, specialists, nurses, physiotherapists, pharmacists).

In addition, the multiplication of inhalation devices and corresponding instructions should be avoided since it can be a source of confusion for healthcare providers who are not specialised in the respiratory area, such as many primary care physicians, even though they care for the majority of patients with asthma or COPD.

CONCLUSIONS

The inhaled route remains crucial for the treatment of bronchial diseases. However, drug deposition and subsequent treatment effectiveness are highly dependent on inhalation technique, which is incorrect in many patients with asthma and COPD. Many inhalation devices are available and others are currently being developed with the aim of simplifying required handling, and thus improving treatment safety. Nonetheless, at present, proper training and regular checking of inhalation technique remain critical to optimise treatment effectiveness. Involved healthcare professionals have to be adequately trained before providing this service. Various educational tools can be used including videos, web-based platforms, and tele-counselling. Optimising treatment effectiveness also requires tailoring the drug-device combination chosen for each individual patient, based on his/her individual characteristics, the specific disease and its severity, the characteristics of devices, and the skills of involved healthcare professionals. There is a need for long term effectiveness studies to identify the training methods/tools that should be used for each inhaler.

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Disclosure

NR has received honorarium for consultancy, participation in advisory boards, symposium presentations, educational activities from Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, MEDA, MSD, Mundipharma, Novartis, Nycomed, Pfizer and TEVA, and research funds from Nycomed, Novartis and Boehringer Ingelheim.

HC has no shares in any pharmaceutical companies. He has received sponsorship to carry out studies, together with some consultant agreements and honoraria for presentation, from several pharmaceutical companies that market inhaled products. These include Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Innovata Biomed, Meda, Napp Pharmaceuticals, Orion, Teva, Truddell Medical International and UCB. Research sponsorship has also been received from grant awarding bodies (EPSRC and MRC).

FL has received fees for participation in advisory boards, symposium presentation as well as reimbursement for attending meetings from MEDA, AstraZenecva, Menarini, Chiesi , Mundipharma.

AA has received honorarium for consultancy, participation in advisory boards, symposium presentations, educational activities from Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, MSD, Mundipharma, Novartis and Takeda, and research funds from GSK, MSD and Takeda.

JCV has lectured for a received honoraria from Asche-Chiesi, AstraZeneca, Avontec, Bayer, Bencard, Bionorica, Boehringer-Ingelheim, Essex/Schering-Plough, GSK, Janssen-Cilag, Leti, MEDA, MSD, Mundipharma, Novartis, Nycomed/Altana, Pfizer, Revotar, Sandoz-Hexal, Stallergens, TEVA, UCB/Schwarz-Pharma, Zydus/Cadila and possibly others and has participated in advisory boards for Asche-Chiesi, Avontec, Boehringer-Ingelheim, Essex/Schering-Plough, GSK, Janssen-Cilag, MSD, Mundipharma, Novartis, Revotar, Sandoz-Hexal, UCB/Schwarz-Pharma and possibly others and has received grants from GSK, MSD, and the Deutsche Forschungsgemeinschaft.

RD has received reimbursements for attending symposia, fees for speaking, organising educational events, funds for research or fees for consulting from AstraZeneca, Boehringer-Ingelheim, Chiesi, Merck Sharp & Dohme, Mundipharma, Novartis, Takeda, Almirall and Teva.

DP has received honorarium for consultancy, participation in advisory boards, symposium presentations, educational activities from Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, MEDA, MSD, Mundipharma, Novartis, Pfizer, Takeda and TEVA, and research funds from Almirall, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, MEDA, MSD, Mundipharma, Novartis, Takeda and TEVA

ADMIT has been sponsored by MEDA pharma and is now sponsored by Chiesi Pharmaceuticals.

BRONCHOSCOPIC NITINOL COIL IMPLANTATION: A NEW LUNG VOLUME REDUCTION STRATEGY IN COPD

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Disclosure: No potential conflict of interest.
Received: 02.09.13 **Accepted:** 30.09.13
Citation: EMJ Respir. 2013;1:72-78.

ABSTRACT

Emphysema is a progressive, debilitating disease characterised by irreversible destruction of lung tissue. The gas trapping within the destroyed alveoli, and resultant hyperinflation, render conventional medical treatment generally of limited benefit, especially in the advanced stages of the disease. Utilisation of bronchoscopic techniques for achieving lung volume reduction has advanced over the past years. Amongst these, lung volume reduction using coils (LVRC) is a promising option. The LVRC are made from preformed Nitinol wire with shape memory. They are bronchoscopically delivered into the desired sub-segmental bronchus and recover to a pre-determined shape upon deployment. Published data so far, with endpoints being safety and feasibility, are promising. The procedure itself seems to be technically feasible and results in significant improvements in pulmonary function, exercise capacity, and quality of life sustained during the follow-up period, and with an overall acceptable safety profile. Current ongoing studies further investigate the feasibility, safety, and efficiency of LVRC. Future research is necessary in order to elucidate whether the patient selection criteria and methodology currently used are amenable to improvements, and to establish the duration of benefit and its cost-effectiveness when compared to optimal medical treatment, before applying this treatment into routine clinical practice.

Keywords: Chronic obstructive pulmonary disease, emphysema, bronchoscopy, lung volume reduction coils.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) continues to be a significant cause of morbidity, mortality, and healthcare costs worldwide. As the global population ages, the burden of COPD will increase in years to come. Due to the number of current and new smokers, emphysema affecting COPD patients is also expected to remain a leading cause of morbidity and mortality.[1] The overall prevalence of stage II or higher COPD is 10.1%, 11.8% for men, and 8.5% for women, with a pooled prevalence of emphysema ranging close to 2%.[2,3]

Emphysema is a progressive, debilitating disease that is characterised by irreversible destruction of lung tissue as a result of inflammation. This inflammation is caused in most patients by exposure to noxious inhaled agents for extended periods of time, the most common being cigarette smoke.[4] The destroyed alveolar tissue results with disease progression in a decrease in lung elastic recoil, hyperinflation, and gas trapping due to premature closure of the small airways of the affected lung tissue. The gas trapping and resultant hyperinflation render conventional medical treatment, consisting of bronchodilators and anti-inflammatory medications, generally of limited benefit, especially in advanced stages of the disease.[5]

TREATMENT OPTIONS

For COPD patients with severe emphysema, therapeutic options are still limited. Lung volume reduction surgery (LVRS) has been applied as a palliative treatment in selected patients, but although the concept was excellent, the referral of patients has been severely influenced by significant perioperative death and complications.[6-8]

Over the past decade, there have been significant improvements in the field of interventional pulmonology, and progress has been made in utilising less invasive techniques for achieving the desired lung volume reduction. Different techniques are currently available. Amongst these endoscopic lung volume reduction (ELVR) treatment methods, the one-way valve placement is the most widespread so far.[9-13] These valves allow air to escape from the distal lung without 'fresh' air entering the segment during inspiration. Total occlusion of the target lobe is desired, and the best results of lung volume reduction are achieved when total atelectasis is evident after the procedure. In order for a patient to be suitable for ELVR with valves, radiologic evidence of an intact fissure, as a marker for lack of collateral ventilation, is required. Absence of intact fissures will inhibit volume reduction of the treated lobe, indicating the need for ELVR treatments that work independently of collateral ventilation.[14,15] Treatment with lung volume reduction coils (LVRC) overcomes this limiting factor and might serve as an alternative choice in this specific group of patients. The aim of this review is to quote the ELVR technique by means of LVRC.

LVRC AND THE LVRC PLACEMENT PROCEDURE

The LVRC are made from preformed Nitinol wire with shape memory (Figure 1a). They are bronchoscopically delivered straight into the desired sub-segmental bronchus and recover to a non-straight, pre-determined shape upon deployment. A possible mechanism that explains how coils work, is by mechanically bending the airway and compressing the diseased lung parenchyma. In this manner, the coil seems to cause tension in the surrounding tissue, increasing elastic recoil and possibly redirecting air to healthier portions of the lung. LVRC exist in three different sizes (100, 125, and 150 mm) to accommodate differently sized airways.

Going through the whole procedure in greater detail, the LVRC placement requires a specific delivery system. This delivery system consists of a guide wire, a delivery catheter, a cartridge, and forceps. First, the airway in the selected segment is identified bronchoscopically and the low stiffness guide wire is advanced into the airway under fluoroscopic guidance. The catheter is passed over the guide wire and aligned with its distal tip at approximately 15 mm from the pleura. The length of the airway is

Figure 1a.

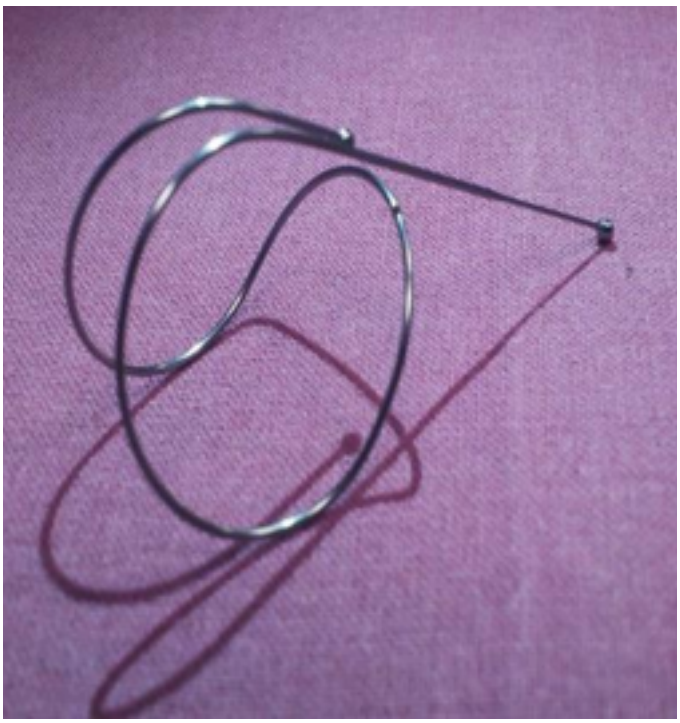

Figure 1b.

Figure 1a. RePneu® Lung Volume Reduction Coil.
Figure 1b. RePneu® Lung Volume Reduction Coil, straightened in cartridge.

measured using radiopaque markers on the guide wire and the desired LVRC length is chosen. The guide wire is then removed and the catheter stays in place. Using the forceps, the endoscopist grasps the LVRC by its proximal end and passes it through the cartridge, which results in the LVRC acquiring a straight form (Figure 1b). The straightened LVRC is then loaded into the distal end of the catheter by coupling the cartridge to the catheter´s hub and the LVRC is then introduced further distally through the catheter with the help of the forceps and under fluoroscopic guidance. Next, the catheter is removed while the proximal end of the LVRC is held in place with the forceps. As the catheter is pulled back, the LVRC assumes its preformed shape, bending the airway and attached parenchyma with it. Finally, the LVRC is released from the forceps and the bronchoscope and forceps are removed (Figure 2). These steps are then repeated in the same sequence for every LVRC to be placed. In the literature, it is mentioned that it is possible to remove or reposition the LVRC by reversing the implantation process,[16] but to our knowledge this can be difficult.

Bronchoscopy can be done under moderate sedation or general anaesthesia according to patient requirements and local practice. Antibiotic prophylaxis according to standard protocols is also common, usually for a total of 7 days. Following recovery from anaesthesia patients must stay in hospital for 1-3 days for observation. Possible post-procedural side-effects, so far observed, include mild to moderate haemoptysis, chest pain, cough, pneumothorax, COPD exacerbation, and pneumonia.

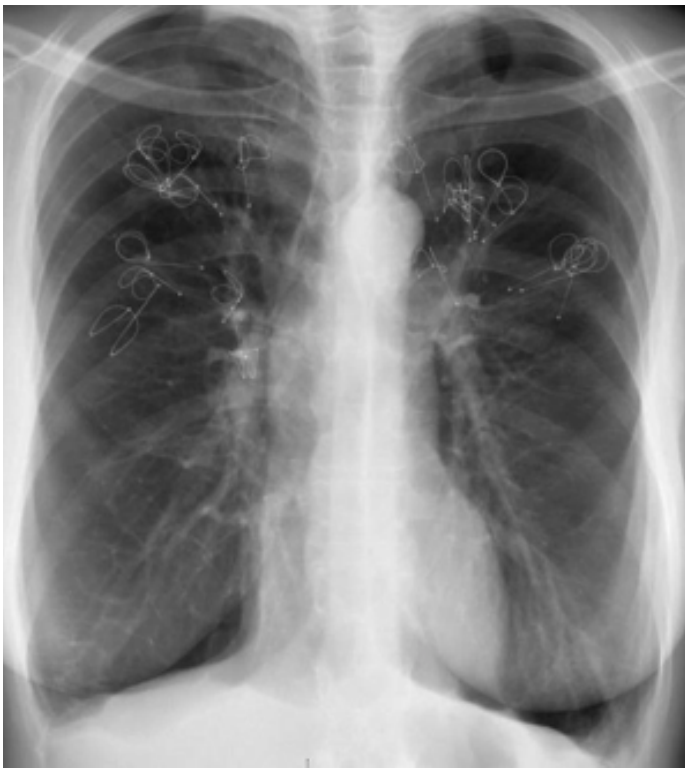

Figure 2. Chest X-ray after bilateral coils insertion. Courtesy of Prof C.P. Heußel.

PUBLISHED DATA

LVRC is a relatively new therapeutic approach and published data so far is meagre. In the first pilot study published, Herth FJ et al.[17] included 11 patients with severe predominantly homogeneous (8 patients) or heterogeneous (3 patients) emphysema and incomplete fissures bilaterally, whose most diseased areas were treated by insertion of coils. These 11 patients underwent a total of 21 procedures. Inclusion and exclusion criteria were modelled on the National Emphysema Treatment Trial (NETT) study and are included in Table 1. The primary endpoints were safety and feasibility, and the secondary endpoints were efficacy outcomes. Safety was measured by the analysis of adverse events. Owing to the sequential treatments and operator discretion, all patients had a total follow-up time of at least 7 and up to 11 months. Each of the 33 adverse events which occurred during the follow-up period, were categorised by severity [mild (36%), moderate (64%), or severe (0%)] and by relationship to device or procedure [not related (42%), possibly related (58%), or probably related (0%)] by the treating physician. Adverse events, rated as possibly related to either the procedure or the device were dyspnoea (10 events), cough (5 events), COPD exacerbation (3 events), and chest pain (1 event). No pneumothorax was noted. Although the study was neither intended nor powered to analyse effectiveness, some interesting trends have emerged, with the predominantly heterogeneous disease group appearing to show substantial improvements in pulmonary function, lung volumes, 6-minute walking test (6MWT), and quality-of-life (QoL) measures.

The study that followed was a prospective cohort pilot study from Slebos DJ et al.,[18] NCT01220908, in which 16 patients were treated (4 unilaterally and 12 bilaterally). This time, the safety and efficacy of LVRC treatment was assessed in patients with severe heterogeneous emphysema, which were followed up for 180 days. Table 1 comprises the inclusion criteria. For safety assessment, all adverse events that occurred were reported. Consequently, they were divided into those occurring during the

Table 1. Main inclusion criteria for three published studies on LVRC treatment of emphysema.

	Herth FJ et al.[17]	Slebos DJ et al.[18]	Shah PL et al.[24]
Age	≥35 years	N/A	≥35 years
Homogenous/heterogeneous emphysema on HRCT	+/+	–/+	+/+
Unilateral/bilateral emphysema on HRCT	+/+	+/+	+/+
FEV1 post-bronchodilation	≤45% pred	<45% pred	≤45% pred
TLC	>100% pred	>100% pred	>100% pred
mMRC score (scale 0-4)	≥2	>1	≥2
Ex-smoker	≥8 weeks	>8 weeks	≥8 weeks
Informed consent	+	NA	+

HRCT: high resolution computed tomography scan, FEV1: forced expiratory volume in the 1 sec, TLC: total lung capacity, mMRC: modified Medical Research Council dyspnoea scale, pred: predicted.

first 30 post-procedural days, rated as possibly related to either the device or the procedure, and those occurring during the follow-up period from 1 to 6 months. In the first instance, the events reported were pneumothorax (1 event), pneumonia (2 events), COPD exacerbation (6 events), chest pain (4 events), and mild haemoptysis (21 events). In the second instance, pneumonia (3 events) and COPD exacerbation (14 events) were reported. All events resolved with standard care. Concerning efficacy, the primary variable assessed was any change in respiratory-related QoL, as estimated by St George's Respiratory Questionnaire (SGRQ); a significant improvement of 14.9 points (±12.1 points, p=.005), and a total score was reported at 6 months compared to baseline. Additionally, pulmonary function testing (PFT) and 6MWT were performed and the initial improvements observed were sustained throughout the 6 month follow-up period with a Δ forced expiratory volume in 1 sec (FEV 1) of +14.9% (±17.0%), Δ functional vital capacity (FVC) of +13.4% (±12.9%), Δ residual volume (RV) of -11.4% (±9.0%), and a Δ 6MWT +84.4 m (±73.4 m), with more than 50% of the patients improving to above the minimal clinical important difference (MCID) for FEV1,[19] 6MWT,[20-22] and SGRQ.[23] (Table 2)

The most recently published trial, up to August 2013, is a prospective, randomised, multicentre trial from Shah PL et al.,[24] which recruited 47 patients with severe emphysema from three centres in the UK. Patients in this study were randomly allocated in a 1:1 ratio to either LVRC treatment (treatment group, 23 patients) or best medical care (usual care group, 24 patients). Inclusion criteria are provided in Table 1. To the patients of the treatment group, LVRC were inserted into the selected lobe or lobes as previously described. Subsequently, patients were reassessed after 1 month, with stable patients (patients with no substantial deterioration of symptoms and on routine medications for at least 14 days) undergoing treatment of the contralateral lung. Treatment group patients were followed by clinic visits at 30 and 90 days after second treatment. The usual care group underwent similar initial clinical assessments and clinic visits to coincide with the two treatment visits. The primary endpoint of the study was the difference in response in the SGRQ between treatment and usual care groups at 90 days after final treatment. Secondary endpoints were changed from the baseline for percentage of change in FEV1, Total Lung Capacity (TLC), RV, 6MWT, and modified Medical Research Council dyspnoea scale (mMRC). The safety outcome of the study was to identify any potential procedure-related and device-related adverse events at 90 days after final treatment. During the initial treatment recovery period (initial 30 days after each treatment or usual care visit), six serious adverse events (2 exacerbations of COPD, 2 pneumothoraces, which responded quickly to intercostal drainage, and 2 lower respiratory tract infections, a total of 15% incidence) were reported in the LVRC group and one in the usual care group (4% total incidence).

Table 2. Results of three published studies on LVRC treatment of emphysema.

	Primary endpoints	Secondary endpoints
Herth FJ et al.[17] *11 patients treated with LVRC* 90 days follow-up	***Safety:*** Total of 33 adverse effects; 36% mild, 64% moderate, 0% severe; 0% probably related to device or procedure, 58% possibly related, 42% not related.	***Efficacy (ΔPFT, ΔmMRC, ΔSGRQ):*** Patients with heterogeneous emphysema trended to achieve better outcomes. The study was neither designed nor powered to evaluate statistical significance.
Slebos DJ et al.[18] *16 patients treated with LVRC* 30 days follow-up	***Safety:*** - pneumothorax, 1 event - COPD exacerbation, 6 events - chest pain, 4 events - mild haemoptysis, 21 events	***Efficacy (ΔPFT, ΔSGRQ, Δ6MWT)*:*** ΔFVC%: + 17.0±14.9, p=.002 ΔFEV1%: + 22.6±21.7, p=.004 ΔRV%: -12.4±9.0, p<.001 ΔRV/TLC%: -8.2±7.1, p=.002 ΔSGRQ: -12.2±13.5, p=.009 Δ6MWT%: +29.8±0.4, p=.006
180 days follow-up	- pneumonia, 3 events - COPD exacerbation, 14 events	ΔFVC%: +13.4±12.9, p=.002 ΔFEV1%: +14.9±17, p=.004 ΔRV%: -11.4±9.0, p<.001 ΔRV/TLC%: -8.0±5.5, p<.001 ΔSGRQ: -14.9±12.1, p<.001 Δ6MWT%: +2.9±36.3, p<.005
Shah PL et al.[24] 90 days follow-up [***2 patient groups:*** ***Treatment group*** (23 patients assigned to LVRC treatment) ***Control group*** (24 patients assigned to control, usual care)]	***Between group difference in SGRQ change from baseline° (intention to treat analysis):*** -8.36 (-16.24 to -0.47), p=0.04	***Between group difference in PFT, 6MWT, mMRC changes from baseline° (intention to treat analysis):*** TLC: -0.11 (-0.29 to 0.07), p=0.22 RV: -0.31 (-0.59 to -0.04), p=0.03 6MWT: 63.55 (32.57 to 94.53) p<0.001 % change in FEV: 10.62 (1.12 to 20.12), p=0.03 mMRC: -0.15 (-0.60 to 0.30) p=0.5

*Data are presented as change from baseline ±SD.

°Corrected for difference between groups at baseline.

LVRC: lung volume reduction coils, PFT: pulmonary function testing, COPD: chronic obstructive pulmonary disease, 6MWT: 6-min walking test, SGRQ: St George's Respiratory Questionnaire, PFT: pulmonary function testing, FEV1: forced expiratory volume in 1 sec, FVC: functional vital capacity, TLC: total lung capacity, RV: Residual Volume, mMRC: modified Medical Research Council dyspnoea scale.

During the next 2 months, and until completion of the follow-up, three serious adverse events were reported in the treatment group and three in the usual care group (exacerbations and lower respiratory tract infections). The results of the analysis included a statistically and clinically significant improvement of SGRQ score (–8.11 [–13.83 to –2.39]) as well as in 6MWT (51.15 m [27.65 m to 74.66 m]) and improvement in the percentage of change in FEV1 (14.19 [6.84 to 21.55]) and reduction of RV (–0.51 L [–0.73 L to –0.30 L]) at 90 days after final procedure. For further results see also Table 2. In all parameters, the changes were greater in the LVRC group than in the usual care group. There was no significant between-group difference observed in the change in mMRC, TLC and also in serious adverse events occurrence.

ONGOING STUDIES

Currently there are several new trials ongoing, or which have recently been completed, that address some of the issues mentioned above. Their data and conclusions should be considered to be preliminary until they have been published in a peer-reviewed journal. Amongst them, a single-arm, open-label study has been recently completed (NCT01421082) which evaluates physiologic parameters directly related to the possible mechanisms of action of LVRC in subjects with homogeneous emphysema. Relative data have been published in abstract form.[25] Several multicentre studies from Germany, France, and the Netherlands have been recently completed (NCT01328899), or are currently recruiting participants (NCT01822795), (NCT01806636), with primary outcomes aiming to validate the safety and clinical efficacy of LVRC as well as its cost-effectiveness. The RENEW study (NCT01608490), a multicentre, randomised, assessor-blinded controlled study of safety and effectiveness of LVRC is expected to follow-up 315 participants from USA and Europe for 1 year. Some of the latest results from research concerning LVRC, presented as abstracts at the European Respiratory Society Congress (2013), further focus on elucidating the mechanism of coils´ action, probably by improving lung compliance[25] and also prove its efficacy in patients with homogeneous emphysema; efficacy sustained for longer periods of time, up to 1 year, both in patients with heterogeneous and homogeneous emphysema has been also addressed.[26,27] In heterogeneous emphysema and bilaterally incomplete fissures, unilateral LVRC showed improvement in exercise capacity, QoL and PFT at 90 days with a tendency to decrease at 6 months post intervention.[28]

DISCUSSION

The above studies show that LVRC, as a novel therapeutic approach of patients with advanced COPD and severe emphysema, seems so far to be promising due to its safety and feasibility. The procedure itself is technically feasible and results in significant improvements in pulmonary function, exercise capacity, and QoL, with an overall acceptable safety profile.

In emphysema patients, as the disease progresses, the lung becomes too large and can neither expand fully nor function effectively within the rigid chest cavity, and this increasing hyperinflation results in reduced exercise capacity. Furthermore, the respiratory muscles are forced to contract at a mechanical disadvantage and consequently, the work of breathing is increased, leading to patients experiencing gradually deteriorating shortness of breath, limited exercise capacity and decreasing quality of life.[29] As Shah et al.[24] pointed out in the RESET trial, the beneficial effects of LVRC could be explained, due to regional compression of the lung and subsequent expansion of better functioning areas of the lungs, and also due to the re-establishment of tethering in the small airways, which improves elastic recoil of the lung. This results in more efficient support of the walls of the small airways, holding them open and preventing premature collapse or narrowing during expiration, resulting in gradual release of trapped gas. This mechanism would also reduce dynamic hyperinflation, explaining the improvement in exercise capacity that has been observed.

Further research is necessary in order to elucidate whether the patient selection criteria and methodology currently used are amenable to improvements. It is also essential to elucidate whether the positive results of LVRC treatment that we have so far, are consistent amongst larger groups of patients, and to also possibly determine which subgroups of patients will have the best outcomes. To date, post-intervention follow-up has been short and long-term results are still not known. We need to establish the duration of benefit and also its cost-effectiveness, when compared to optimal medical treatment before

applying this treatment into routine clinical practice. LVRC treatment remains currently an expensive procedure, performed in highly specialised centres from experienced bronchoscopists.

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COPD NOCTURNAL DESATURATOR PATIENTS WITH OBESITY AND PULMONARY HYPERTENSION: CROSS-TALK BETWEEN ADIPOCYTE TISSUE SYSTEMIC HYPOXIA AND LUNG-TO-BLOOD TRANSLOCATION OF INFLAMMATORY MEDIATORS

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Disclosure: No potential conflict of interest.
Received: 12.04.2013 **Accepted:** 26.07.2013
Citation: EMJ Respir. 2013;1:79-85.

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is often accompanied by other chronic diseases associated with systemic inflammation, such as chronic heart failure, diabetes, and atherosclerosis. Nocturnal oxygen desaturation waxing and waning plays a central role in conditions leading to systemic inflammation in COPD obese patients. Obesity and metabolic syndrome (MetS) represent two different metabolic abnormalities that may be linked by the presence of underlying systemic inflammation. Alveolar hypoxia and consequent hypoxaemia increase in prevalence as the severity of COPD also increases. Chronic hypoxaemia contributes to the development of adverse sequelae of COPD such as pulmonary hypertension (PH), secondary and systemic inflammation. The innovation of COPD phenotyping is defined as COPD desaturators. These sleep-related changes predispose to nocturnal cardiac dysrhythmias, PH and potentially nocturnal death, particularly during acute exacerbations. In patients with COPD, systemic inflammatory phenotype likely reflects pulmonary inflammation, which results from lung-to-plasma spillover of inflammatory mediators. However, obesity-related hypoxia evokes local inflammatory response within adipose tissue per se, and systemic hypoxaemia likely contributes to the presence of adipose tissue inflammation. The nocturnal hypoxic insult occurring during sleep-disordered breathing may also contribute to chronic vascular remodelling. Consequently, these mechanisms may result in endothelial dysfunction and vascular damage, leading to increased risk of PH in COPD. In patients with COPD and concurrent obesity, we have proposed that three factors can play a role in the systemic inflammatory syndrome: the severity of pulmonary impairment, the degree of obesity-related adipose tissue hypoxia, and the severity of systemic hypoxia due to reduced pulmonary function.

Keywords: Chronic obstructive pulmonary disease (COPD), metabolic syndrome (MetS), oxygen desaturation waxing and waning, pulmonary hypertension (PH).

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a complex, multi-factorial, heterogeneous disease, whose clinical and functional presentation greatly varies from patient to patient, despite similar degree of airflow limitation.[1,2] Comorbidities such as chronic heart failure, cardiovascular disease, depression, diabetes, muscle wasting, weight loss, lung cancer, and osteoporosis can frequently be found in patients with COPD and are considered to be part of the commonly prevalent non-pulmonary sequelae of the disease.[3,4] Sleep-disordered breathing and COPD are among the most common pulmonary diseases. The severity of COPD also influences the degree of oxygen desaturation. The lower the FEV1/FVC ratio (≤70%), the more likely that significant desaturation occurs during sleep.[5,6] Systemic inflammation is considered a hallmark of COPD and one of the key mechanisms that may be responsible for the increased rate of comorbidities, including cardiovascular complications, obstructive sleep apnoea syndrome (OSAS), muscle dysfunction, osteoporosis, clinical depression, and anxiety.[7,8] The metabolic syndrome (MetS) was assessed according to International Diabetes Federation criteria (Table 1). Recently, the correlation between MetS and COPD has been confirmed, MetS being a risk factor for increased number of exacerbations in COPD patients due to the shared inflammatory cytokine burden.[9,10] A high prevalence (61%) of MetS was found in COPD patients participating in a respiratory study, while another study reported a lower prevalence (44%) in patients without COPD.[11,12]

This brief review focuses on COPD and nocturnal hypoxaemia with obesity and MetS that can be the cause of systemic inflammation. In patients with COPD and concurrent obesity and MetS, we propose (Figure 1) that at least three factors play a role in the systemic inflammatory syndrome: the severity of pulmonary impairment, the degree of obesity-related adipose tissue hypoxia, and the severity of systemic hypoxia due to reduced pulmonary functions.

Table 1. Different inflammatory mechanisms involved in COPD/disease process/obesity and comorbidities.

1	Epidemiological data suggest that nocturnal symptoms and nocturnal oxygen desaturation with symptomatic sleep disturbance is common, and may exceed 43% among patients with chronic obstructive pulmonary disease (COPD).
2	COPD patients with a T_{90} ≥30% with mean nocturnal SaO_2 ≤90% and a Nadir $SatO_2$ ≤85% are defined as desaturators (D); all others are defined as non-desaturators (ND).
3	Nocturnal oxygen desaturation waxing and waning in COPD patients is a major inflammatory stimulus: the desaturation–reoxygenation sequence is a typical pattern coupled with the majority of nocturnal respiratory events.
4	It has been shown that 50% of COPD patients have one or more MetS defining criteria.
5	Obesity is diagnosed when body mass index is ≥30 kg/M^2.
6	Metabolic syndrome (MetS) defining criteria are: central obesity (waist circumference: men ≥94 cm; women ≥80 cm); plus any two of the following four factors: triglyceride levels ≥150 mg/dL; high-density lipoprotein cholesterol levels of ≤40 mg/dL in men and ≤50 mg/dL in women; systolic BP ≥130 mm Hg or diastolic BP ≥85 mm Hg; fasting plasma glucose levels of ≥100 mg/dl or previously diagnosed type 2 diabetes.
7	The principal contributor to hypoxaemia in COPD patients is ventilation/perfusion (V/Q) mismatch resulting from progressive airflow limitation and emphysematous destruction of the pulmonary capillary bed.

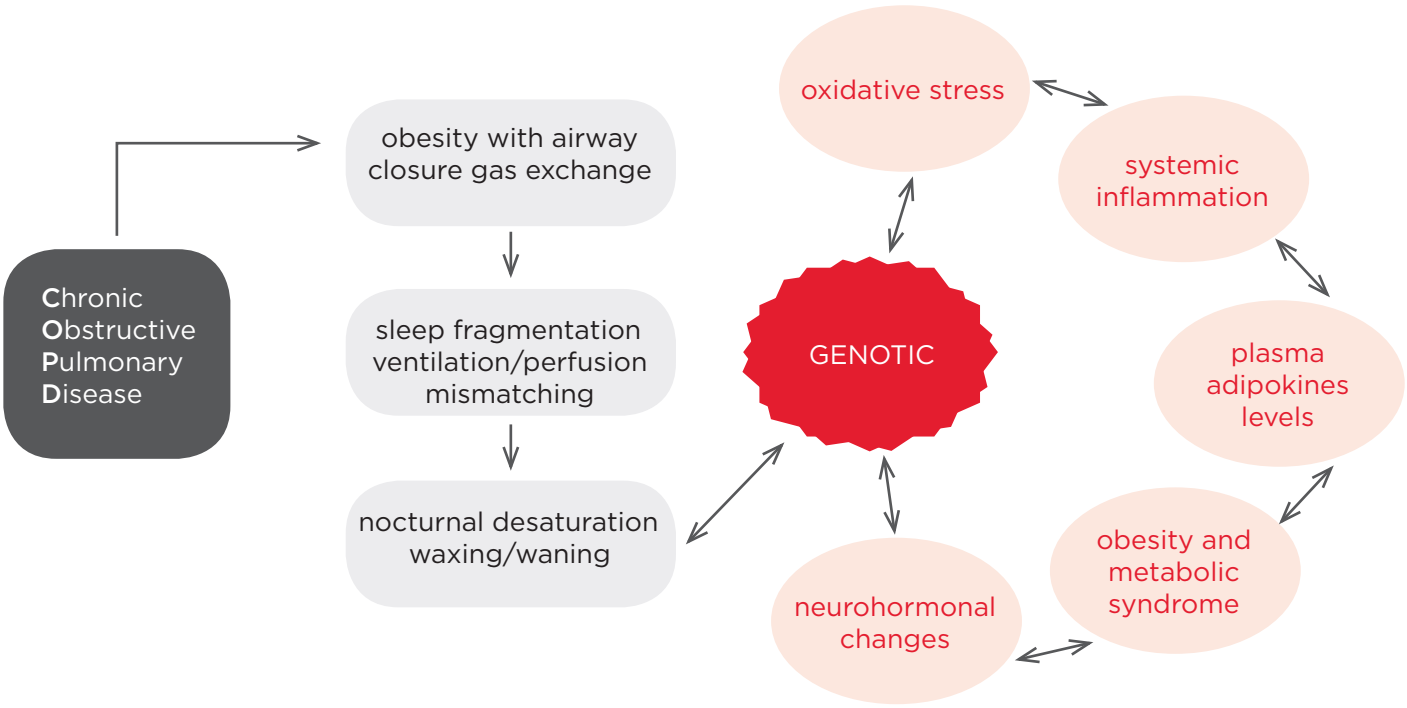

Figure. 1. Biological mechanisms for the development of various inflammatory processes in obese COPD patients with metabolic syndrome.
The diagram shows the pathophysiological effects of chronic nocturnal hypoxaemia and obesity in COPD patients.

SLEEP FREQUENTATION IN COPD

Nocturnal Oxygen Desaturation Waxing-Waning with Hypoxic Vascular Remodelling and Pulmonary Hypertension

Patients with COPD may experience changes in sleep and clinical symptoms. Evidence indicates that as many as 43% of patients with chronic bronchitis or emphysema have sleeping difficulties.[13] More than just the diagnosis of COPD, the presence of COPD symptoms, such as a cough or sputum production or wheezing, strongly correlated with difficulty in falling or staying asleep.[14] Other investigations have objectively confirmed poor sleep quality, with decreased total sleep-time and decreased sleep efficiency in patients with respiratory problems.[15,16]

Nocturnal oxygen desaturation in COPD is likely to be the consequence of the combined effects of physiological hypoventilation during sleep. However, there is evidence that some patients with awake arterial oxygen tension (PaO_2) levels in the mildly hypoxaemic range can also develop clinically significant nocturnal oxygen desaturation, which may predispose to pulmonary hypertension (PH).[17] Potential causative mechanisms for this reduction include respiratory muscle hypotonia, cephalic displacement of the diaphragm and a decrease in lung compliance.[18] Sleep-related hypoventilation has been demonstrated in COPD, particularly during rapid eye movement (REM), with associated oxygen desaturation.[19] There is a close relationship between the awake PaO_2 and nocturnal oxygen saturation ($SatO_2$) levels,[20] although hypercapnia is associated with a more pronounced nocturnal oxygen desaturation than normocapnia for any given level of waking $SatO_2$.[21]

Nocturnal hypoxaemia has been defined as a $SatO_2$ of ≤90% for at least 5 minutes with a Nadir $SatO_2$ of ≤85%. The percentage of total recording time (TRT) has been defined as the time spent in bed minus sleep latency plus intrasleep wakefulness. A TRT with a $SatO_2$ of ≤90% has been defined as T_{90}%. The minimal TRT required for a satisfactory analysis of nocturnal recordings was 2 hours. COPD patients with a T_{90} of ≥30% and a Nadir $SatO_2$ of 85% have been defined as desaturators (D) and all others as non-desaturators (ND).[22-24] In this study, as revealed by cluster analysis, authors

showed that clinical parameter predictors, when awake from nocturnal desaturation, were different. COPD D patients may be identified by a clinical pattern of variables such as T_{90}, mean pressure artery pulmonary and $PaCO_2$ values, rather than by T_{90} alone, with the latter two variables being predictors of nocturnal desaturation severity.

This study has revealed the complexity of the nocturnal desaturation. Alveolar hypoventilation probably accounts for most of the nocturnal oxygen desaturation and hypoxic vasoconstriction and vascular remodelling. Several authors[25,26] measured minute ventilation during wakefulness, non-REM sleep, and REM sleep in normal subjects and patients with COPD. The greater drop in minute ventilation in subjects with COPD may reflect increased dependence on accessory muscles that become hypotonic during sleep, particularly during REM sleep. An alternative explanation comes from the work by O'Donoghue and colleagues[27] who have found an even greater drop in minute ventilation during non-REM sleep in hypercapnic COPD patients.

The exact prevalence of PH in patients with COPD is unclear.[28] PH is a complication of advanced COPD observed in patients who show severe longstanding hypoxaemia. Even if PH is generally mild to moderate in most COPD patients, it may markedly worsen during acute exacerbations, sleep and exercise, and these acute increases in PH could facilitate the development of right heart failure. Diagnosis of PH in COPD patients is difficult: published studies differ not only in their definition but also for the conditions under which PH has been reported (rest, exercise, and exacerbation). According to the European Society of Cardiology and the European Respiratory Society,[29] PH has been defined as an increase in mean pulmonary arterial pressure (PAP) ≥25 mmHg at rest as assessed by right heart catheterisation (RHC). The definition of PH under exercise conditions as a PAP ≥30 mm Hg, assessed by RHC, is not supported by published data, and healthy individuals can reach much higher values.

The incidence of PH in COPD patients has been evaluated by Kessler and colleagues.[30] In this longitudinal study on 131 patients with COPD, serial RHCs were performed at baseline and at follow-up (mean follow-up was 6.8±2.9 years). All subjects had normal mean PAP at rest (≤20 mm Hg). They have been divided into two groups based on the presence or absence of elevated mean PAP under exercise (≥30 mm Hg). On follow-up, 25% of patients had mild PH according to haemodynamic criteria (mean PAP 26.8±6.6 mm Hg).

Nocturnal oxygen desaturation appears to contribute to the development of PH even in the absence of significant awake hypoxaemias.[31] REM-associated drops in $SatO_2$ are associated with increases in PAP during sleep that can be reversed by supplemental oxygen, although most COPD patients with sustained PH are also hypoxaemic during the daytime. Various arrhythmias are also reported during episodes of nocturnal desaturation.[32] These consequences might help explain why nocturnal oxygen desaturation is recognised as a marker of increased mortality, and why COPD patients are reported to die more frequently at night than expected.[33]

Tissue hypoxia is another mechanism that can contribute to systemic inflammation in COPD. It has previously been demonstrated that TNF-α and receptor levels have been shown to be significantly higher in patients with COPD, but significantly correlated with the severity of arterial hypoxaemia.[34] These results suggest that arterial hypoxaemia in COPD is associated with activation of the TNF-α system *in vivo*. The systemic effects of inflammation may significantly contribute not only to respiratory abnormalities, respiratory symptoms and functional impairment (e.g. exercise intolerance) associated with COPD, but also to its chronic marked changes of vasomotor and endothelial function as pulmonary vascular remodelling.[35]

The nocturnal desaturation–reoxygenation sequence, waxing and waning sequence, is a typical pattern coupled with the majority of respiratory events. This sequence (Figure 2) of waxing and waning desaturation, differently from nocturnal desaturation of OSAS, leads to oxidative stress and the production of reactive oxygen species.[36] Hypoxia-induced pulmonary vasoconstriction is a protective response to maintain an optimised ventilation-perfusion ratio by shunting blood away from the hypoxaemic areas. The traditional vascular hypoxic model of PH is based on the hypothesis that chronic hypoxia initiates vascular remodelling leading to permanent changes in pulmonary vasculature.

Barbera et al.,[37] evaluated COPD patients undergoing lung resection and demonstrated that vascular changes contribute to vascular remodelling and may have an effect on vascular dynamics leading to PH. Nocturnal hypoxia may induce endothelial cells to release proliferation-stimulating cytokines,

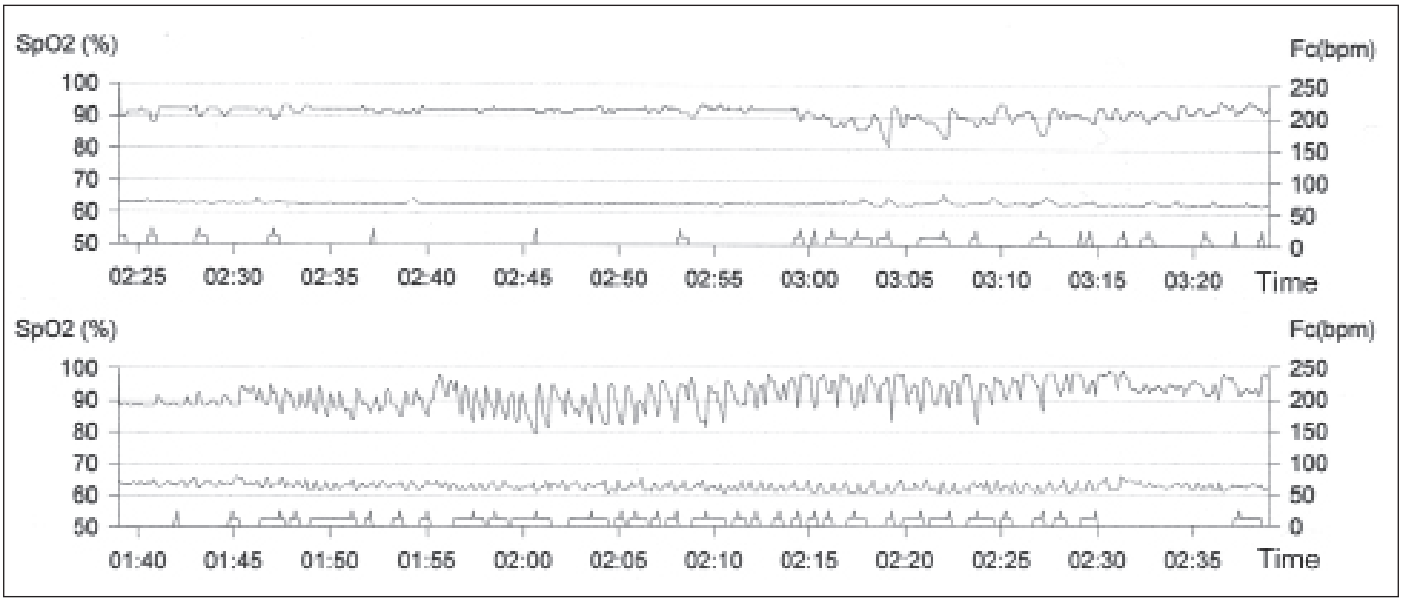

Figure 2. Simultaneous recording of nocturnal oximetry and heart rate in a patient with COPD desaturator (above) and in patient with OSAS (below).

Oxygen nocturnal desaturation with waxing/waning morphology in a COPD patient and the morphology of cyclical recurrence aspect with short, rapid intermittent nocturnal hypoxia in an OSAS patient, are shown.

leading to cellular hypertrophy in the vessel wall and an increase in extra-cellular matrix. Another intriguing possibility reported in that study is that nocturnal desaturation in COPD may contribute to an increased incidence of COPD exacerbations, which may accelerate lung-function decline and be associated with greater mortality.[38,39]

EXCESS BODY WEIGHT AND CHANGES IN METABOLISM

Obesity-Related Systemic Hypoxia

A potentially important source of inflammation in obese patients with COPD with nocturnal hypoxaemia is white adipose tissue. Obesity and MetS are increasingly prevalent in modern COPD, and may contribute to abnormalities in gas exchange. In patients with COPD, obesity is characterised by an absolute abundance of fat mass, similar to other diseases associated with excessive adiposity.[40] The prevalence of obesity is highest among patients with milder forms of the disease, and lowest in patients with the most severe lung function impairment.[41] It has been demonstrated that one or more components of MetS are present in almost 50% of COPD patients.[42] High adiposity and fat tissue accumulation impair pulmonary functions and exercise performance.[43]

The study by Trayhurn et al.[44] suggests that insulin resistance is aggravated by both high body mass index and increases in circulatory inflammatory mediators, such as IL-6, in this set of patients. Indeed, inflammatory mediators TNF-α, IL-6, and leptin were significantly higher while plasma adiponectin levels were reduced in overweight COPD patients. Chronic low-grade adipose tissue inflammation in obesity may represent a specific response to relative hypoxia of adiposities.[45]

Several factors may contribute to cell hypoxia within adipose tissue in association with high adiposity: (a) blood flow per unit of adipose tissue mass is reduced in obese humans resulting in decreased blood supply to the tissue; (b) large adipocytes are further from the vasculature than the normal diffusion distance for O_2. Adipocyte tissue hypoxia has detrimental effects on cell metabolism and function, as evidenced by *in vitro* studies and animal models. *In vitro* studies have shown that hypoxia results in enhanced TNF-α production, increased expression of PAI-1 and reduced adiponectin and peroxisome proliferators-activated receptor gamma, or PPARγ, expression.[46,47]

Even in the absence of COPD, obesity is associated with small airways dysfunction, decreased chest wall compliance, V/Q mismatch, and increased peripheral oxygen consumption, all potentially

leading to relative hypoxaemia. Risk of sleep-disordered breathing and consequent nocturnal hypoxaemia correlates with the degree of obesity[48] and, in extreme cases, morbid obesity can lead to profound alveolar hypoventilation with chronic hypercapnic respiratory failure. Dysregulated ventilatory control is another factor contributing to the occurrence and persistence of hypoxaemia in COPD patients.[49]

CONCLUSIONS

The hypoxic insult occurring during sleep-disordered breathing in COPD patients varies from one condition to another. However, there are cardiovascular and metabolic morbidities which are common among different conditions. Major differences are found in continuous hypoxia, suggesting specific pathways originating from the occurrence of oxidative stress and inflammatory cascade activation. In addition, the hypothesis that adipose tissue may contribute to the overall systemic inflammatory phenotype in patients with early stages of COPD and obesity or relative abundant fat mass is novel.[50] The potential links between night-time symptoms and long-term clinical outcomes will have to be explored in order to ensure that any interventions aimed at acutely improving night-time symptoms and/or sleep disturbance in COPD patients are also aimed at improving or stabilising the long-term health of COPD patients.

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BRONCHIAL ALLERGEN CHALLENGES IN ASTHMA RESEARCH

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Disclosure: No potential conflict of interest.
Received: 21.06.13 **Accepted:** 10.10.13
Citation: EMJ Respir. 2013;1:87-91.

ABSTRACT

In the development of new antiallergic or antiasthmatic therapies, mouse models have helped to identify novel therapeutic agents. Before a medication is evaluated for potency in phase II and phase III studies in humans, different bronchial challenge models are used to test the efficacy and mode of action in small sample sizes. Most published trials follow a classical approach in which allergic subjects are challenged with the same amount of allergen before and after treatment with a specific agent. Repeated challenge models are designed either to imitate natural allergen exposure or to induce significant asthma symptoms and airway inflammation. Although the available literature is less abundant, repeated models promise insights into the action of agents and the mechanisms of airway inflammation.

Keywords: Allergic asthma, bronchial allergen challenge, low-dose challenges, high-dose challenges.

INTRODUCTION

Specific bronchial allergen provocation is an established tool in asthma research that can increase our understanding of the pathological mechanisms responsible for allergic asthma and can offer key information concerning the therapeutic potential of new agents. Asthma research allows for the evaluation of antiallergic and antiasthmatic agents in relatively small sample sizes.[1-3] Subjects are typically challenged with an allergen before and after treatment with antiallergic or antiasthmatic drugs, and they are selected to develop reproducible early and late asthmatic responses (EAR and LAR, respectively). Another provocation model imitates natural allergen exposure. In the low-dose provocation model, a small amount of an allergen is inhaled to induce bronchial inflammation on consecutive days.[4-6] Recently, studies have described high-dose challenge protocols[3,7] as an interesting alternative to the existing protocols.

ANIMAL MODELS

Animal models have tremendously advanced the understanding of allergic disease development and have helped to identify novel therapeutic agents.[8] The mouse is now the species of choice,[9] and the BALB/c strain exhibits a genetically determined tendency to develop Th2-biased immune responses.[10] In mouse models, allergic asthma is typically induced by intraperitoneal injection of an antigen. After the sensitisation period, the mice are challenged with aerosol inhalation or nasal application of the antigen.[8] In recent years, several groups have developed mouse models that can reproduce many of the features of the remodelled asthmatic airway.[11] Blyth et al.[12] noted a reduction in subepithelial reticulin and an almost complete depletion of airway eosinophilia, when given an anti-IL-5 antibody before allergen challenges. During chronic repetitive allergen challenges, IL-5 gene deletion suppresses lung eosinophilia and tissue remodelling, simultaneously.[13] These animal studies strongly support the hypothesis that eosinophils contribute to the airway remodelling.[11] In mice, intranasal-challenged with house dust mite, both prophylactic and therapeutic treatment with an anti-IL-13 mAb significantly inhibited the generation and maintenance of chronic airway cellular inflammation, peribronchial collagen deposition, and epithelial goblet cell up-regulation.[14]

In comparison with human models, the mouse model has several disadvantages. Mice do not display spontaneous symptoms consistent with asthma.[15] In mice, bronchial hyperresponsiveness (BHR) is only transient and does not, as in asthmatic subjects, appear during clinical remission since mouse airways do not contain smooth muscle bundles.[11,15] The majority of studies have been performed with ovalbumin, but ovalbumin is not a clinically relevant allergen in humans.[8] Challenges in mice predispose to nasal and alveolar response rather than directed to the lower conducting airways. In humans, the EAR alerts the individual to natural exposure to inhaled allergens.[16] In contrast, in the LAR, eosinophils are important, and in response to IgE binding to the Fc[epsilon]RI receptor, eosinophil cytoplasmic granules and a number of cytokines are synthesised and released by degranulation.[17] In mice, it is only in some models that allergen challenges involve either EAR or LAR.[18] Allergen-specific IgE significantly predicts the LAR and EAR in mite-allergic asthmatic children and adolescents.[19] However, in mice, IgE and mast cells are unnecessary for the generation of allergic asthma.[20] Moreover, allergen-driven murine models disregard other environmental factors of asthma, such as oxidant stress, viral infection, obesity, exposure to tobacco smoke, and pollutants.[15]

CLASSICAL CHALLENGE MODELS

The most common method of testing pharmaceutical agents is to challenge patients with the same dose of allergen after receiving treatment with the test drug and again after receiving treatment with placebo.[16] Alternatively, randomised, placebo-controlled, parallel designs have been used[21] in which allergen challenge is performed according to a standardised protocol. The allergen concentration that causes a 20% drop in FEV_1 (PC_{20} allergen) is predicted from the PC_{20} methacholine and skin test sensitivity, which is derived from a multi-dose skin prick test (SPT). Starting three concentrations below the predicted PC_{20} allergen, consecutive doubling concentrations of allergen are aerosolised for 2 minutes using a DeVilbiss 646 nebuliser.[22] The endpoint measurements in such studies are the maximal early and late percentage decreases in the FEV_1 and the areas under the curve in the EAR (0–2 hours post-challenge) and the LAR (3–7 hours post-challenge).[16] The practical use of the protocols has been shown in numerous clinical trials.[22-25] Allergen challenge studies can be of value to predict efficacy or lack of efficacy of asthma controller therapies because agents that inhibit the LAR and allergen-induced inflammation are generally effective in asthma therapy.[16] In the classical method, concerns exist because the predictive value of the SPT is limited. In adults allergic to cats, a positive SPT (wheal size 3 mm) failed to discriminate between challenge-positive and challenge-negative patients.[26] In house dust mite allergies, the skin sensitivity did not significantly contribute to the prediction of an EAR.[27] Consecutive doubling concentrations of allergen might not allow for the exact and equivalent timing of allergen administration between subjects, particularly in trials in which repeated allergen challenges are necessary to study the kinetics of antiallergic drugs.[2]

LOW-DOSE CHALLENGE MODELS

Low-dose allergen challenges are designed to induce airway inflammation. Useful markers of inflammation are the induction of eosinophils and the eosinophilic cationic protein (ECP) in sputum and exhaled nitric oxide (eNO).[4-6] In an initial incremental challenge with doubling doses or concentrations of an allergen, the dose/concentration is increased until the FEV_1 has fallen by 20% or more from baseline (PD/$PC_{20}FEV_1$). The dose that causes a 5% fall in the FEV_1 is determined during the screening allergen challenge. This dose is administered as a single challenge on 5 consecutive days.[4] Trials with similar procedures have reported different outcomes, and not all trials have reported the presence of asthma inflammation caused by 'silent' chronic allergen exposure (Table 1). In repeated low-dose allergen provocations, night-time asthma symptoms and night-time β2-agonist use significantly increased during the challenge period, and the PC_{20} methacholine levels were significantly reduced.[4] In a placebo-controlled study with inhaled steroids, 26 patients with mild asthma and mite allergy performed repeated inhalations of the PD_5 allergen for 2 consecutive weeks. Due to increased β2-agonist use at day 2, the use was significantly elevated in the placebo group, whereas there were no significant differences in the total daily symptom scores. In the placebo group, the PC_{20} methacholine levels did not decrease significantly after 2 weeks of allergen exposure.[5] In a similar protocol, our working group showed that the participants did not require any β2-agonists during the challenge period with house dust mite allergen despite the induction of allergic airway inflammation. The PD_{20} methacholine levels decreased in

Table 1. Low dose challenges and symptoms.

Clinical trial	Author	Subjects (n)	Inhaled allergen	Duration of challenge	Allergen-dose	Cough	ß2-agonist	BHR
Effect of n-3 polyunsaturated fatty acids in asthma after low-dose allergen challenge	Schubert et al., 2009	23	Mite	10 days	PD_5	↔	↔	↔
Comparison of the effects of repetitive low-dose and single-dose antigen challenge on airway inflammation	Liu et al., 2003	8	Mugwort, Mite, Cat	4 days	PD_5	NA	NA	NA
Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma: protection by inhaled steroids	de Kluijver et al., 2002	26	Mite	10 days	PD_5	↔	↑	↔
Airway inflammation and altered alveolar macrophage phenotype pattern after repeated low-dose allergen exposure of atopic asthmatic subjects	Lensmar et al., 1999	8	Birch, Grass	7 days	PD_{10}	↔	NA	↑
Repeated aerosol exposure to small doses of allergen. A model for chronic allergic asthma	Arshad et al., 1998	9	Mite	12 days	0.4 ng	↑	↑	↑

BHR: bronchial hyperresponsiveness.

the placebo group, but the difference failed to reach significance.[6] All reported studies[4-6] have shown significant changes in sputum eosinophils and ECP during allergen challenges, and the eNO has been reported to increase stepwise to a peak level.[6] Therefore, the low-dose allergen challenge is a perfect model for testing antiasthmatic or antiallergic agents in humans, and it has been used for budesonide[5] and n-3 polyunsaturated fatty acids.[6] In clinical asthma studies, the reduction of symptoms is one of the primary outcomes. The main point of criticism is that low-dose allergen challenges do not cause asthma symptoms.

HIGH-DOSE CHALLENGE MODELS

In a real-life setting, individuals may be exposed repeatedly to symptomatic doses of allergen,[7] for example cladosporium allergy,[3] and so, in mouse models, acute sensitisation protocols include multiple systemic administrations of the allergen.[18] To support previous findings, these mouse models should be validated against human responses.[7] In the development of high-dose challenge protocols, Grainge and Howarth[7] and our group[3] independently designed very similar protocols. The Aerosol Provocation System (APS) dosimeter technique (Cardinal Health, Hoechberg, Germany) allows the computer-controlled production of an aerosol using a jet-type nebuliser to define an individual dose. The integrated pressure calibration procedure, associated with the compressor, ensures a highly constant and reproducible nebuliser output. In the incremental provocation, rather than doubling concentrations, a single allergen dilution with predefined doses is used. Both working groups chose the PD_{15} allergen to

challenge mild asthmatics who were allergic to house dust mites. Whereas Grainge and Howarth[7] used a more attenuated protocol with three consecutive challenges at 48 hour intervals, we hypothesised that four consecutive challenges in 1 week may be more likely to induce symptoms and allergen-driven asthma exacerbation in diseased volunteers. In both protocols, subjects developed significant asthmatic symptoms and rescue medication use. In the attenuated protocol, the pre and post-FEV_1 did not differ significantly and there were no serious adverse events, such as significant worsening of asthma requiring oral corticosteroids or hospital admission. In contrast, in our study the overall FEV_1 dropped significantly, and seven subjects had to stop the protocol prematurely; five patients experienced decreases in FEV_1 that were greater than that defined in the study protocol, and two had asthma attacks and required prednisolone during the night.

One of the primary outcomes of our study was sputum induction and the measurement of sputum cell counts and cytokines. We observed significant increases in the total eosinophil count, percentage of eosinophils, levels of ECP, and IL-5, which is a key mediator of eosinophil activation. In addition, transcription factor Foxp3 was significantly increased. In parallel, bronchial hyperresponsiveness, measured by methacholine challenge, and eNO demonstrated highly significant changes.[3]

In a bronchoalveolar lavage (BAL) study, the numbers of CD69+ and Foxp3+ lymphocytes were higher in the BAL fluid post-allergen provocation in asthmatic patients compared to pre-allergen provocation. To the best of our knowledge, we are the first group to demonstrate that Foxp3 is expressed in sputum cells after bronchial allergen challenge. The appearance of Foxp3 suggests the involvement of CD25+CD4+ Treg cells and a modulating role of Treg cells after allergen exposure, as Foxp3 CD4+CD25+Treg cells contribute to the control of allergen-specific immune responses in several major ways (e.g. the regulation of effector Th-1 and Th-2 cells).[28]

High-dose challenge models are suitable to induce significant asthma symptoms in diseased volunteers. Sputum cell counts and cytokine levels are promising parameters for understanding new mechanisms in asthma and allergy regulation, and they are more precise than the measurement of bronchial hyperresponsiveness or eNO.[29,30]

It is possible that the protocol we used was too intense because subjects developed severe asthma symptoms and decreases in pulmonary function. However, three high-dose challenges at 48 hour intervals are safe and repeatable.[7] The high-dose challenge is a model for proof-of-concept studies in clinical settings to reduce the risk of severe asthma exacerbations.[3]

CONCLUSIONS

Different provocation models may answer different questions regarding the antiallergic or antiasthmatic action of new agents. Classical bronchial allergen challenges sufficiently demonstrate the efficacy of asthma controller therapies. Repeated low-dose allergen challenges cause airway inflammation and they are suitable for demonstrating the effects of medications in everyday life. As demonstrated in mouse models, high-dose challenge models validate the findings of basic research by both demonstrating the efficacy of a new agent and its antiasthmatic potency and by investigating its impact on airway inflammation, as represented by sputum cell counts and sputum cytokine levels.

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ASTHMA IN PREGNANCY

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Disclosure: No potential conflict of interest.
Received: 29.05.13 **Accepted:** 31.08.13
Citation: EMJ Respir. 2013;1:92-100.

ABSTRACT

Asthma is a common disease among pregnant women. Uncontrolled asthma may increase the risk of maternal and foetal complications, thus optimal asthma management is elemental during pregnancy. Therapy must aim to control the disease; asthmatic women with controlled asthma should continue taking their medications during pregnancy, and maintenance therapy should be increased if asthma is not controlled. Most of the asthma medications have no effects on foetal growth. Although oral corticosteroids may confer an increased risk of lower birth weight and congenital malformations, benefit-risk considerations still favour their use in patients with asthma exacerbations during pregnancy. This review summarises immunological changes characterising asthmatic pregnancy and the clinical implications of asthma management during pregnancy.

Keywords: Asthma, clinical implications, immunology, management, pregnancy.

INTRODUCTION

Asthma is one of the most common chronic diseases complicating pregnancy, affecting 3.7–13.9% of all pregnancies.[1,2] Asthma influences the outcome of pregnancy, posing a risk for several maternal and foetal complications,[3-8] and pregnancy also affects the natural course of asthma, causing deterioration of symptoms in one third of patients. Fortunately, both maternal and neonatal risks decrease with maintenance of optimal asthma control during pregnancy,[9,10] thus appropriate asthma management is essential in these patients.

IMMUNOLOGICAL CHANGES IN HEALTHY PREGNANCY

In healthy pregnancies, the semi-allograft foetus is protected from maternal immune responses by physiological immune tolerance (Figure 1).[11] A trimester-dependent increase in regulatory T (CD4+CD25 high+; Treg) cell number has a key role in this process;[12] moreover, this increase is associated with physiological growth of the foetus.[13] These cells inhibit the activation of effector T lymphocytes and natural killer (NK) cells, as well as the differentiation of antigen-presenting cells.[14] Diminished numbers of Tregs in pregnancy were associated with immunological rejection of the foetus, low foetal birth weight or preeclampsia.[15] Of note, the inhibitory effect of proliferating Treg cells on NK cells[16] contributes to increased susceptibility to viral infections during pregnancy, as observed in the H1N1 influenza pandemic in 2009.[17] Healthy pregnancy is characterised by a subtle shift in T lymphocyte balance towards T helper (Th)2 phenotype, as a predominance of the Th1 cytokines causes spontaneous abortion.[18,19] The circulating number of the proinflammatory Th17 cells was shown to be unaffected by healthy pregnancy,[20] but increased in pregnancies with adverse outcomes such as preterm labour[21] and recurrent spontaneous abortion.[22] Alterations of immune state during pregnancy may cause changes in the course of autoimmune diseases, e.g. systemic lupus erythematosus or rheumatoid arthritis.[23]

IMMUNOLOGICAL CHANGES IN ASTHMATIC PREGNANCY

In the background of the usually transient and reoccurring symptoms, there is a persistent

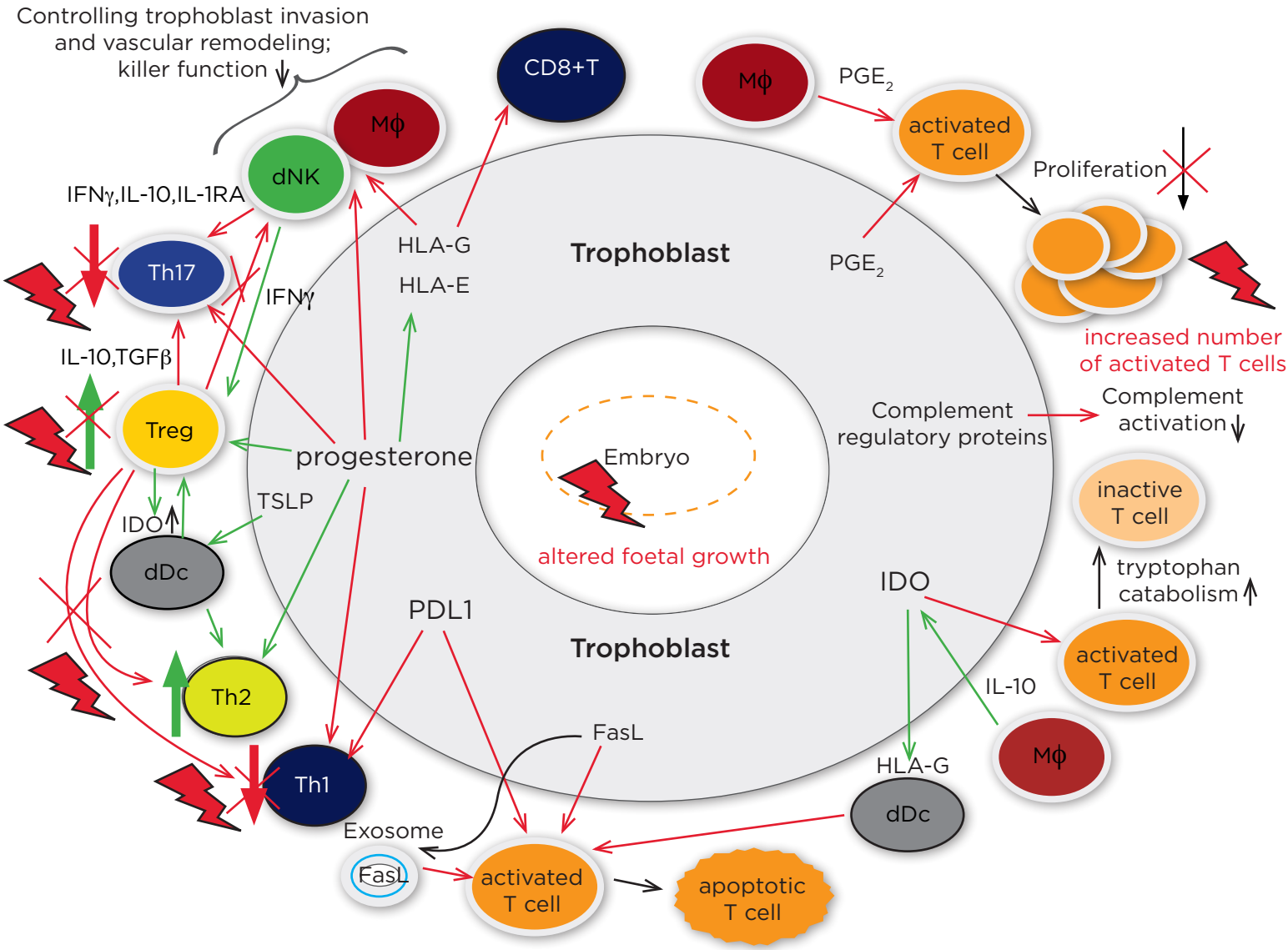

Figure 1. Alterations in the cellular mechanisms of maternal-foetal immune tolerance in asthmatic pregnancy.

(Th – T helper; Treg – regulatory T; dNK – decidual natural killer cell; Mφ – macrophage; dDC – decidual dendritic cell; CD - cluster of differentiation; PGE_2 – prostaglandin E_2; HLA – human leukocyte antigen; IL – interleukin; IDO – indoleamine 2,3-dioxygenase; FasL – Fas ligand; PDL1 – programmed cell death 1 ligand 1; TSLP – thymic stromal lymphopoietin; TGFβ – transforming growth factor β; IFNγ – interferon gamma; → stimulation/increase; ⊣ inhibition/decrease).

chronic inflammation in asthma,[24] in which Th2 type inflammation is elemental. Also abnormal Th17 immunity contributes to disease pathology, primarily in uncontrolled moderate to severe patients.[25] Expansion of these cells is accompanied by decrease in circulating Treg cell prevalence,[26,27] which promotes airway eosinophilia, mucous hypersecretion, and airway hyperresponsiveness.[28]

If the disease is well controlled, the physiological maternal immune suppression may attenuate the allergic response in asthmatic pregnant women, but not in patients with uncontrolled asthma. Activated pools within CD4 and CD8 T cells were larger, and the number of natural killer T (NKT) cells was increased both in non-pregnant asthmatic and in healthy pregnant subjects (compared with non-pregnant healthy controls). However, in the mostly well controlled pregnant asthmatics, no further lymphocyte activation was observed, suggesting that the immunosuppressive effect of uncomplicated pregnancy may blunt the

lymphocyte activation that characterises asthma.[29] In contrast with this, in mostly uncontrolled asthmatic pregnant women, a substantial number of peripheral interferon (IFN)-γ and IL-4 producing T cells were detected, and a negative correlation was revealed between the numbers of these cells and birth weight of newborns, suggesting that foetal growth restriction (intrauterine growth restriction - IUGR) may be related to active, asthma-associated maternal immune reactions.[30] Similarly, circulating levels of heat shock protein Hsp70, which is an inflammatory marker, were higher in asthmatic than in healthy pregnant women, and foetal birth weight was lower in pregnancies complicated with asthma.[31] Again in uncontrolled, symptomatic asthmatic women the Th1/Th2 cell ratio increased during pregnancy compared to healthy pregnant women, but remained unaltered in gestations of mostly well or partially controlled patients.[20] The physiologic elevation of peripheral Treg numbers was blunted during gestation of either symptomatic or asymptomatic asthmatic women; furthermore, the positive correlation between Treg numbers and birth weight of newborns was absent.[13] In asthmatic pregnant women, an abnormal asthma-dependent Th17 elevation was also detected[20] (Figure 1, Table 1). In a recent *in vitro* study, a reduction in adaptive antiviral immunity was found in pregnant women with well controlled asthma, which may be related to the susceptibility to respiratory virus infections.[32]

In healthy pregnancies, the normally increased oxidative stress is counterbalanced by increased antioxidant mechanisms;[33] however, in placentae of asthmatic women, more increased protein oxidation and lipid peroxidation were found together with more increased antioxidant capacity.[34] Also, circulating levels of antioxidants were elevated in moderate/severe asthmatic pregnant patients, suggesting a response to the high oxidative load induced by asthma during pregnancy.[35] Offset of this sensitive balance potentially contributes to the altered placental function and reduced foetal growth.

THE EFFECT OF MATERNAL ASTHMA ON PREGNANCY OUTCOMES

Due to the immunological and clinical changes, the risk of several maternal and foetal complications is higher in asthmatic pregnancy compared to healthy pregnancy; thus pregnancies in women with asthma need to be considered as high-risk pregnancies.[36] Perinatal mortality increases by 35% according to a database cohort of 13,100 pregnant asthmatics,[5] and prematurity and/or low birth weight seem to be the major contributing factors, which are associated with uncontrolled asthma, maternal obesity, or smoking.[6] However, optimal asthma management may prevent the adverse events.[6] The incidence of preterm delivery was higher among patients with inadequate asthma symptom control during the first part of pregnancy compared with patients with adequate asthma control, and it was higher among patients who were hospitalised for asthma during pregnancy compared with asthmatic women without a history of hospitalisation.[9] Severe and moderate asthmatic pregnant women have a higher risk for small gestational age babies than those with mild asthma, according to a population-based cohort of 13,007 pregnancies in asthmatic women.[37] Maternal asthma was also associated with a higher risk of spontaneous abortion, and uncontrolled asthma increased the risk by 26%.[38] A recent meta-analysis of 40 publications (involving 1,637,180 subjects) found an increased risk of low birth weight, small for gestational age, preterm delivery, and preeclampsia associated with maternal asthma, however, active asthma management reduced the relative risk of preterm delivery.[39]

Maternal asthma may increase the risk of congenital malformations in the nervous, respiratory and digestive systems;[40,41] however, there are also population-based data that did not detect any teratogenic effect of maternal asthma.[42,43] Finally, a multicentre, prospective, observational cohort study found that asthma also affects newborns' morphometry, as asthma severity was associated with an increased head circumference/birth weight ratio.[44] Asthma exacerbations during pregnancy are a particularly unfavourable issue because an exacerbation itself raises the risk of low birth weight[45] and congenital malformations.[41] Maternal asthma also has long-term consequences for offspring health. In a recent cohort study, asthma was associated with an increased risk of infectious and parasitic diseases, diseases of the nervous system, ear, respiratory system and skin, and potentially (not confirmed in secondary analyses) of endocrine and metabolic disorders, diseases of the digestive system, and malformations in the offspring during childhood.[46]

Table 1. The already established immunological changes in asthmatic pregnancy.

Authors	Chemical	Level of asthma control	Alteration	How they affect the situation
Bohács A et al. 2010[29]	CD4 and CD8 T cells, B cells, NK and NKT cells	Controlled	No further lymphocyte activation in AP compared either with ANP, or HP women.	Lower average birth weight in the AP than in the HP group.
Tamási L et al. 2005[30]	IFN-γ+ and IL-4+ T cells	Mostly uncontrolled	Culminating proliferation of IFN-γ+ and IL-4+ T cells in AP group. Increased IFN-γ+/IL-4+ T cell ratio in AP compared with HP group.	Numbers of IFN-γ+ and IL-4+ T cells correlated negatively with maternal PEF as well as birth weight. Patients on higher doses of maintenance therapy had higher numbers of IFN-γ+ and IL-4+ T cells. Pregnancy-related change in asthma severity was not associated with any T cell subsets.
Tamási L et al. 2010[31]	Hsp-70	Satisfactory level of asthma control (mean ACT score 20.66±2.24)	Increased serum levels in AP than in HP group.	Lower average birth weight in the AP than in the HP group. ACT scores showed a trend towards an increase in Hsp70 levels with the loss of asthma control.
Toldi G et al. 2011[20]	Th1, Th2, Th17, Treg, IL-17-producing CD8+ and NK cells	Well controlled in most cases (median ACT score: 22)	Lower prevalence of Th1 cells and elevated prevalence of Th2 cells in HP, ANP and AP than in HNP group. As a result, similar Th1/Th2 ratio in HP and AP groups. Higher Th17 prevalence in ANP than in HNP as well as in AP than in HP group. Higher prevalence of Treg cells in HP than in HNP or AP groups. As a result, higher Th17/Treg ratio in AP than in HP group.	Similar median birth weights in AP and HP groups. No correlation between any of the lymphocyte subsets and ACT values, FeNO levels or neonatal birth weight in any group.
Bohács A et al. 2010[13]	effector/memory and naive CD4+ T cells, Treg, NK, NKT and iNKT cells	Median ACT scores (quartiles): 20.5 (18-24) in AP and 19 (17-22) in ANP group	Lower prevalence of Treg and higher prevalence of iNKT cells in AP compared with HP women. Higher naive and lower NK and effector/memory T cell prevalence in AP than in ANP group.	Positive correlation between Treg prevalence and foetal birth weight in HP, but not in AP group. Only 3 newborns to healthy and 14 newborns (mostly girls) to asthmatic mothers had birth weight below 3 kg. Lower PEF, ACT scores, and birth weight in AP patients with female foetuses. In the AP goup, lower prevalence of naive T cells in obese patients.
Vanders RL et al. 2013[32]	IFN-γ, IL-10 and IL-17 from culture supernatant of PBMCs stimulated *in vitro* by PHA or a strain of the 2009 pandemic swine influenza	Well controlled	Following PHA stimulation: enhanced IL-17 response in AP, HP and ANP than in HNP group; reduced IFN-γ response in AP than in HNP group. Following infection with influenza: reduced IFN-γ and IL-10 response in AP and HP than in HNP group. Differences in IFN-γ, IL-10 and IL-17 persisted for at least 6 months post-partum.	Asthma is suggested to lead to an impaired adaptive immune response during pregnancy.

AP: asthmatic pregnant; HP: healthy pregnant; ANP: asthmatic non-pregnant; HNP: healthy, non-pregnant; CD: cluster of differentiation; Th: T helper; Treg: regulatory T; NK: natural killer; iNKT: invariant NKT; IFNγ: interferon gamma; IL: interleukin; Hsp: heat shock protein; PBMC: peripheral blood mononuclear cell; PHA: phytohemagglutinin; PEF: peak expiratory flow; ACT: Asthma Control Test; FeNO: fractional exhaled nitric oxide.

PREGNANCY-INDUCED CHANGES IN ASTHMA CONTROL

As a consequence of pregnancy-associated immunological and clinical changes, asthma improves in approximately one-third, remains the same in another one-third, and worsens in one-third during gravidity, but the underlying immunological mechanisms are mostly unknown and biomarkers predicting deterioration are lacking. However, there are some clinical signs that can draw the attention of the treating physician. The risk of asthma worsening during pregnancy increases with disease severity before pregnancy, and there is a concordance between the courses of asthma during consecutive pregnancies.[47] Similarly, asthma-specific quality of life in early pregnancy is related to subsequent asthma morbidity during pregnancy.[48] Asthma exacerbations are more common and more severe in pregnant women who smoke.[49] Interestingly, female foetuses also cause greater risk for worsening symptoms[13,50] and IUGR.[13,51] Obesity is associated both with asthma exacerbations and non-pulmonary complications during pregnancy (e.g. preeclampsia, gestational diabetes, and gestational hypertension).[4] Lower prevalence of naive T cells observed in obese compared to non-obese asthmatic pregnant patients may be a sign of dysfunctional pregnancy-induced immune tolerance in obese patients.[13]

MANAGEMENT OF ASTHMA DURING PREGNANCY

Diagnosis and Monitoring

The diagnosis of asthma is usually known before pregnancy; however, there are a further proportion of pregnant women who possibly have asthma.[2] In the latter case, reduced forced expiratory volume in one second (FEV1), or ratio of FEV1 to forced vital capacity (FVC), and a 12% or greater improvement in FEV1 after inhalation of rapid acting beta-agonist confirm the diagnosis. Lacking safety data, the bronchial hyperresponiveness test is contraindicated during pregnancy, thus women with a clinical picture of new-onset asthma without spirometric confirmation of the diagnosis should be treated for asthma during pregnancy. Skin prick tests are not recommended due to risk of systemic reactions, but blood tests for specific IgE antibodies to suspected allergens may be evaluated.[52]

Monthly assessments are required in all asthmatic pregnant women. Beyond asthma control evaluation, physical examination and spirometry, evaluation of arterial oxygen saturation is important: at least 95% measured by pulse oximetry is recommended[52] because, due to pregnancy, induced physiological hyperpnoea[53,54] and even mild maternal hypoxaemia may represent respiratory compromise during pregnancy. Fractioned concentration of nitric oxide present in exhaled breath (FENO) reflects airway inflammation in asthma.[55] This method has been shown to be applicable also in asthmatic pregnant patients;[60] contrarily, a recent longitudinal study found large intrasubject variability in pregnant asthmatics, regardless of the degree of asthma control.[57] However, asthma exacerbation rate, such as neonatal hospitalisations, could be reduced by treating pregnant patients according to a FENO-based treatment algorithm.[58]

Regarding obstetrical care, ultrasonographic examinations are recommended in the first trimester to confirm the accuracy of the estimated due date, anytime after recovery from a severe exacerbation and serially from the 32nd gestational week (together with nonstress testing), in case of suboptimally controlled or moderate to severe asthma, to monitor foetal growth and wellbeing.[59]

Treatment

Patient education

Maintaining control of the disease is essential but many patients do not use any reliever or prophylactic medications during pregnancy, even if their asthma is poorly controlled.[2] Therefore, pregnant asthmatics should be better educated about their disease and possible risks regarding maternal and foetal outcomes, such as their avoidance by reducing exposure to allergens, regular visits, proper treatment, and correct use of devices; furthermore, they need to be equipped with a written self-treatment action plan.[8,52,60]

Approximately one third of pregnant asthmatic women smoke,[61] which increases exacerbations,[49] asthma symptoms, foetal growth abnormalities,[54] and neonatal asthma as well.[62] Moreover, asthma combined with cigarette smoking increases the risk of preterm birth and urinary tract infections to a greater degree than with either exposure alone.[63] Thus, smoking cessation during pregnancy is indispensable.

Pharmacological therapy

Because safety data of asthma medications in pregnancy are, in general, reassuring,[52] pregnant women with well controlled asthma should continue taking their medications in order to reduce the risk of loss of control. If asthma is poorly controlled, therapy should be increased by one or more steps similarly to the treatment of non-pregnant asthmatic patients.[52] The required doses are, in general, also similar to that of non-pregnant patients.

Based on current data in human pregnancies, albuterol is the reliever medication of choice, and budesonide is the preferred controller treatment.[64,65] However, no increase in adverse pregnancy outcomes has been reported with beclomethasone or with fluticasone.[66] In addition, recent cohort showed no association between any dose of inhaled corticosteroid (ICS) use and perinatal mortality;[67] thus any ICS that achieved optimal control before pregnancy should be pursued during gestation.[66] Observational studies of inhaled corticosteroids and inhaled beta-agonists showed no increase in perinatal risks or congenital malformations.[68-72] Long-acting inhaled beta-agonists (formoterol and salmeterol) can be used as add-on therapy if symptoms persist in spite of the already received ICS treatment. In a large Canadian cohort of pregnant women with asthma, no increased prevalence of low birth weight, preterm birth or small for gestational age was found for LABA use and ICS doses <125 μg/day.[73] Leukotriene-receptor antagonists also seem to be safe during gestation; in one study enrolling 180 asthmatic pregnant women taking montelukast, no increase in the rate of major congenital malformations was observed.[74]

However, there are also data about possible adverse effects of asthma medication during pregnancy. The use of bronchodilators was associated with an increased risk of gastroschisis,[75] cardiac defects,[76] oesophageal atresia and omphalocele,[77] and anti-inflammatory use was associated with anorectal atresia and omphalocele.[77] But the role of asthma itself could not be excluded; hence these findings may be a consequence of maternal asthma severity and related hypoxia rather than medication use.[77] But systemic corticosteroid use may indeed adversely affect pregnancy outcomes, as it was associated with gestational hypertension, preeclampsia, preterm birth, lower birth weight, and congenital malformations.[72,78,79]

Acute asthma exacerbations

Acute asthma exacerbations may be prevented with optimal treatment, avoidance of trigger factors (e.g. viral infection),[45] and greater perceived control of asthma,[80] which is extremely important during pregnancy. Even so, asthma exacerbations occur in about 20% of asthmatic pregnant women primarily in the late second trimester, with approximately 6% of women needing hospitalisation.[45]

Therapy of acute asthma during pregnancy is similar to that in non-pregnant state. In the first 48 hours of an exacerbation, 120–180 mg/day of oral prednisone (or equivalent) are recommended in 3 or 4 divided doses, then 60–80 mg/day until PEF reaches 70% of predicted or personal best, followed by 7-14 days of tapering. For outpatient burst, 40-60 mg/day for 3–10 days may be sufficient, followed by 7-14 days of tapering.[59] Effective, rigorous treatment is important for the health of both the mother and foetus;[53] however, in everyday clinical practice, pregnant asthmatics are less likely to receive appropriate treatment with corticosteroids.[81] Status asthmaticus is a life-threatening disorder in obstetric patients; however, there are reports of excellent pregnancy outcomes after mechanical ventilation started due to severe respiratory acidosis in acute asthma exacerbation during pregnancy.[82]

CONCLUSION AND SUMMARY

Asthma is one of the most common chronic diseases complicating pregnancy. Although uncontrolled asthma may increase the risk of maternal and foetal complications, women with adequately-treated and well-controlled disease during pregnancy do not appear to be at increased risk. The difference is caused by the physiological function of pregnancy-induced immune tolerance that may attenuate inflammation in controlled asthmatic pregnant patients. Thus, controlling asthma during pregnancy with appropriate therapy is essential. Effective patient consultations with treating physicians and frequent communication between obstetricians, asthma specialists, and general practitioners are important. Most of the asthma medications have no effects on foetal growth. Although taking oral corticosteroids during pregnancy may confer an increased risk of lower birth weight and congenital malformations; benefit-risk considerations still favour their use in patients with asthma exacerbations.

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RECOMBINANT ALLERGENS IN DIAGNOSIS AND THERAPY OF ALLERGIC DISEASES

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Disclosure: No potential conflict of interest.
Received: 18.07.13 **Accepted:** 02.10.13
Citation: EMJ Respir. 2013;1:101-107.

ABSTRACT

The component-resolved diagnosis use in routine clinical and laboratory practice has increased in recent years. Recombinant allergens can be produced with high purity by using controlled procedures, obtaining molecules with known molecular, immunologic, and biological characteristics; they can help clinicians to treat patients with multiple pollen sensitisations. Recombinant allergens are useful in respiratory allergies such as: grass pollen, birch pollen, parietaria pollen, olive pollen, and dermatophagoides in food allergies, especially milk, eggs and peanuts. Recombinant allergens constitute an important tool in diagnosis and therapy of allergic diseases, which allows a better characterisation of the allergic patient.

Keywords: Recombinant allergens, allergy, diagnosis, immunotherapy.

BACKGROUND

In recent years, there has been an increasing use of recombinant allergens in routine clinical and laboratory practice, allowing a diagnostic approach of allergy at component level,[1] concurrently to the development of allergen microarrays. The ImmunoCAP® Solid-phase Allergen Chip (ImmunoCAP ISAC; Phadia, Upssala, Sweden) is the most common microarray that allows the detection of specific immunoglobin E (IgE) against a large variety of molecular components belonging to inhalant, food allergens and hymenoptera,[2,3] including: as species-specific components, as pan-allergens, or cross-reacting molecules, such as profilins, lipid transfer proteins, calcium binding proteins, storage proteins, tropomyosin, and serum albumins.[4,5]

Recombinant allergens can be produced with high purity by using controlled procedures that yield defined molecules with known molecular, immunologic, and biological characteristics.[6,7] Traditional allergen extracts, used for diagnosis and therapy, are prepared from natural allergen sources as a mixture of different species, which contain mixed allergenic components in undefined amounts of non-allergenic materials.[8-10]

Recombinant allergens are molecules that exactly mimic the properties of the natural allergens, or modified variants with advantageous properties, such as reduced allergenic activity or increased immunogenicity, or alternatively as hybrid molecules resembling the epitopes of several different allergens to include the relevant epitopes of complex allergen sources.[6,10]

The component-resolved diagnosis designates diagnostic tests based on pure allergen molecules, either produced by purification from natural allergen sources (designated according to the Allergen Nomenclature with the prefix 'n') or by recombinant expression of allergen-encoding cDNAs (designated with the prefix 'r').[11] These tests include marker allergens to diagnose the genuine sensitisation of patients towards a given allergen source or cross-reactive molecules that point to cross-sensitisation to several allergen sources.[12-14] This allows the accurate prescription of Sublingual Immunotherapy (SIT) for birch pollen,[12,13] grass pollen,[12] house dust mites,[15] and cats,[16,17] and includes

marker allergens for important Mediterranean pollen sources, including olive[18] and parietaria.[19,20]

Although allergenic source materials can contain just one major allergen (e.g. Bet v 1 from birch pollen), often several allergens are involved; 11 different allergens are characterised and cloned from sweet grasses, including Timothy-grass (*Phleum pratense*) and Ryegrass (*Lolium perenne*), while for the house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), the number is in excess of 20. Major allergens that account for the larger part of the IgE reactivity are at the basis of the development of therapeutic preparations.[21]

Recombinant allergens can help the clinicians in patients with multiple pollen sensitisations, who are frequently sensitised to several taxonomically unrelated allergens. Calcium-binding proteins and profilins are cross-reacting pollen pan-allergens: markers of multiple pollen sensitisation. Clinicians, if considering allergen-specific immunotherapy, have to establish whether sensitisation to several pollens is the result of co-sensitisation to different allergen proteins, co-recognition of homologous allergens, or both. So, detection of IgE reactivity to pan-allergens, and to major specific pollen allergens, is essential.[22]

GRASS POLLEN ALLERGY

Approximately 40% of allergic patients show IgE reactivity to grass pollens: one of the most important causes of IgE-mediated allergic disease in the world.[23,24] The most important source of grass pollen allergens in northern and central Europe is Timothy-grass (*Phleum pratense*).[25,26] Molecular and biochemical characterisation of *Phleum pratense*[25] has revealed several allergen components as rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11 and rPhl p 12: rPhl p 1 and rPhl p 5 are considered 'Species-Specific Allergens'.[27,28] The profilin rPhl p 1225 and the calcium-binding protein rPhl p 7 are the main cross-reactive components.[10]

The grass group 1 allergens are acidic glycoproteins (27–35-kDa), localised in the exine and cytoplasm of the pollen grain, with unknown function. Extensive immunologic cross-reactivity among the group 1 allergens from taxonomically-related grasses was established, and over 95% of allergic subjects were highly reactive to the respective group 1 allergens. The group 5 allergens include nine allergens with similar molecular mass to the group 1 (27–38 kDa); Phl p 5 has a ribonuclease activity, and its IgE-binding determinants are localised in the N-terminal and C-terminal ends. Approximately 80% of grass pollen allergic patients react with group 5 allergens, and together with the group 1, account for most of the IgE binding of most grass allergic sera: thus, they are considered major allergens.[12,27] Calcium-binding proteins such as Phl p 7 (8.6 kDa) are responsible for cross-reactivity between pollens of grasses, trees and weeds: antibodies against calcium-binding protein family were detected in 5-10% of pollen allergic individuals. Profilins as Phl p 12 (12-15 kDa) in the *Phleum* pollen are another group of allergenic molecules responsible for cross-reactions between various species of plants: Phl p 12 specific IgE antibodies account for cross-reactions with homologous profilins in many other allergens in both pollens and foods, and can be detected in 20–50% of grass-sensitive subjects.[27,29]

The predominant role of Phl p 1 and Phl p 5 in grass pollen allergy is demonstrated by many studies. Casquete-Román et al.[8] detected, in a paediatric population, IgE against rPhl p 1 and rPhl p 5 in 99.4% of the patients sensitised to grass pollen, while against rPhl p 7 and rPhl p 12, allergens were detected in 46% of them. Rossi et al.[27] found that in 77 adult patients (with a mean age of 21.6 years): rPhl p1 = 93.5%; rPhl p 5 = 72.7%; rPhl p 7 = 7.8%; and rPhl p 12 = 35.1%.[27] Successively, Rossi detected in 33 patients (with an age range of 9–62 years) rPhl p 1 in 100% of patients, rPhl p 5 in 76%, rPhl p 7 in 3%, and rPhl p 12 in 45%.[30] A study by Mari[28] found rPhl p 1 in 83%, rPhl p 5 in 50%, rPhl p 7 in 7%, rPhl p 12 in 15%, and isolated reactivity to rPhl p 1 in 6% in sera of 749 grass-sensitised patients (selected on a population of 4,606 unselected subjects, with an age range of 2-70 years).[10] Recently, Scaparrotta et al.[10] observed IgE against rPhl p 1 in 99% (205/207) of grass pollen-allergic children, to rPhl p 5 in 67% (139/207), to rPhl p 12 in 32% (66/207), and to rPhl p 7 only in 5% (10/207), with sensitisation only to 'Species-Specific' (rPhl p1, rPhl p5) allergenic molecules detected in 65% (135/207) of children. This study shows the predominant role of rPhl p 1 in paediatric populations as the most relevant sensitising allergen detectable at all ages and at all levels of Timothy-grass pollen-specific IgE antibodies, demonstrating that the importance of rPhl p 5 rises with the increase of patients' age and with grass pollen IgE levels.[10]

These observations confirm that the assessment of sensitisation to grass pollen allergenic molecules gives a better characterisation of allergic

sensitisation in grass pollen allergy in children, allowing a more specific and effective immunotherapy based on sensitisation to allergenic molecules.

The recombinant wild-type allergens and genetically modified hypoallergenic allergen derivatives can be used for immunotherapy, as for complex allergen sources with only one predominant allergen, if the relevant allergens have been included in the vaccine.[6] Although the first clinical immunotherapy study with recombinant allergen preparations used two different hypoallergenic derivatives of the major birch pollen allergen Bet v 1,[31] successively also *Phleum* recombinant allergens were successfully used.[32-35] Jutel and colleagues[32] demonstrated that a recombinant allergen vaccine with recombinant grass pollen, can be an effective and safe treatment to ameliorate symptoms of allergic rhinitis, associated with modification of specific immune response with IgG4 promotion and IgE reduction, consistent with the induction of IL-10–producing regulatory T cells.[32] Other authors confirmed these data, observing that patients with rhino conjunctivitis diagnosed using skin prick testing with a grass mix allergen extract and treated with a short course of SIT, based on a single species of *Phleum pratense* allergen extract, were able to develop an immune response that targets not only the immunising species, but also the grass mix allergen extract.[33]

BIRCH POLLEN ALLERGY

As in cold and temperate regions, birch pollen allergy affects approximately 20% of the population, e.g., in central and northern Europe,[36] the major birch pollen allergen, Bet v 1, is one of the first recombinant allergens extensively studied for allergy vaccination. Bet v 1 is the disease-eliciting allergen in approximately 90% of birch pollen sensitised patients and Bet v 1-cross-reacting allergens also cause symptoms to related tree pollen and foods.[37] IgE reactivity to the major birch pollen allergen Bet v 1 allows us to distinguish patients who are genuinely sensitised to birch pollen, while patients who exhibit positive skin tests to birch pollen extracts, but who have not been exposed to birch, have IgE to cross-reactive allergens, such as Bet v 2. So, the use of rBet v 1 is recommended in order to confirm the diagnosis of birch pollen allergy, before initiating immunotherapy.[38]

Bet v 1 vaccines, based on hypoallergenic recombinant rBet v 1, have demonstrated an improvement in allergic symptoms and favourably modify the immune response to Bet v 1. Vaccination with rBet v 1, formulated as tablets for sublingual administration, revealed clinically-relevant efficacy in rhino conjunctivitis patients, reducing symptoms and rescue medication compared to placebo.[37,39-43]

The profilin Bet v 2 is an actin-binding protein firstly identified as an allergen in birch, with homologous counterparts in a high number of pollens from phylogenetically-distant botanical families.[44] Detecting IgE reactivity to a single marker protein such as Bet v 2 is sufficient to diagnose or exclude sensitisation to profilins. Detecting IgE to multiple homologous, cross-reacting allergen proteins is not clinically more informative and increases the risk of confusion and misinterpretation.[45]

PARIETARIA POLLEN ALLERGY

Parietaria profilin Par j 2 might not share IgE-binding epitopes with profilins from other seasonal airborne allergens. Skin prick tests to *Parietaria* pollen is often negative in patients showing multiple pollen sensitisations, suggesting that Par J 2 might not always cross-react with profilins from other plant species. One study demonstrated that only less than 50% of patients hypersensitive to birch and grass profilins recognise this cross-reacting, ubiquitous allergenic protein in *Parietaria* pollen, and most of those who react to *Parietaria* profilin are sensitised also to the major, specific pellitory allergens, with practical relevance when the prescription of specific immunotherapy is considered.[46]

González-Rioja et al.[47] demonstrated that rPar j 2 displayed a 100% sensitivity and specificity among *Parietaria judaica*-allergic patients, supporting that *in vivo* and *in vitro* diagnosis could be simplified using rPar j 2, with comparable IgE response and skin prick reactivity of this protein with those produced by pollen extract.

A recent study demonstrated that a mutant hybrid, expressing genetically engineered forms of the major *Parietaria judaica* allergens (Par j 2/Par j 1), displayed reduced allergenicity and retained T cell reactivity for the induction of protective antibodies in vaccination approaches for the treatment of *Parietaria* pollinosis.[48]

OLEA POLLEN ALLERGY

Olive (*Olea europaea*) pollen allergy is one of the most significant pollinoses depending on geographical location. Although 10 allergens have been described from olive tree pollen, individual frequency of sensitisation can vary with the geographical area. Ole e 1 is the most prevalent allergen, observed in more than 70% among olive-sensitive patients, and the single major allergen in regions with low pollen counts, whereas other allergens such as Ole e 6, Ole e 7, and Ole e 9 also affect more than 50% of patients in locations with a high count.[49]

Ole e 1 is a single-polypeptide chain glycoprotein of 145 amino acid residues that constitutes more than 10% of the total protein content of pollen of the *Olea europaea* tree, but it does not exist in fruit, leaf, or stem. It has been demonstrated that the epitopes of Ole e 1 are only present in *Oleaceae* pollens[18] and subsequent studies demonstrated that sensitisation to Ole e 1 indicates primary sensitisation to *Oleaceae* pollens. Ole e 2 is a profilin and Ole e 3 is a calcium-binding protein with an amino acid sequence highly conserved in both taxonomically-related and non-related species: so, they are known as pan-allergens: markers of polysensitivity.[49]

Recombinant biotechnology offers most of the olive pollen allergens, with production of some hypoallergenic derivatives of Ole e 1: some of these molecules have been proven in a mouse model of allergy with promising results.[49,50]

HOUSE DUST MITE ALLERGY

rDer p 1 and rDer p 2 are the major recombinant allergens of house dust mite, and strongly correlate with *Dermatophagoides pteronyssinus* IgE. The lack of Der p 1 and Der p 2 IgE may help with differential diagnosis.[51] Both of these are proposed as promising hypoallergenic vaccine candidates for safer immunotherapy against house dust mite allergy.[52,53]

Der p 10 serum IgE prevalence and level suggest different patterns in food and mite-related tropomyosin sensitisation.[51] Der p 10 may be a diagnostic marker for patients with house dust mite allergy and additional sensitisation to other allergens. Such patients may require attention when allergen-specific immunotherapy is considered.[54]

FOOD ALLERGY

The use of recombinant allergens also represents a useful tool in food allergy. At first, its ability to reveal the exact allergen to which patients are sensitised (species-specific allergens or pan-allergens) is important in the evaluation of the potential danger of sensitisation and the risk of reaction on exposure. Sastre[55] focuses upon another area of research which looks to establish whether information can provide an indication as to the chances of tolerance development or if the allergy will be persistent.[55]

Milk contains more than 40 proteins. Casein (or nBos d 8) is a major allergen in milk and the main protein constituent of cheese.[56] It makes up about 75-80% of all milk proteins and is heat stable. nBos d 8 is subdivided into a number of families, αs1-, αs2-, β-, κ- and γ-caseins.[57] These are rapidly and extensively degraded by proteolytic enzyme during digestion. There is now growing evidence that casein seems to be a major allergen component to test for in the treatment of a patient with cow's milk allergy: it best discriminates between persistent and transient allergy,[58] it was often the cause of allergic reactions in patients with cow's milk allergy who eat so-called non-dairy products,[59] and in patients with a positive challenge to milk, nBos d 8 was the milk allergen component against which they most frequently had IgE.[60]

α-lactalbumin (or nBos d 4) represents about 25% of lactoserum (whey) proteins and approximately 5% of cow's milk proteins. It is the protein in highest concentration in human milk.[61] β-lactoglobulin (or nBos d 5) is the most abundant protein in whey, accounting for 50% of total protein in the lactoserum fraction and approximately 10% of cow's milk. The molecule nBos d 5 possesses 2 disulphide bridges and 1 free cysteine; this structure is responsible for the relative resistance of nBos d 5 to acid hydrolysis, as well as to proteases, which allows some of the protein to remain intact after digestion.[62] It has no homologous counterpart in human milk that does not contain β-lactoglobulin.[63]

Serum albumin (or nBos d 6), heat-labile protein, and lactoferrin are minor allergens.[64] The main allergens in egg are found in the egg white, but egg yolk also contains a large portion of specific IgE binding allergens. Gal d 1, Gal d 2, Gal d 3, and Gal d 4 are the most important allergens in egg white. Gal d 1 (ovomucoid) makes up approximately 10% of egg white and is often regarded the major allergen. Its allergenic potential is thought to

depend on its heat-stability and protease digestion. IgE binding activity to pepsin-digested ovomucoid have diagnostic value for distinguishing the challenge-positive subjects from the negative subjects: subjects with high IgE-binding activity to pepsin-treated ovomucoid are unlikely to outgrow egg white allergy.[64] Another study[65] concluded that patients with persistent egg allergy develop IgE antibodies against more-sequential and conformational epitopes of ovomucoid and ovalbumin, and that the presence of serum IgE antibodies to specific sequential epitopes of ovomucoid may be used as a screening instrument for persistent egg allergy.[66]

Gal d 2, also known as ovalbumin and albumin, is a major allergen of hen's egg white and is the most abundant of egg white proteins. A more recent study indicated that heated and ovomucoid-depleted egg white was less allergenic that heated egg white.[67] Gal d 3, also known as conalbumin, ovotransferrin, is a glycoprotein which is a present in egg white, egg yolk and plasma. Lysozimes (Gal d 4) are small globular proteins found in animal tissues and have concentration in egg albumen of about 0.5%. It is used as an additive and through this route may uncommonly induce symptoms of food allergy in sensitised individuals.

In children, positive IgE antibodies test results to Tri a gliadin indicate primary wheat sensitisation with a low risk of pollen cross-reactivity: in children, IgE antibodies to ω-5 gliadin (Tri a 19) are associated with a risk of immediate reactions to wheat.[68] In adults, they are associated with a risk of excercise-induced reactions in connection with wheat ingestion, although the presence of the antibodies is not specific to this disorder in patients allergic to wheat.[69]

Several proteins have been identified as peanut allergens, and the use of recombinant allergens has offered improved possibilities for a more specific and simplified peanut diagnosis.[70,71] Ara h 1, h 2, h 3 are seed storage proteins and have been designated as major allergens. Ara h 6 is a 2S albumin and shares several IgE epitopes with Ara h 2.[72,73] The minor peanut allergen Ara h 5 shows homology with pollen profilins and it is reported to be recognised by around 10% of peanut-sensitive individuals. Ara h 8, a Bet v 1-homologous pathogenesis-related (PR)-10 protein, has been shown as a major allergen for patients with concurrent birch pollen and peanut allergies. Lipid transfer protein (LTP), a pan-allergen with a degree of cross-reactivity comparable to profilin, is present in peanuts as Ara h 9. Measurement of IgE antibodies to rAra h 1, 2, and 3 is useful in the diagnosis of peanut allergy and in the investigation of reactions to raw and roasted peanuts. Some studies demonstrated that IgE antibodies to rAra h 2 are superior markers in their ability to differentiate between children in the allergic and tolerant groups, with a sensitivity and specificity of 88% and 84%, respectively (cut-off, 0.35 kUA/l). By combining the rAra h 2 and the rAra h 1 and rAra h 3 ImmunoCAP tests, it was possible to obtain an even higher specificity (94%).[74]

Peach is a well-documented and common cause of allergy in children, resulting in oral allergy and systemic reactions such as urticaria, asthma and anaphylactic shock, following the ingestion of fresh or processed fruit. Several peach allergens have been detected: Pru p 1 (PR-10 protein), Pru p 3 (a non-specific lipid transfer protein, Pru p 4 (a profilin) and Pru p glucanase. Pru p 3 is involved in allergenic relationship with other fruits from the family *Rosaceae*. A high level of cross-reactivity occurs among fruits and vegetables containing lipid transfer proteins, including sweet chestnut, cabbage, walnut, lettuce, and hazelnut.

CONCLUSIONS

In conclusion, there are strong data concerning the usefulness of recombinant allergens in diagnosis and the therapy of allergic diseases. These are two important tools that allow us to obtain a better characterisation of the allergic patient, resulting in a tailored treatment, specific for each patient.

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PHARMACOLOGICAL TREATMENT OF IDIOPATHIC PULMONARY FIBROSIS - AN UPDATE

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Disclosure: No potential conflict of interest.
Received: 27.06.2013 **Accepted:** 05.09.2013
Citation: EMJ Respir. 2013;1:108-121.

ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is the most common and most lethal fibrosing interstitial pneumonia, with a mortality rate that exceeds that of many cancers. Currently, there is no standard treatment recommended by the guidelines. A number of high-quality clinical trials evaluating novel potential therapies have recently been concluded. While the results have mostly been disappointing, some compounds appear promising in reducing disease progression. In this regard, pirfenidone is the most advanced molecule for IPF treatment, having been approved in Europe, Japan, India, and Canada. However, due to the complexity and uncertainties intrinsic to IPF, it is essential that each therapeutic strategy be tailored to the individual patient, after evaluation of potential benefits and pitfalls. Randomised controlled trials represent a valid choice for IPF patients. Many agents with high potential are being tested and many more are ready to be tested in clinical trials. Their completion is critically important to achieve the ultimate goal of curing IPF.

Keywords: Idiopathic pulmonary fibrosis, clinical trials, pirfenidone, treatment.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive fibrosing interstitial pneumonia of unknown cause, limited to the lung and associated with the histopathological (evidence of patchy involvement of lung parenchyma by fibrosis/architectural distortion, honeycombing in a predominantly subpleural/paraseptal distribution, presence of fibroblastic foci; Figure 1) and/or radiological (subpleural, basal-predominant honeycombing and reticular abnormality, with or without traction bronchiectasis; Figure 2) pattern of usual interstitial pneumonia (UIP).[1] The diagnosis of IPF is established in the presence of a UIP pattern on high-resolution computed tomography (HRCT) of the chest and/or surgical lung biopsy (SLB) specimen, in the appropriate clinical setting (commonly a current or ex-smoker male of >60 years of age) and after the exclusion of all known causes of pulmonary fibrosis.[1] The disease, which primarily affects older adults, carries a dismal prognosis with a median survival time in retrospective longitudinal studies of 2 to 3 years after diagnosis,[2-5] although recent data from placebo arms of large clinical trials, which recruited patients with mild to moderate disease, have reported a longer survival.[1]

In the last decade, the pharmacological treatment of IPF has changed considerably, mirroring the evolving understanding in disease pathogenesis. Initially, the prevailing hypothesis was that a persistent inflammation eventually triggered scarring of the lung. As such, early studies evaluated the potential efficacy of drugs that primarily

suppress inflammatory or immune responses, such as corticosteroids and immunomodulatory agents; the results of these trials have all been uniformly disappointing. Over the last decade the perspective on IPF pathogenesis has profoundly changed, and current concepts suggest that there is an initial alveolar epithelial cell damage followed by an aberrant healing response resulting in the migration, proliferation and activation of mesenchymal cells, accompanied by focal accumulation of myofibroblasts, known as fibroblast foci. Progressive laying down of extracellular matrix proteins and destruction of lung architecture complete the histopathological picture.[6] Accordingly, more recent randomised controlled trials have shifted their focus to molecules with anti-fibrotic and anti-proliferative properties. However, the pathogenesis of IPF remains incompletely understood and the rationale for evaluating the efficacy of specific compounds has often derived from post-hoc analyses of previous studies. Drugs approved for the treatment of other diseases, but with some evidence of potential efficacy in fibrotic disorders, have also been evaluated in IPF clinical trials.

Available therapeutic options for IPF have recently been systematically assessed according to the GRADE methodology (Table 1).[1] Thus, for the very first time, clinicians confronted with a patient with IPF can base their clinical decisions on the evidence derived from data of randomised-controlled trials.

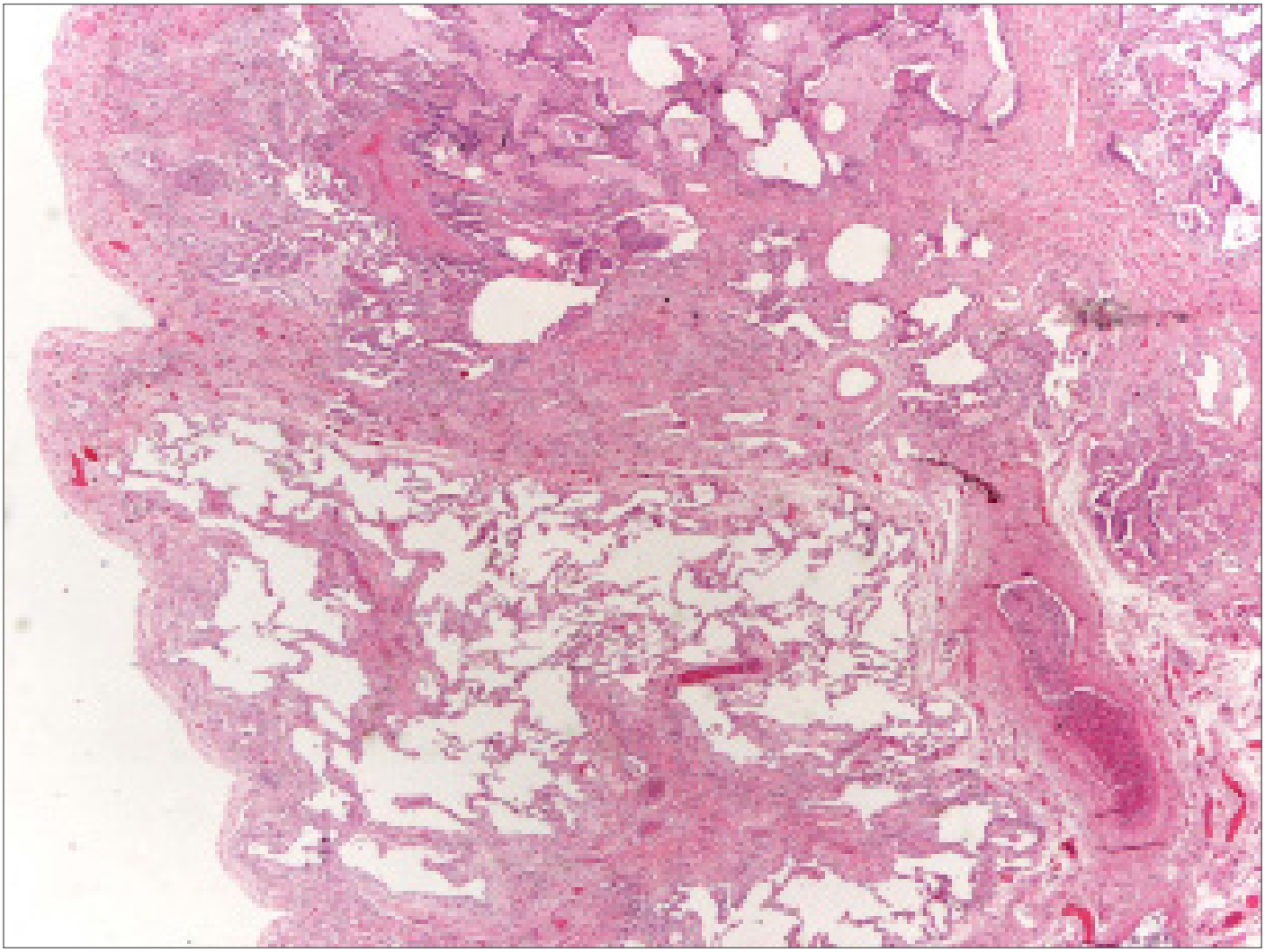

Figure 1. Surgical lung biopsy showing usual interstitial pneumonia (UIP) pattern, characterised by the abrupt juxtaposition of scarred lung with honeycombing (top) and nearly normal lung (bottom). Several pale fibroblastic foci are also seen.

Haematoxylin-eosin, 20x. Courtesy Alberto Cavazza, Reggio Emilia, Italy.

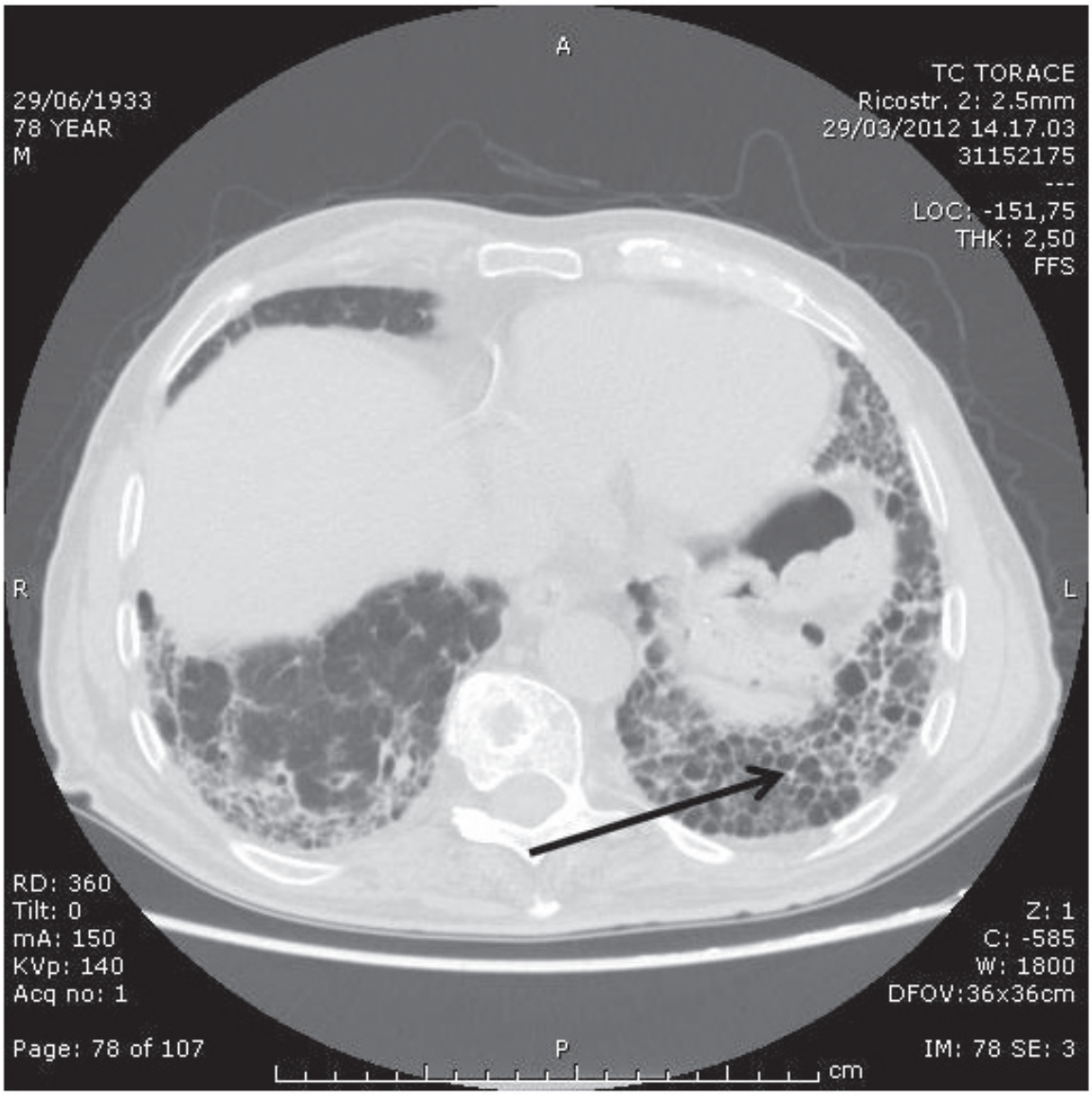

Figure 2. High-resolution computed tomography (HRCT) image of usual interstitial pneumonia (UIP) pattern showing basal and peripheral predominant reticular abnormality with subpleural honeycombing (more extensive at the left lung base; arrow).

ANTI-INFLAMMATORY AND IMMUNOMODULATORY DRUGS

Early studies in IPF largely focused on the effects of corticosteroids because of their anti-inflammatory effects and wide use in clinical practice in any fibrotic lung disorder. However, these studies were mostly conducted prior to the international guidelines published in 2000 and likely included patients with idiopathic interstitial pneumonias other than IPF, such as nonspecific interstitial pneumonia (NSIP), which would be more responsive to anti-inflammatory therapies.[7] Yet, two recent systematic reviews did not identify any high-quality trial evaluating the efficacy of corticosteroids in IPF.[8,9] On the other hand, long-term corticosteroid treatment is associated with significant morbidity and potentially severe side-effects. Accordingly, current evidence-based guidelines make a strong recommendation against the use of corticosteroid monotherapy in IPF, despite the absence of any randomised placebo-controlled trial.[1] Similarly, limited and low-quality evidence of efficacy is available for non-steroid immunomodulatory drugs, such as colchicine, cyclosporin A, cyclophosphamide or azathioprine, either alone or in combination with corticosteroids,[10] and current guidelines place a strong recommendation against their use in IPF.[1]

Azathioprine, an antimetabolite, blocks most T cell functions, inhibits primary antibody synthesis, and decreases the number of circulating monocytes and granulocytes.[11] In a prospective, double-blind, placebo-controlled trial, 27 patients were randomised in a 1:1 ratio to prednisone (1.5 mg/kg/day for 2 weeks, with a bi-weekly taper until a maintenance dose of 20 mg/day) plus either placebo or azathioprine (3 mg/kg/day to a maximum of 200 mg/day).[12] After 1 year, changes in lung function, as measured by resting P[A-a]O_2, forced vital capacity (FVC), and diffusing capacity of the lung for carbon monoxide (DLCO), were all slightly better in the azathioprine/prednisone group than in the prednisone/placebo group, although none of these comparisons were statistically significant. Yet, azathioprine in combination with low dose corticosteroids has long represented the standard of care in IPF.[7]

Table 1. Summary of the current evidence-based recommendations on pharmacological treatment of patients with IPF.

	Recommendation							
	For				Against			
Strength	**Weak**		**Strong**		**Weak**		**Strong**	
Quality of evidence	L/VL	M/H	L/VL	M/H	L/VL	M/H	L/VL	M/H
Corticosteroids alone							X	
Colchicine							X	
Cyclosporin A							X	
Cyclophosphamide + corticosteroids							X	
Azathioprine + corticosteroids							X	
Azathioprine + corticosteroids + NAC*					X			
NAC alone					X			
Interferon-γ-1b								X
Bosentan								X
Etanercept								X
Anticoagulants*					X			
Pirfenidone					X			
Long-term oxygen			X					
Lung transplant			X					
Mechanical ventilation					X			
Pulmonary rehabilitation	X							
Treatment of pulmonary hypertension					X			
Steroids in acute exacerbation of IPF	X							
Asymptomatic gastro-oesophageal reflux	X							

(Modified from Raghu G. et al.[1])

L: low; VL: very low; M: medium; H: high

NAC: N-acetylcysteine.

*Recommendations on these drugs are likely to change in the near future based on the results from recently published clinical trials.

Note: official recommendations are not available for sildenafil and imatinib, as the results of clinical trials evaluating these drugs have been published after the publication of the ATS/ERS/JRS/ALAT 2011 guideline document.[1] See text for details.

ANTIOXIDANTS

The IFIGENIA (Idiopathic Pulmonary Fibrosis International Group Exploring N-Acetylcysteine), a double-blind, randomised, placebo-controlled multicentre study, assessed the efficacy over 1 year of a high oral dose (600 mg three times daily) of N-acetylcysteine (NAC), a precursor of the antioxidant glutathione (GSH) synthesis that has been shown to be reduced in the lungs of patients with IPF,[13] added to standard therapy, i.e. a combination of prednisone and azathioprine.[14] NAC had previously been shown to increase the GSH levels in the bronchoalveolar lavage fluid (BALF) and improve lung function in patients with fibrosing alveolitis.[15] In comparison to prednisone plus azathioprine (the 'placebo' arm), the so-called triple therapy significantly slowed the decline of both vital capacity (VC) and DLCO (the primary endpoints). Specifically, at 12 months, the absolute differences in the change from baseline between patients taking NAC and those taking placebo were 0.18 litres or a relative difference of 9%, for VC (p=0.02), and 0.75 mmol per minute per kilopascal or 24%, for DLCO (p=0.003).

Weaknesses of this study related mainly to the lack of a true placebo arm (i.e. patients not taking any potentially effective drug), the lack of a survival benefit, the high rate (about 30%) of patients lost to follow-up at 12 months due to death or withdrawal and the consequent statistics utilised, and the least squared last observation carried forward for imputations approach, which tends to preserve the sample size from high drop-out rate but may make unwarranted assumptions about the missing data, potentially resulting in either underestimation or overestimation of the treatment effects. Due to these drawbacks, and in spite of the positive results of the study, recent guidelines make a weak recommendation against the use of this combination therapy, i.e. the majority of patients with IPF should not be treated with the triple therapy, although this may represent a reasonable therapeutic option in a minority of patients.[1]

The National Heart, Lung and Blood Institute (NHLBI)-sponsored IPFnet consortium designed a placebo-controlled, randomised three-arm trial, the PANTHER-IPF (Prednisone, Azathioprine, and N-acetylcysteine: A Study That Evaluates Response in IPF), to confirm the efficacy of N-acetylcysteine in IPF.[16] In this study patients were randomly assigned in a 1:1:1 ratio to prednisone, azathioprine and NAC (combination therapy), NAC alone, or placebo. The primary outcome was the change in longitudinal FVC measurements over a 60 week period. Secondary outcomes included mortality, time to death, frequency of acute exacerbations, and time to disease progression as defined by a composite endpoint of death or relative drop in FVC ≥10%. Unexpectedly, a pre-specified efficacy and safety interim analysis, planned at approximately 50% of data collection, showed that the combination therapy, as compared to placebo, was associated with a statistically significant increase in all-cause mortality (11% versus 1%), all-cause hospitalisations (29% versus 8%), and treatment-related severe adverse events (31% versus 9%). These observations, coupled with the lack of evidence of physiological or clinical benefit for the combination therapy, prompted the data and safety monitoring board to recommend termination of the combination therapy group at a mean follow-up of 32 weeks, while the NAC alone and the placebo arms continue to enroll patients. These largely unexpected results not only provide evidence against the use of this combination of drugs in patients with IPF but also underscore the importance of placebo-controlled trials in areas where the effects of treatment are largely based on limited evidence or low-quality data.

INTERFERON-GAMMA-1B

Interferon gamma-1b (IFN-γ-1b), a protein with antifibrotic and immunomodulatory properties, is secreted primarily by T cells (CD4 T cells, CD8 T cells, and natural killer cells). A pilot study by Ziesche and co-workers[17] showed that the association of IFN-γ-1b and prednisolone (as compared with prednisolone alone) improved lung function and partial pressure of arterial oxygen at rest in patients with IPF. However, in a subsequent large randomised, double-blind, placebo-controlled phase III trial, in which IPF patients were randomly assigned to receive subcutaneous IFN-γ-1b 200 μg three times weekly (n=162) or placebo (n=168), the primary endpoint of progression-free survival, defined by time to disease progression or death, was not achieved.[18] Similarly, no significant treatment effect was observed on lung function, gas exchange, extent of fibrosis on HRCT, or quality of life. However, post-hoc analyses suggested that patients with mild-to-moderate impairment in lung function at study entry might be more likely to benefit from IFN-γ-1b treatment. Moreover, a reduced mortality (10%) was observed in the IFN-γ-1b arm as compared with the placebo arm (17%), although this difference was not statistically significant. A subsequent meta-analysis, involving

390 patients, confirmed that treatment with IFN-γ-1b significantly reduced mortality in patients with IPF.[19] Based on these findings, a larger randomised-controlled trial of over 800 patients (International study of Survival outcomes in idiopathic Pulmonary fibrosis with InteRfEron gamma-1b: the INSPIRE trial) was specifically designed to assess the efficacy of IFN-γ-1b on survival time in IPF patients with mild-to-moderate impairment in baseline pulmonary function.[20]

However, a protocol-defined interim analysis revealed that the hazard ratio for mortality among patients treated with IFN-γ-1b crossed the predefined stopping boundary for lack of minimal benefit. After a median treatment duration of 77 weeks, 14.5% of patients in the IFN-γ-1b group had died compared to 12.7% of patients in the placebo group (p=0.497). As such, the guidelines make a strong recommendation against the use of IFN-γ-1b in patients with IPF.[1]

DRUGS ACTING ON THE PULMONARY VASCULATURE

Data from basic science, animal, and translational studies suggest that the endothelin system, and endothelin (ET)-1 in particular, is a potential contributor to the pathobiology of several fibrotic disorders, including IPF.[21] In fact, ET-1 has been shown to modulate matrix production and turnover, leading to increased collagen synthesis and decreased interstitial collagenase production.[22]

Bosentan

In a double-blind, placebo-controlled study (Bosentan Use in Interstitial Lung Disease: BUILD-1), 158 IPF patients were randomly assigned to receive either bosentan, a dual ET receptor antagonist (ET_A and ET_B), or placebo.[23] Bosentan did not meet its primary endpoint (change in 6 minute walk test distance [6MWD] by month 12). However, a post-hoc analysis revealed a trend in favour of bosentan in time to death or disease progression in patients with limited honeycombing on HRCT whose diagnosis had been obtained by surgical lung biopsy. This finding formed the basis for a second, prospective, randomised (2:1), double-blind, placebo-controlled trial (BUILD-3) that enrolled patients with IPF (n=616) of less than 3 years' duration, diagnosed histologically, and with <5% of honeycombing on HRCT.[24] Unfortunately, the primary endpoint (death or disease progression defined by a decline ≥10% in FVC and ≥15% in DLCO or an acute exacerbation of IPF at month 12) was not met. Bosentan was well-tolerated, but its lack of efficacy makes it a non-viable treatment option for IPF.[1]

Ambrisentan

Ambrisentan is a selective antagonist of the ET_A receptor, approved for the treatment of pulmonary arterial hypertension.[25] Endothelin-1 induces lung fibroblast proliferation and contractile activity via the ET_A receptor.[26] Importantly, preclinical studies have shown that both the phenotypic and transcriptional responses to ambrisentan are different from bosentan, suggesting that clinical effects in IPF may also be different.[27] The ARTEMIS-IPF (Randomised, Placebo-Controlled Study to Evaluate Safety and Effectiveness of Ambrisentan in IPF) was a randomised, double-blind, placebo-controlled, multi-national trial, evaluating effectiveness of ambrisentan in reducing disease progression (defined as death, respiratory hospitalisation or decline in lung function). The study was terminated earlier, after enrolment of 492 patients (75% of intended enrolment), following an interim analysis indicating a low likelihood of efficacy for the primary endpoint.[28] Indeed, ambrisentan was associated with an increased risk of disease progression and respiratory hospitalisations.

Macitentan

Macitentan, a dual endothelin receptor antagonist, has been shown to prevent the development of lung fibrosis in a mouse model.[29] The MUSIC (Macitentan USe in Idiopathic pulmonary fibrosis Clinical) trial, a prospective, randomised, double-blind, multicentre, parallel-group, placebo-controlled, phase II proof-of-concept study evaluated the efficacy and safety of macitentan in IPF patients.[30] Of the 178 randomised patients, 119 were allocated to macitentan and 59 to placebo. The study did not meet its primary endpoint (change from baseline up to month 12 in FVC). Similarly, no differences were observed between treatment groups in any of the secondary or exploratory measures including time to IPF worsening or death.

Sildenafil

Sildenafil, a phosphodiesterase-5 inhibitor that induces pulmonary vasodilatation by stabilising the second messenger of nitric oxide (cyclic guanosine monophosphate), is approved for the treatment

of pulmonary arterial hypertension.[31,32] In a small open-label study, the oral administration of sildenafil at the dose of 25-50 mg three times daily improved the 6MWD by a mean of 49 metres.[33] These observations prompted a large phase III double-blind, placebo-controlled study (Sildenafil Trial of Exercise Performance in Idiopathic Pulmonary Fibrosis: STEP-IPF) in which 180 subjects were randomised to sildenafil (20 mg three times daily) or placebo for 12 weeks, with a subsequent 12-week open label phase in which all patients received the active drug.[34] The difference in the primary outcome was not significant, with 9 of 89 patients (10%) in the sildenafil group and 6 of 91 (7%) in the placebo group having an improvement of ≥20 meters in the 6MWD (p=0.39). However, significant differences favouring sildenafil were observed in the change in PaO_2, DLCO, degree of dyspnoea, and quality of life. The presence of some positive secondary outcomes creates clinical equipoise for further research. At present, there is no evidence to support the routine use of sildenafil in IPF.

Etanercept

Etanercept is a recombinant soluble monoclonal antibody that binds and neutralises tumour necrosis factor (TNF)-α receptor. The rationale for its use in IPF comes from the observations that TNF-α has inflammatory and fibrogenic properties, and elevated levels of this cytokine have been detected in the lungs of patients with IPF.[35,36] In addition, in mouse models, TNF-α antagonists diminish bleomycin-induced pulmonary inflammation and fibrosis,[37] suggesting a potential beneficial effect in patients with IPF. A randomised, prospective, double-blind, placebo-controlled, multicentre phase II trial evaluated the safety and efficacy of subcutaneous etanercept (25 mg twice weekly).[38] After 48 weeks of treatment, no significant differences in any of the efficacy endpoints (changes in the percentage of predicted FVC or DLCO, and in the $P(A\text{-}a)O_2$ at rest from baseline) were observed between the groups. As such, the use of etanercept in IPF is not recommended.[1]

Pirfenidone

Pirfenidone, an orally administered pyridine with antifibrotic, anti-inflammatory and antioxidant properties,[39] is the only drug approved for clinical use in the treatment of IPF.[40] In an open-label study, 54 IPF patients were treated with pirfenidone and followed for mortality, change in lung function, and adverse effects.[41] Pirfenidone appeared to slow the decline in lung function and enabled corticosteroid dosage to be reduced to discontinuation in the majority of patients. In a subsequent larger multicentre, randomised, double-blind, placebo-controlled phase II trial, 107 Japanese patients were assigned to receive either an escalating dosage of pirfenidone or placebo.[42] The primary endpoint (change in the lowest blood oxygen saturation (spO_2) during a 6 minute exercise test) was not met. However, positive treatment effects were observed in change in VC at 9 months and rate of acute exacerbations, which occurred exclusively in the placebo group, although this latter effect has not been replicated in subsequent studies. Pirfenidone was associated with significant adverse events - with skin photosensitivity, gastro-intestinal symptoms, and liver function test abnormalities being the most common - although there was no significant difference in the treatment discontinuation rate between the two groups at 9 months.

In a subsequent larger multicentre, double-blind, placebo-controlled phase III study, 275 Japanese patients with IPF were randomly assigned in a 2:1:2 ratio to high-dose (1,800 mg/day) or low-dose (1,200 mg/day) pirfenidone, or placebo over a 52 week period.[43] The study met its primary endpoint, change in VC. In fact, the rate of decline of VC was higher in the placebo arm (-0.16 L) compared to both the high-dose (-0.09 L; p=0.042) and low-dose pirfenidone arms (0.08 L; p=0.039). Significant differences were also observed in progression-free survival time between the high-dose and the placebo arms (p=0.028) and in the changes in total lung capacity (TLC) between the low-dose and the placebo arms (p=0.040). A limitation of this study is the change of the primary endpoint before unblinding, which could possibly have hampered the integrity of the study. An exploratory analysis of this same study revealed that patients with a baseline VC ≥70% and oxygen saturation <90% had a greater benefit from pirfenidone.[44] Similar to the previous study, photosensitivity was the most common drug-related adverse event (observed in 51% of patients in the high-dose group and 53% in the low-dose group), but not a major reason for discontinuation of the study.

The CAPACITY studies (CAPACITY 1 – PIPF 006 and CAPACITY 2 – PIPF 004) are two almost identical randomised, double-blind, placebo-controlled, multinational phase III clinical trials that evaluated

the efficacy of oral pirfenidone over 72 weeks.[45] In the 004 trial, patients were assigned in a 2:1:2 dosing ratio to pirfenidone 2,403 mg/day, pirfenidone 1,197 mg/day, or placebo, while in study 006, patients were assigned in a 1:1 ratio to pirfenidone 2,403 mg/day or placebo. The primary endpoint was change in percentage predicted FVC at week 72. In study 004, mean FVC change at week 72 was -8·0% in the pirfenidone 2,403 mg/day group and -12·4% in the placebo group (p=0.001). Conversely, in the 006 study, the change in FVC at week 72 was not significant between the treatment and placebo arms (p=0.501). Of note, while the magnitude of decline over time was similar in the two active arms, in the two placebo groups it differed. Specifically, in the study PIPF 006 the placebo arm displayed an attenuated FVC decline (-9.6%) as compared to the placebo arms of other large clinical trials of IPF.[46]

All these trials had sufficient methodological quality to be included in a Cochrane systematic review and meta-analysis, which confirmed that pirfenidone reduces both the rate of decline of lung function and the risk of disease progression (as measured by progression-free survival) as compared to placebo.[10] Some limitations to the interpretation of these data still apply, mainly related to a certain degree of methodological heterogeneity across studies with regard to reporting of lung function data.

Pirfenidone has been granted marketing authorisation for the management of patients with mild to moderate IPF in Japan in 2008, and in Europe in 2011. Despite this, the use of pirfenidone has not been approved by the Food and Drug Administration (FDA) due to a perceived lack of efficacy as measured by change in FVC, and lack of survival benefit.[47] A new phase III trial of pirfenidone aiming to confirm the positive effect on FVC is therefore underway in the US (the ASCEND trial, clinicaltrials.gov; identifier NCT01366209). Current guidelines, considering the cost of pirfenidone and the potentially relevant side-effects make a weak recommendation against its use in IPF. Regardless, patients willing to receive pirfenidone should be fully informed on the available evidence for its efficacy as well as on the possible side-effects.

ANTICOAGULANTS

Anticoagulants have been evaluated in IPF based on evidence of their efficacy in ameliorating pulmonary fibrosis in animal models when given either prophylactically or therapeutically.[48,49] Based on this pathogenetic hypothesis, 56 Japanese patients with IPF were randomly assigned to receive prednisolone alone or prednisolone plus anticoagulant therapy (oral warfarin, which was switched to low-molecular-weight heparin in case of hospitalisation for acute deterioration) in an open label study.[50] While the incidence of acute exacerbations (AE) did not differ between the groups, there was an increased mortality associated with AE in the non-anticoagulant group compared to the anticoagulant group (71% versus 18%, respectively; p=0.008). Limitations of the study included lack of blinding; patient recruitment (e.g. there may have been a selection bias toward more advanced and rapidly progressive disease); substantial withdrawal rate in the anticoagulant group (26%) (e.g. it is likely that patients who left the study were more ill and would have had higher mortality); failure to exclude pulmonary embolism as a potential cause of acute deterioration (e.g. mean plasma levels of D-dimer were significantly higher in patients who died from AE). As such, treatment with anticoagulants is not recommended for routine use in patients with IPF (weak recommendation against, very low-quality evidence).[1]

To further investigate the utility of anticoagulation in IPF, the NHLBI sponsored the AntiCoagulant Effectiveness in Idiopathic Pulmonary Fibrosis (ACE-IPF) trial.[51] In this study patients were randomly assigned in a 1:1 ratio to warfarin or matching placebo for a planned treatment period of 48 weeks. Due to excess mortality in the warfarin arm (14 warfarin versus 3 placebo deaths; adjusted HR=4.85), the study was terminated after 145 of the planned 256 subjects were enrolled (72 warfarin, 73 placebo). Similar trends favouring placebo were observed in all-cause hospitalisations, respiratory-related hospitalisations, and AE of IPF. In partial accordance with the current guideline recommendations, the results of this study strongly argue against the routine use of warfarin for the treatment of IPF. As such, recommendations on this drug are very likely to change in the near future.

TYROSINE KINASE INHIBITORS

Tyrosine kinases regulate a variety of physiological cell processes, including metabolism, growth, differentiation and apoptosis, and aberrant tyrosine kinase activity has been shown to promote the development and progression of both neoplastic and non-neoplastic diseases.[52,53] Signalling

pathways activated by tyrosine kinases have also been suggested to be implicated in the pathogenesis of lung fibrosis.[54]

Nintedanib (BIBF 1120)

The TOMORROW (To Improve Pulmonary Fibrosis With BIBF 1120) study, a 12-month, randomised, double-blind, placebo-controlled trial, evaluated the safety and efficacy of BIBF 1120,[55] a tyrosine kinase inhibitor that suppresses pro-angiogenic intracellular signalling by targeting the proliferative growth factor receptors on platelets (PDGFR), vascular endothelium (VEGFR) and fibroblasts (FGFR).[56] Four different oral doses of BIBF 1120 (50 mg once a day, and 50 mg, 100 mg, or 150 mg all twice a day) were tested. BIBF 1120 at a dose of 150 mg twice daily showed a trend toward a reduction in the decline in FVC, the primary endpoint. Specifically, in the group receiving 150 mg of BIBF 1120 twice a day, FVC declined by 0.06 litres per year, as compared to 0.19 litres per year in the placebo group; a 68.4% reduction in the rate of loss. In addition, patients treated with 150 mg of BIBF 1120 twice daily had a lower incidence of AE and an improvement in quality of life (small decrease in St. George's Respiratory Questionnaire score as compared with an increase with placebo). Overall, BIBF 1120 showed an acceptable safety profile, although diarrhoea, nausea, vomiting, and increases in levels of liver aminotransferases - the most common drug-related side-effects - were more frequent in the group receiving 150 mg of BIBF 1120 twice daily than in the placebo group. These results warranted the investigation of BIBF 1120 in phase III clinical studies, with results expected in 2014.

Imatinib

Imatinib, a tyrosine kinase inhibitor with activity against several fibrogenic factors (including PDGFR-α and β), has been investigated in IPF based on its ability to inhibit lung fibroblast-myofibroblast transformation and proliferation as well as extracellular matrix production in animal models of pulmonary fibrosis.[57] In a phase II, randomised, double-blind, placebo-controlled study, 119 patients with mild or moderate IPF were randomly assigned to receive imatinib (600 mg orally once daily; n=59) or placebo (n=60) for 96 weeks.[58] The study found neither a survival benefit nor an effect on FVC, the primary outcome. Similarly, no differences in any of the predefined secondary endpoints were observed between the imatinib and the placebo groups. As such, imatinib does not represent a therapeutic option for patients with IPF.

Co-trimoxazole

Following a pilot study of 20 patients with idiopathic interstitial pneumonias (IIP), in which co-trimoxazole treatment improved FVC, shuttle walk distance and Medical Research Council (MRC) dyspnoea score,[59] a larger randomised placebo-controlled double-blind parallel group clinical trial was designed to assess the efficacy and safety of the addition of 12 months of oral co-trimoxazole (960 mg twice daily) to usual treatment in fibrotic IIP (definite or probable IPF, n=170; definite or probable NSIP, n=11).[60] No significant difference was observed between treatment groups for change in FVC, the primary outcome. However, co-trimoxazole reduced mortality (a tertiary endpoint), a finding somewhat unexpected and possibly related to a reduction of respiratory infection. In fact, patients receiving immunosuppressive treatment were more likely to die if they were in the control group, whereas baseline immunosuppressive therapy did not have an effect on mortality in the intervention group. In addition, the difference between groups in survival was observed with the per-protocol analysis but not with the intention-to-treat analysis. Drawbacks of the study include the high dropout rate because of side-effects among patients receiving co-trimoxazole, the lack of a true placebo arm, the inclusion of both IPF and NSIP patients, and poorly defined diagnostic criteria.

ANTI-GASTRO-OESOPHAGEAL REFLUX DRUGS

Abnormal acid gastro-oesophageal reflux (GER) is common in patients with IPF and is considered a risk factor for the development of the disease.[61,62] Retrospective studies have shown longer survival in patients given anti-acid treatment.[63] A recent study analysed the change in FVC in patients randomly assigned to the placebo arms in three large randomised controlled trials. After adjustment for gender, baseline FVC, and baseline DLCO, patients taking anti-acid treatment at baseline (proton-pump inhibitors or H2 blockers) had a smaller decrease in FVC at 30 weeks compared to those not taking anti-acid treatment (p=0.05).[64] Anti-acid treatment could be beneficial in patients with IPF, and current guidelines recommend the treatment of asymptomatic GER

in patients with IPF (weak recommendation, very low-quality evidence). However, controlled clinical trials of anti-acid treatments are now needed.

EMERGING TREATMENTS

Treatment of IPF has always been challenging and, for more than 20 years, patients have been given treatments that were not appropriate (if not harmful). The future however looks bright owing to a continuous flow of information that provides new insights in disease pathogenesis. This has resulted in an exponentially increasing number of potential therapeutic targets, and currently there are more than 60 clinical trials in IPF that are either recruiting or about to start patient recruitment (www.clinical trials.gov).

Transforming Growth Factor β

Transforming growth factor β (TGF-β) is considered to play a key role in pulmonary fibrosis as it interferes with almost all the processes involved in its development, such as chemotaxis and proliferation of fibroblasts, differentiation of fibroblasts into myofibroblasts, which represent the major source of extracellular matrix, epithelial-mesenchymal transition, and inhibition of myofibroblasts apoptosis.[65,66] At present, there are three known isoforms of the protein: TGF-β 1, 2 and 3. Multiple strategies to inhibit TGF-β activities exist. The first is to directly block TGF-β by using human monoclonal antibodies. Antibodies against TGF-β1, 2 and 3 have been developed (GC1008 Genzyme) and a phase I trial has been completed, but the data has yet to be published. Another way is by interfering with the activation of the protein. Before the protein becomes active, cleavage of the latency associated protein is necessary, which is performed by the integrin αvβ6. A potential agent directed against αvβ6 has been identified in animal models (STX-100). This is an important intermediate in the activation of latent TGF-β.[67]

Connective Tissue Growth Factor

Connective tissue growth factor (CTGF) is thought to be a central mediator of tissue remodelling and fibrosis.[68] Following promising results in animal models, a phase II trial evaluated safety and efficacy of anti-CTGF antibodies (FG-3019; clinicaltrials.gov identifier NCT01262001) in patients with IPF. Preliminary data showed improvement or stability of fibrosis (as determined by HRCT) after 24 weeks of treatment, with this improvement being positively associated with changes in FVC. However, full results of this study are yet to be published in a peer-reviewed publication.[69]

Interleukin-13

Interleukin-13 (IL-13) is a profibrotic protein found to be increased in BALF of patients with IPF.[70] IL-13 induces TGFβ, PDGF and other profibrotic agents such as insulin-like growth factor and connective tissue growth factor. A human anti-IL-13 antibody has been developed (QAX576), and is currently being tested in a phase II trial. The results are eagerly awaited.

Lysyl Oxidase-Like 2

Lysyl oxidase-like 2 (LOXL2) plays a role in cross-linking monomeric collagen fibers, that are secreted by fibroblasts. This maturation process, which makes the extracellular matrix stiffer, impacts on the progression of fibrosis.[71] LOXL2 has been shown to be upregulated in patients with IPF.[6] In the bleomycin-induced pulmonary fibrosis mouse model a monoclonal antibody against LOXL2 induced a reduction in inflammatory cytokines, and activated fibroblasts and fibrillar collagen.[72] A study evaluating the efficacy of an anti-LOXL2 antibody has recently started patient recruitment.

Chemokine (C-C Motif) Ligand 2

An important, but for a long time neglected, cell in pulmonary fibrosis is the macrophage. When macrophages become polarised to the M2 phenotype, they may promote collagen synthesis. Chemokine (C-C motif) ligand 2 (CCL2) regulates monocyte and macrophage recruitment via the CCR2 receptor.[73] Recently, a study with anti-CCL2 antibody (CTNO888) has been completed and the results are expected soon.

Miscellaneous

Phosphodiesterase-4 (PDE4) is involved in cAMP metabolism, and cAMP elevation has been shown to reduce both fibroblast proliferation and matrix synthesis.[74] Roflumilast, a PDE4 inhibitor, diminishes intracellular cAMP breakdown and has been tested in other chronic lung diseases.[75] Viruses such as cytomegalovirus or Epstein-Barr virus have also been implicated in progressive pulmonary fibrosis.[76] Indeed, it has been shown that the use of intravenous ganciclovir for 2 weeks in patients with advanced IPF improved 6 minute walk test

and symptoms.[77] There are data suggesting also a potential role for antibiotics in the treatment of IPF. For instance, azithromycin has been demonstrated to slow the progression of pulmonary fibrosis in animal models. Interestingly, the effect is induced by a dose way below the antibiotically active one, thus suggesting an immunomodulatory effect.[78] Another crucial mechanism in chronic inflammatory lung disorders is the adaptive arm of the immune response, involving T_H1 and T_H2 cells, inducible regulatory T (iTreg) cells, and IL-17 producing CD4+(TH17) cells. Excessive T_H17 cell activation is observed in chronic inflammatory disorders such as psoriasis, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, and rejection after transplantation; yet, its role in interstitial lung disease remains unclear.[79]

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) have the ability to home to sites of injury and contribute to epithelial restoration. As such, they have been suggested as a novel therapeutic strategy in IPF, where loss of epithelial integrity and abnormal alveolar re-epithelialisation are thought to be critical.[80] A recent phase Ib, non-randomised, clinical trial demonstrated an acceptable profile of endobronchially-administered autologous adipose-derived stromal cells.[81] Carefully designed future clinical trials will clarify whether MSC could regenerate and repair diseased IPF lungs.

CONCLUSION

Over the past 10 years, substantial advances have been made in our understanding of the pathobiology of IPF. In parallel, the last decade has witnessed a steady increase in the number of high quality clinical trials being designed, undertaken and completed. This massive effort of both the medical and industry community has produced the approval for clinical use (at least in Japan, India, Europe and Canada) of the first drug for IPF: pirfenidone. In addition, the well-characterised patient populations enrolled in these studies have provided valuable insights into the natural history of the disease. Crucial information has also been gained by the lack of efficacy of specific drugs. For instance, the failure of both anticoagulants and endothelin receptor antagonists to show any benefit in patients with IPF suggests that pathways involving the coagulation cascade or the endothelin system are not as critical, with regard to disease pathogenesis, as previously thought.

The (mostly disappointing) results of recent clinical trials in IPF highlight the challenge of identifying the 'ideal' patient population to study. Thus far, clinical studies in IPF have enrolled subjects with mild-to-moderate disease, as assessed by FVC. However, the possibility to identify individuals at highest risk of disease progression - the ones more likely to respond to any given treatment - would allow a targeted enrichment in the study population with a corresponding reduction in the required sample size.[82] There is a continuing debate on what constitutes a clinically meaningful endpoint in clinical trials in IPF.[83-86] While all-cause mortality is undoubtedly the most robust primary endpoint, measuring this outcome could be prohibitive because of the (large) number of patients and (long) study duration required for adequate power (particularly for patients with limited disease). As such, a number of surrogate markers for survival benefit have been proposed. Of these, change in FVC (either absolute or relative) is now the preferred primary endpoint since it closely fulfils the ideal characteristics of being reliable, reproducible, easy-to-measure, and applicable to all IPF patients,[87,88] although not a proven surrogate of mortality.[1]

A drug or drug regimen that provides a universally agreed standard of care for patients with IPF has yet to emerge. Therefore, the role of the clinician is of utmost importance in helping patients to make an informed treatment decision. Owing to the plethora of pathways potentially involved, future treatment of IPF will likely require a combination of drugs targeting diverse components of disease pathogenesis (injury, inflammation, if any, and fibrosis). Nonetheless, the current momentum in this area of research, together with experience gained and emerging insights from genetic studies, provides hope for the development of effective therapies for this devastating disease.

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NOVEL TREATMENT OPTIONS FOR AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS

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Disclosure: No potential conflict of interest.
Received: 03.06.2013 **Accepted:** 30.08.2013
Citation: EMJ Respir. 2013;1:122-128.

ABSTRACT

Pulmonary alveolar proteinosis (PAP) is a diffuse pulmonary disease, characterised by the accumulation of lipoproteinaceous material in the distal air spaces, which results in impaired gas transfer. Autoimmune PAP accounts for the vast majority of cases in humans and is caused by autoantibodies directed towards granulocyte-macrophage colony-stimulating factor (GM-CSF), which causes a defect in the function of alveolar macrophages linked to the disruption of surfactant homeostasis. Whole lung lavage (WLL) is the current standard of care for PAP patients and although it is effective in the majority of cases, disease persistence is not an unusual outcome, even if airspace accumulation is well controlled by WLL. Even though WLL remains the current standard therapy for PAP, in this review we focus on novel treatment approaches for autoimmune PAP.

Keywords: PAP, autoantibodies, WLL, GM-CSF.

INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare respiratory disease, first described by Rosen et al. in 1958,[1] and characterised by the accumulation of lipoproteinaceous material in the alveoli, which may lead to impaired gas-exchange and progressive respiratory failure. The pathogenesis of the disease has been widely reconsidered over the last 20 years, with the conclusion that the acquired form is an autoimmune disease, in which autoantibodies directed against neutralising granulocyte-macrophage colony-stimulating factor (GM-CSF), lead to defective maturation of alveolar macrophages. The latter contain giant secondary lysosomes filled with the same material that accumulates within the alveoli, and cause defects in chemotaxis, adhesion, phagocytosis, microbicidal activity, and phagolysosome fusion.[2]

Alveolar macrophages and type II pneumocytes are responsible for the re-uptake and clearance of pulmonary surfactant, a lipid-protein complex, which is synthesised, packaged, and secreted by alveolar type II cells. The lipid portion constitutes approximately 90%, whereas the protein portion constitutes approximately 10% by weight.[3] Pulmonary surfactant plays a pivotal role in reducing the surface tension at the air-liquid interface of the alveolar wall, thus preventing alveolar collapse and transudation of fluid into the lumen of the alveolar capillary.[4]

The amount of surfactant is tightly regulated by mechanisms controlling its synthesis, recirculation and catabolism.[5] The results of ultrastructural, biochemical, and functional analyses, together with studies in genetically modified mice, strongly support the hypothesis that the basis for PAP is a defect in the clearance of surfactant rather than due to its overproduction.[6]

Evidence for adult PAP as an autoimmune disease was first presented by Kitamura et al.,[7] who noted that circulating anti-GM-CSF autoantibodies neutralised GM-CSF biological activity, and thus resulted in a virtual GM-CSF deficiency. Subsequent studies in idiopathic adult PAP patients confirmed the existence of anti-GM-CSF antibodies and demonstrated that

autoantibody measurement could be a clinical tool for diagnosis.[8,9]

PAP THERAPY

Different treatment options have been utilised since PAP was first described. As far as the therapeutic aspects of the disease are concerned, in the pre-lavage era the death rate was approximately 30% of cases[10], but the introduction of the whole lung lavage (WLL) in the mid-1960s has changed the natural history of PAP, with a dramatic reduction of the mortality, at least where the WLL is performed, with an immediate positive outcome in >90% of cases, but with a recurrence rate ranging from 30 to 70%, according to different series reported. WLL for idiopathic pulmonary alveolar proteinosis is currently a safe procedure in an experienced setting, and yields durable benefit in the majority of patients.[11] Little is known about the intimate mechanisms of action of WLL. Presumably WLL is able not only to remove the surfactant excess, but also the autoantibodies, interfering with surfactant homeostasis, as it was suggested several years ago.[12] Even though WLL remains the current standard therapy for PAP, in this paper we focus on novel treatment approaches for autoimmune PAP.

RITUXIMAB

Rituximab is a monoclonal antibody directed against the CD20 antigen on B lymphocytes. In B cell lymphoproliferative disorders, the mechanism of action is mediated by eradication of the malignant CD20 positive B cells. Subsequently, rituximab therapy has been applied in the treatment of autoimmune disease with clinical benefit.[13-15]

Based on data indicating autoantibody involvement in PAP, Borie et al.[16] described a 41-year-old non-smoker who refused WLL; the patient exhibited clinical, functional and radiographic improvement after 1,000 mg of rituximab was delivered intravenously on day 1 and day 15. In particular, the treatment resulted in a decrease in anti-GM-CSF concentration. 9 months after treatment, dyspnoea improved; similarly, diffusing capacity of the lung for carbon monoxide (DLCO), CT scan and alveolar–arterial oxygen gradient ($P(A\text{-}a)O_2$) at rest improved. This response was sustained at a 12-month follow-up.

Amital et al.[17] described a 40-year-old female, non-smoker with severe dyspnoea and hypoxaemia. Despite WLL and subcutaneously delivered GM-CSF, a year later the patient's condition deteriorated. Rituximab at 375 mg/m^2 was administered weekly over 4 weeks, with improved DLCO and oxygen saturation at rest and during exercise, as well as chest CT and X-ray.

Recently, the first prospective, open label, proof of concept trial of rituximab has been carried out in 10 PAP adult patients.[18] The intervention consisted of two intravenous infusions of rituximab (1,000 mg), 15 days apart. The primary study end-point was improvement in oxygenation, as assessed by the $P(A\text{-}a)O_2$; in seven out of nine patients (one patient dropped out of the study for unknown reasons) partial pressure of oxygen in arterial blood (PaO_2) and $P(A\text{-}a)O_2$ were significantly improved at 3 and 6 months. Improvements were also noted in total lung capacity (TLC), high-resolution CT (HRCT) scans and transitional dyspnoea index (TDI).

The data also indicated that a single course of rituximab therapy was well tolerated, with no major adverse reactions in this PAP cohort. The major limitation of this study was the absence of a placebo group. Moreover, as the trial was conducted recently, with a 32±6 month post-therapy follow-up, the durability of response and the need for longer-term therapy remains an open question.

Mechanisms responsible for rituximab-mediated improvement in PAP are unclear; this trial demonstrated that rituximab effectively depleted circulating B lymphocytes for a period of 3 months, but the potential role of B cells in PAP, is not well defined. In fact, the majority of antibody-forming B cells are plasma cells, which do not express CD20 and are, therefore, not susceptible to rituximab depletion. Importantly, neither the total serum anti-GM-CSF, nor serum GM-CSF neutralising capacity, were reduced following rituximab therapy. On the contrary, reduction in anti-GM-CSF levels in bronchoalveolar lavage (BAL) fluid from the lung correlated with disease changes, suggesting that disease pathogenesis is related to autoantibody levels in the target organ.

Starting from these results, the same research group demonstrated that the clinical improvement in rituximab-treated PAP patients might be due to restoration of alveolar macrophage lipid homeostasis, associated with reduced GM-CSF autoantibodies in the pulmonary compartment.[19] Utilising BAL samples from the original cohort of PAP patients treated with rituximab, confirmed the positive therapeutic effect of rituximab on

PAP lung, by enhancing alveolar macrophage functional activity and expression of lipid regulatory genes, PPARγ, ABCG1, and LPLA2. Compared to baseline, both PPARγ and ABCG1 were significantly upregulated by rituximab treatment. An unexpected finding was the LPLA2 deficiency in untreated PAP patients, a situation that was significantly reversed by rituximab therapy. In conclusion, these data indicate that rituximab is able to reconstruct lipid homeostasis in PAP alveolar macrophages.

GM-CSF

Based on the role of neutralising anti-GM-CSF autoantibodies in autoimmune PAP, treatment aimed at relieving functional GM-CSF deficiency by administering exogenous GM-CSF has been utilised. Several studies have reported a favourable response with systemic (subcutaneous) or localised (aerosol) GM-CSF. Prompt improvement with resumption of GM-CSF in patients who relapsed suggests that disease resolution was not attributable to spontaneous remission and that GM-CSF does have therapeutic activity.

Subcutaneous GM-CSF

Evaluation of GM-CSF supplementation as a potential therapy in autoimmune PAP was initially prompted by the demonstration that this cytokine exhibits restorative activity on impaired surfactant metabolism and innate immunity in GM-CSF-deficient mice.[20] Seymour et al.[21] were the first to treat 14 autoimmune PAP patients with subcutaneous GM-CSF. Patients not responding to an initial dose of 5 μg/kg/d GM-CSF underwent stepwise dose escalation until a therapeutic response (represented by improvement in oxygenation at the lung level) was obtained. The treatment showed efficiency in 43% of patients with a median treatment time of 39 weeks. Among responders, there was a significant effect on PaO_2, $P(A\text{-}a)O_2$, DLCO, CT scan and the 6-minutes walking test.

In 2000, Kavuru et al.[22] published the preliminary results on four patients treated with subcutaneous GM-CSF for moderate exacerbation of PAP. GM-CSF was self-administered once daily for 12 weeks (dose escalation from 3 to 9 mg/kg/d). In three of the four patients with idiopathic PAP, administration of GM-CSF improved oxygenation and conferred symptomatic benefit.

Subsequently, in an open-label study on 25 patients, using escalating doses of GM-CSF from 5 to 18 mg/kg/day, GM-CSF treatment was associated with improvements in $A\text{-}aDO_2$ and other clinical parameters in 48%, with relapse rates of 25% among responders.[23] Nevertheless, in this PAP series, only subjects with moderate symptomatic disease were enrolled, thus we cannot speculate on the role of this treatment in severe forms of PAP.

Inhaled GM-CSF

Since it is conceivable that the alveolar space is the site of GM-CSF signal disruption, with the impairment of surfactant catabolism in autoimmune PAP, then it is reasonable to propose that local GM-CSF supplementation would result in better treatment outcome. In 2006, Wylam and coworkers[24] reported a retrospective case series of 12 idiopathic PAP patients, elected to receive aerosolised GM-CSF (250 mg b.i.d. every other week). All patients except one showed mean improvements in arterial oxygen tension, $P(A\text{-}a)O_2$, DLCO, and forced vital capacity. Two patients made a complete recovery and were disease-free 1 and 2 years after discontinuing treatment. 4 patients showed complete response to both the initial course or when treated again for recurrence after discontinuation of treatment. One patient required dose escalation (500 mg b.i.d.) and achieved a complete response. Importantly GM-CSF was well-tolerated without late toxicity.[24] Subsequently, Tazawa et al.[25] treated 35 stable PAP patients with an induction dose (recombinant GM-CSF (rGM-CSF) 250 mg/day b.i.d. every other week for 6 weeks) followed by a maintenance dose (125 mg/day b.i.d. for 4 days every 2 weeks for 6 cycles). The positive response rate, in terms of decrease in $P(A\text{-}a)O_2$ and DLCO, was 62%, and no adverse events were recorded. A total of 29 of the 35 remained stable without further therapy during the follow-up period. The Tazawa group also demonstrated that GM-CSF inhalation therapy decreased markers of surfactant accumulation in BALF of high responders.[26]

Combination Therapy

The cumulative response rate of GM-CSF is lower than that described for WLL age; in fact it seems only to limit disease progression.[27] For this reason, it has been proposed as standalone therapy in PAP patients with less severe disease and as a supplementary therapy to WLL in patients with more advanced autoimmune PAP.

Yamamoto et al.[28] described a 9-year-old girl with autoimmune PAP, who was initially refractory to inhalation therapy (250 mg of GM-CSF daily).

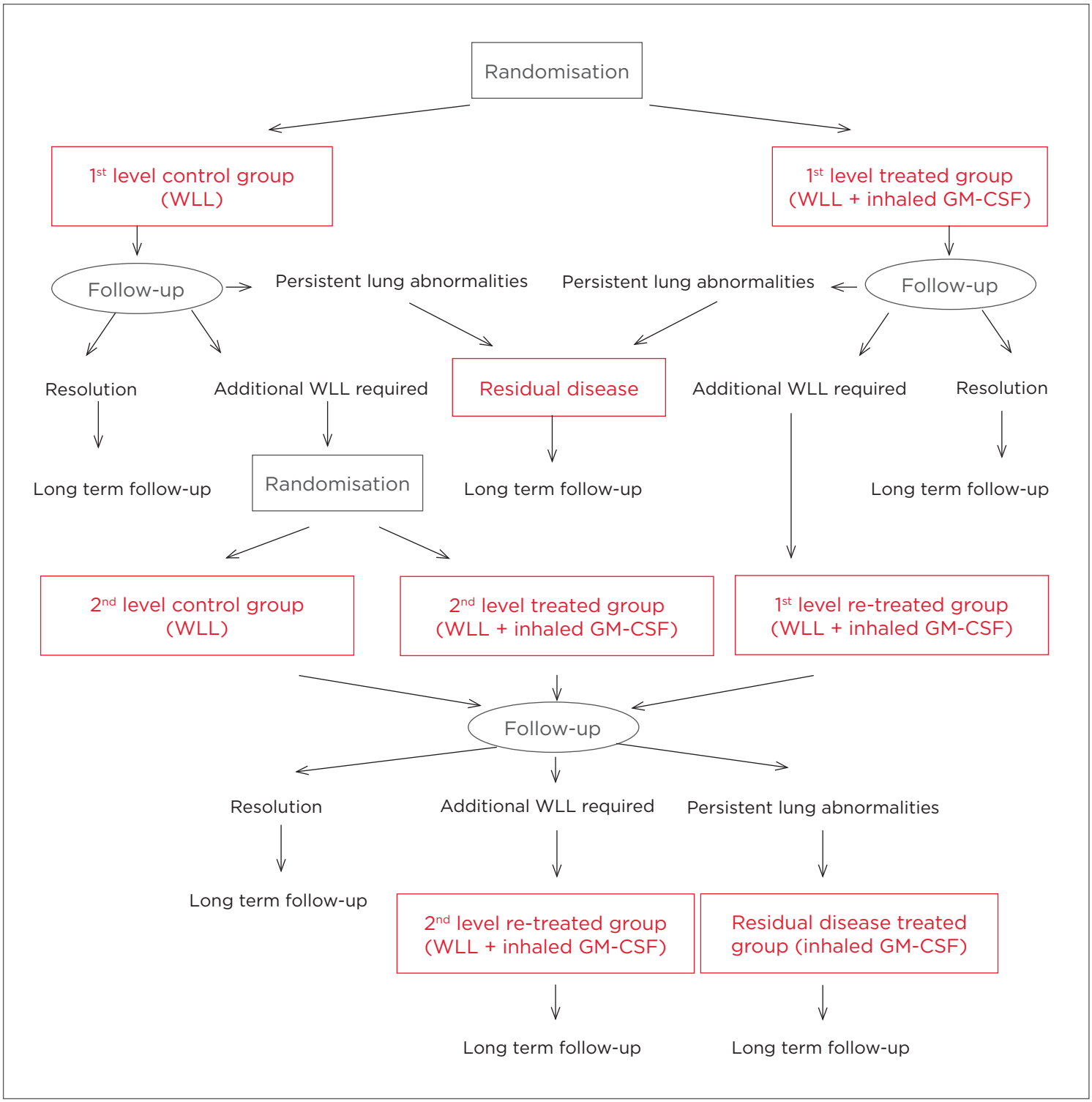

Figure 1. Flow Chart of the Pavia Centre Study.
PAP patients requiring a first WLL will be randomised 1:1 to receive either WLL followed by inhaled GM-CSF (first level treated group) or WLL alone (first level control group). PAP patients requiring a second WLL, if previously treated by WLL alone, are randomised 1:1 to receive either WLL followed by inhaled GM-CSF (second level treated group) or WLL alone (second level control group); if previously treated by WLL followed by inhaled GM-CSF, these subjects will be submitted to WLL followed by another course of inhaled GM-CSF (first level re-treated group). PAP patients requiring a third WLL, irrespective of the previous treatment, will be submitted to WLL followed by inhaled GM-CSF (second level re-treated group). In case of PAP patients not requiring additional WLL, but with persistent lung abnormalities and without severe functional impairment, this group will be submitted to a course of inhaled GM-CSF (residual disease treated group), since the main objective of the study is 100% resolution of PAP lesions.

Initial failure of the GM-CSF inhalation seems to be due to inefficient access of GM-CSF to the alveolar spaces because of densely accumulated surfactant. Unilateral WLL was performed three times and subsequent GM-CSF inhalation therapy yielded marked physiological and radiological improvements.

Recently, a study protocol for the treatment of autoimmune PAP, with WLL followed by inhaled GM-CSF (Sargramostin), has been started by our research group (AIFA FARM7MCPK4). We designed an experimental, phase II, parallel randomised trial with two cohorts. We plan to identify the best treatment schedule for PAP patients, looking at the evaluation of the superiority of the combination WLL/inhaled rGM-CSF versus WLL alone in PAP patients. The working hypothesis is that inhaled GM-CSF might speed recovery after WLL and reduce the frequency of disease recurrence in autoimmune PAP patients.

The study protocol is to be continued over a period of 36 months, with an expected target enrollment of 18 patients. Patients are divided in two groups (Figure 1): the control group, in which patients are treated with standard WLL, and the study group, where WLL is followed by inhaled GM-CSF. The treatment involves an acute phase lasting 12 weeks (250 mcg/day every second week) and a maintenance period of 6 months, 4 weeks after the completion of the acute treatment (250 mcg/day for 2 days every 14 days). The primary objective is to obtain complete and lasting regression of pulmonary infiltrates.

At scheduled visits, the PAP patients are evaluated by questionnaire, respiratory function testing, severity score, quality of life, clinical chemistry serum biomarkers, and CT-assisted lung profusion score. The latter is a method that, although originally developed for scoring interstitial pneumonitis, has been successfully applied to score changes in PAP lung infiltrates. The CT scan is performed at baseline and then 3 and 10 months after WLL (corresponding, in patients of the study group, to the end of the acute treatment and the maintenance treatment, respectively). Physiology and CT scoring data so far collected are encouraging, suggesting that the trial will be helpful in identifying the optimal sequence of treatments, to gain durable resolution of lung infiltrates in PAP patients.

Plasmapheresis

The presence of systemic anti GM-CSF antibodies in idiopathic PAP led to the hypothesis that PAP could be an autoimmune disease and hence the rationale for plasmapheresis as a therapeutic be an option. This should reduce autoantibody levels sufficiently to restore surfactant catabolism in alveolar macrophages. A 41-year-old non-smoking woman, with a 5-year history of non-resolving pulmonary infiltrates, was the first case of PAP treated with plasmapheresis. She was refractory to three WLLs and subcutaneous GM-CSF. She underwent low intensity plasmapheresis on ten separate sessions, resulted in a reduction of the levels of plasma autoantibodies, and improvement in symptoms, blood oxygen saturation, and radiographic appearance of the lungs[29]. The clinical course was complicated by a Gram-negative sepsis; however, the patient subsequently recovered, thus the plasmapheresis schedule was terminated.[30]

Subsequently, a patient with autoimmune PAP refractory to three WLLs, performed in less than 12 months, was submitted to 10 sessions of low-intensity plasmapheresis, which lowered the serum autoantibody level, but did not improve respiratory impairment. Further WLL therapy was required, but it was transiently effective, with increased length of symptom-free periods between subsequent WLLs.[31]

CONCLUSION

WLL is the current standard treatment for PAP patients and, although it is effective in the majority of cases, disease persistence is not an unusual outcome with a substantial portion of patients needing more than one or even repeated WLL. Even if the mechanism leading to the development of autoimmune antibodies towards GM-CSF is poorly understood, in the past two decades efforts made by researchers have contributed to the development of more refined and specific treatment options for PAP. While with rituximab therapy questions remain concerning the durability of response and the need for longer-term therapy,[18] GM-CSF, both systemically and targeted directly to the lungs, seems to be ineffective in about one-third of patients treated.[32] In this context, combination therapy with WLL followed by aerosolised GM-CSF would represent the best strategy.

Acknowledgement

This paper was, in part, supported by the Italian Agency for Medicines (AIFA) project for Independent Research 2007 (FARM7MCPK4), from E-Rare Project 2009 (EuPAPNet), and from the Scientific Direction of the San Matteo Hospital Foundation of Pavia.

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ADVANCES IN THE DIAGNOSIS OF TUBERCULOSIS AND DRUG RESISTANCE

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Disclosure: No potential conflict of interest.
Received: 26.04.2013 **Accepted:** 12.08.2013
Citation: EMJ Respir. 2013;1:129-134.

ABSTRACT

Countries in which tuberculosis (TB) is common are countries with lower economical conditions and less laboratory opportunities, so diagnostic tests for tuberculosis should not only be rapid and sensitive, but they should also be cheap, reliable and easily applicable. Current initiatives targeting the development of new diagnostic tests have increased the pace of identification and testing of a number of potentially useful innovations. Novel diagnostic methods for use in TB epidemiological studies are highly desirable. Detection of mycobacterial species (excluding *Mycobacterium tuberculosis*) using molecular methods is cheaper and simpler than conventional cultural detection, however, culture is still the gold standard in the diagnosis of *Mycobacterium tuberculosis* (MTB); even molecular and non-molecular tests cannot replace it. Results of molecular and non-molecular methods should be evaluated together with culture, microscopy, and clinical findings. When using these tests (conventional methods for example microscopy and culture; phagotypic and chromatographic methods; molecular methods for example nucleic acid amplification techniques (NAAT) and solid-phase hybridisation assays; serology) in a country with limited economical resources, cost/effectiveness analysis should be made carefully. This review examines the recent advances in the diagnosis of MTB in humans.

Keywords: Tuberculosis, diagnosis, treatment, drug resistance.

INTRODUCTION

Tuberculosis (TB) continues to be one of the most important causes of disease worldwide. Studies on diagnosis, treatment and prevention of disease have been increasing because of this. In 2011, there were an estimated 8.7 million incident cases of TB (range, 8.3 million–9.0 million) globally, equivalent to 125 cases per 100,000 in the population. Most of the estimated number of cases in 2011 occurred in Asia (59%) and Africa (26%); smaller proportions of cases occurred in the Eastern Mediterranean Region (7.7%), the European Region (4.3%) and the Region of the Americas (3%). Of the estimated 8.7 million incident TB cases in 2011, only 66% were diagnosed and notified to national TB control programmes, due, in part, to inadequate laboratory capacity in many low and middle income countries.[1]

TB is caused by a bacterium called *Mycobacterium tuberculosis* (MTB). Once inhaled, the bacteria reach the lungs and grow slowly over several weeks. In over 80% of people, the immune system kills the bacteria and they are removed from the body. In a small number of cases, a defensive barrier is built around the infection but the TB bacteria are not killed and lie dormant. This is called latent tuberculosis; the person is not ill and is not infectious. If the immune system fails to build the defensive barrier, or the barrier fails later, latent tuberculosis can spread within the lung (pulmonary tuberculosis), into the lymph glands within the chest (intrathoracic respiratory tuberculosis), or develop in any part(s) of the body to which it has spread (extrapulmonary tuberculosis). Because TB can affect many sites in the body, there can be a wide range of symptoms, some of which are not specific and may delay diagnosis.[2] Typical symptoms of pulmonary TB include chronic persistent cough

(of 3 weeks or more), sputum production (sometimes with haemoptysis), chest pain (TB pleurisy), and shortness of breath.[2,3]

TB, in parts other than the lungs, has symptoms which depend on the site, and may be accompanied by intermittent fever or weight loss. TB is a possible diagnosis to be considered in anyone with intermittent fever, weight loss, night sweats, fatigue or weakness, and other unexplained symptoms. Latent tuberculosis without disease, however, has no symptoms. The diagnosis of TB is suspected from a combination of context, symptoms, clinical signs and investigations. TB is diagnosed in a number of ways. Tissue samples from biopsies may show changes which suggest TB, as do certain X-ray changes, particularly on chest X-rays. Definite diagnosis is achieved by culturing the TB bacterium from sputum or other samples. This not only confirms the diagnosis, but also shows to which of the TB drugs the bacterium is sensitive.[2] However, low sensitivity of microscopic examination, and 2 to 6 weeks duration of culturing has resulted in a search for more rapid and sensitive diagnostic methods.

Polymerase chain reaction (PCR) is a method for reproducing specific DNA chains in a tube, which was an advancement for the diagnosis of agents, and which can hardly be cultured or which cannot be cultured at all.[4] Countries in which TB is most common are countries with lower economical conditions and less laboratory opportunities, so diagnostic tests for tuberculosis should not only be rapid and sensitive, but they should also be cheap, reliable, and easily applicable.[5] Novel diagnostic methods for use in TB epidemiological studies are highly desirable.[6,7] Current initiatives targeting the development of new diagnostic tests, new drugs, and new vaccines have increased the pace of identification and testing of a number of potentially useful innovations.[8,9] Detection of mycobacterial species (excluding *Mycobacterium tuberculosis*) using molecular methods is cheaper and simpler than conventional cultural detection, however, culture is still the gold standard in the diagnosis of TB; even molecular tests cannot replace it.[1,4,5] This review examines the recent advances in the diagnosis of MTB in humans.

LATENT TB INFECTION

Accurate identification of latent TB infection (LTBI) is the key to prevention of the disease among persons at risk. The tuberculin skin test (TST) was, until recently, the only tool available for detecting LTBI. Although the TST has proven to be useful in clinical practice, it has several major limitations.[10] Another advanced method to detect cellular immune response is the measurement of IFN-γ (interferon-gamma), excreted by T cells stimulated with MTB antigens (Interferon-gamma release assays [IGRAs]). New version antigens (early secreted antigen target-6 [ESAT-6] and culture filtrate protein-10 [CFP-10]) were coded by genes located on the RD1 (the region of difference 1) segment of MTB genome and are more specific for MTB than PPD.[11,12] Two IFN-γ assays, based on RD1 antigens, are available as commercial kits: the QuantiFERON®-TB Gold assay and the T-SPOT®.TB. As there is still no gold standard for the diagnosis of LTBI, these assays potentially may serve as a routine diagnosis test other than TST to identify people with LTBI.[13]

QuantiFERON®-TB Gold assay (for diagnosing both latent infection and active disease) is a whole-blood, ELISA-based test, whereas the T-SPOT®. TB test uses peripheral blood mononuclear cells and ELISPOT technology. The QuantiFERON®-TB Gold assay comes in two formats: a 24-well culture plate format and a newer, simplified, in-tube format. QuantiFERON®-TB Gold In-Tube (which also includes, in addition to ESAT-6 and CFP-10, the antigen TB7.7) test (Cellestis Limited, Carnegie, Victoria, Australia, 2007) and the T-SPOT®.TB test (Oxford Immunotec Limited, Abingdon, United Kingdom, 2008) are both newer tests.[14] A recent study has compared QuantiFERON®-TB Gold In-Tube, T-SPOT®.TB and TST in 373 HIV-infected patients, reporting that IGRAs were more sensitive than TST for the diagnosis of MTB infection in this category of patients.[15] The world Health Organization (WHO) advises against the use of IGRAs over TSTs as a diagnostic test in low and middle-income countries with typically high TB and/or HIV burdens.[16] Specificity of both tests for the determination of LTB in BCG-vaccinated are higher than TST.[17] Because of this high specificity, IGRA can be helpful in cases with cross reactivity due to BCG as the interpretation of TST could be difficult. IGRA may help to decrease false-positive results and can thereby increase the efficacy of LTB screening.[18,19]

MICROBIOLOGICAL DIAGNOSIS IN TUBERCULOSIS

Conventional Methods

Though studies to discover new diagnostic tests for TB have gained speed worldwide, detection of TB cases are still based on sputum acid-fast bacilli (AFB) culture, radiological findings and clinical symptoms. At present, 57% of all TB cases are

diagnosed bacteriologically, therefore the quality of presently applied methods needs to be increased. Some success has been achieved in this subject.[20]

Microscopy

Staining of AFB is widely used all over the world as it is fast, simple, inexpensive and an easy laboratory method. Globally, the rate of treatment success for the 2.7 million new cases of sputum smear-positive pulmonary TB who were treated in the 2010 cohort was 87%. It is also impressive that as the size of the global treatment cohort grew from 1.0 million in 1995 to 2.7 million in 2010, the treatment success rate progressively improved. Globally, the rate of treatment success was 85% in 2010.[1] Although carbol fuchsine staining is preferred routinely, more sensitive fluorochrom stains are also used, which enable a more rapid screening of the preparations. Using these methods, called 'flourenscent AFB staining' or 'fluorochrome AFB staining', a wider field can be screened with a lower magnification power, and time needed to screen all preparations can be decreased up to approximately ten times. They are therefore advised for laboratories examining a large number of samples daily for diagnosis and follow-up of patients during treatment.[21] Recent literature reviews have confirmed that it may also be beneficial in ordinary microscopy. This technique could be further improved by attaching a stronger light source called an ultra-bright light-emitting diode.[20]

Culture examinations

Despite the development of various new techniques for rapid diagnosis, the gold standard for diagnosis nowadays is still culturing[22] as, for example, 2 live Bacilli ml-[1] was reported to be enough for culture positivity.[23] A systematic review demonstrates that these liquid cultures (BACTEC™ 460TB, Septi-Chek™ AFB) are more rapid and sensitive than solid medium cultures (Lowenstein-Jensen, Middlebrook7H-10,7H11). The mean time to detection was 12.9 days by BACTEC™ MGIT™ 960, and 15 days with BACTEC™ 460TB, compared with 27 days with Lowenstein-Jensen solid medium.[20] An Indian study showed that the BACTEC™ 460TB radiometric method obtained 87% of the positive results within 7 days and 96% within 14 days. Therefore, the BACTEC™ 460TB method is considered cost-effective in countries endemic for TB.[24] BACTEC™ 460TB (Becton Dickinson, Sparks, MD, USA) has been considered the best method for rapid testing of susceptibility of MTB to major anti-tuberculous drugs such as rifampicin, isoniazid, ethambutol, pyrazinamide, and streptomycin.[25]

Phagotypic Methods

Novel diagnostic tests using mycobacteriophages to identify MTB from biological specimens, require only 2 days of turnaround time in the laboratory. There are two methods: luciferase reporter phage assay (LRP) and the phage amplified assay (PhaB or MAB). Both are simple, rapid and rather cheap techniques requiring few pieces of equipment.[19,20] They have a high specificity, but lack sufficient sensitivity to conventional culture techniques.[19,20]

Phage-based assays are available as commercial kits. For diagnosis, the FASTPlaque-TB® (Biotec Laboratories Ltd., Ipswich, UK) assay can be directly used on sputum specimens. A variant, the FASTPlaque-TB-MDRi® kit, is designed to detect rifampicin resistance in culture isolates. An advanced version of this kit, FASTPlaque-TB-Response®, has been developed for the detection of drug resistance (e.g. rifampicin) directly from sputum specimens.[26]

Chromatographic Methods

Direct MTB identification from clinical samples has been attempted by using different chromatography methods to detect tuberculostearic acid (TBSA) alone or in combination with other structural components of the mycobacterial cell wall. Fast gas chromatography mass spectrometry (GC-MS) and new immunochromatographic assays (based on the MPT-64 antigen) are used,[27] but they do not yet represent a significant alternative for the rapid diagnosis of TB from clinical specimens.

Molecular Methods

Many fast and easy molecular methods with high sensitivity and specificity have been developed in the last 25 years to detect and identify mycobacteria directly in the specimens or by cultivation. These methods are nucleic acid amplification (NAA), nucleic acid hybridisation and nucleic acid chain analysis.[28]

Nucleic acid amplification techniques (NAAT)

These are molecular systems aimed to determine MTB complex from the clinical specimens.[20] Nucleic acid amplification tests are used in smear-positive cases to differentiate TB from atypical mycobacteria in developed countries with a low incidence of TB and in regions with high HIV infection rates.

PCR is the first developed method. There are two commercially available molecular systems based on NAA for AFB positive airway specimens by direct observation of fresh specimens. One is Amplicor® MTB (Roche Diagnostic Systems) based on PCR. The other is Amplified MTB® Direct Test (AMTD) based on GenProbe-Transcription-mediated amplification (TMA). Amplicor MTB® is used in smear-positive airway specimens and AMTD is used both in smear-positive and negative airway specimens. In addition to these, there are a lot of commercially available molecular tests based on NAA. Some methods based on strand-displacement amplification (SDA) are BD ProbeTec™ (BD Diagnostic assay), Abbott LCx MTB assay (Ligase chain reaction-LCR; Abbott Laboratorıes, Chicago, IL), commercial kits for Real-Time PCR (LightCycler®-Roche, iCycler®-BioRad, ABI PRISM7000®-Applied Biosystem) instrument, COBAS TaqMan® MTB test, and nucleic acid chain amplification (NASPA) based methods produced by DNA•STRIP® technology, which includes GenoType® Mycobacteria Direct Test (Hain Lifescience, Germany).[23]

A half automatic method based on SDA principle is BD ProbeTec™, which has a primary amplification target of IS6110 insertion chain and 16S rRNA gen. Sensitivity and specificity of this test is good in smear-positive airway samples.[29] Commercial systems such as GenoType® Mycobacteria Direct Test (Hain Lifescience, Nehren-Germany) and Real-Time PCR (LightCycler™ system-Roche Diagnostics, Indianapolis, IN) are also molecular methods which have been used more and more in rapid diagnosis of tuberculosis. Special probes such as fluorescence resonance energy transfer (FRET) are used in some of the methods and are specific for MTB.[23] NAA-based tests are rapid tests with high sensitivity and specificity in positive direct observed fresh airway specimens and high specificity and low sensitivity in negative fresh specimens.[30-32]

In addition to the developments in clinical laboratory tests, DNA fingerprint methods are helpful with showing laboratory contaminations and epidemiological studies.[19,20]

Rapidly developed drug resistance can also be shown with molecular methods. Phenotypic (culture-based) and genotypic (nucleic acid amplification testing–based) methods have been developed to detect drug-resistant TB, however, first-generation tests were rarely available in TB-endemic areas, were poorly standardised, and had slow turnaround times. Genotypic drug-susceptibility testing (DST) for firstline agents is accurate for RIF and isoniazid (INH) but less reliable for streptomycin, ethambutol, and pyrazinamide.[33,34] Automated liquid culture systems and molecular line probe assays are recommended by WHO as the current gold standard for first-line DSTs.[34] The most important drug for the development of rapid drug resistance is probably rifampicin. Strains resistant to rifampicin are detected to be also resistant to many drugs because most rifampicin-resistant isolates are also isoniazid-resistant. Detection of rifampicin resistance in the early stages can be important for the management of treatment.[35] There is an instrument (GeneXpert® Xpert MTB/RIF, Cepheid, Sunnyvale, CA, U.S.A) detecting rifampicin resistance using PCR directly without a prior procedure of the specimen. In this assay, high sensitivity and specificity are obtained for the detection of MTB, and the few studies performed to date have also observed a good response as regards resistance to rifampin.[36,37] The Xpert MTB/RIF assay was rapidly endorsed by WHO in December 2010 for use in TB, multidrug-resistant TB, and TB/HIV-endemic regions using a risk-based approach to testing.[38]

Solid-phase hybridisation assays

Species detection of mycobacteria using probe technology can also be done; AccuProbe® (Gen-Probe-San Diego, CA), line probe assays (LiPA, Innogenetics, Ghent, Belgium ve Bayer Diagnostics, Tarrytown), and GenoType® MTBC (Hain Lifescience, Nehren-Germany) are the most commonly used products for this. Line probe assays are developed for the identification of cultured mycobacteria and for the detection of drug-resistant mutants. The first version of reverse hybridisation is LiPA mycobacteria assay (Innogenetics); probes for 16S and 23S sites of mycobacteria are used in this method, and MTB complexes, MAI complexes, *M. avium, M. intracellulare, M. kansasii, M. chelonae, M. gordonae, M. xenopi, and M. scrofulaceum* can be detected. The test takes approximately 3 hours. It was reported that PCR, following reverse hybridisation, gave very successful results in smear-positive direct clinical specimens.[39] In addition to identification of species, genetic determinants of rifampicin resistance can also be detected with reverse hybridisation of direct clinical specimens.[40] Rifampicin resistance can be examined using commercially available INNO-LiPA Rif TB (Innogenetics, Ghent, Belgium) and GenoType MTBDR (Hain Lifescience, Germany) kits.[41] Duration of these tests is approximately 3 to 4 hours and the results are 90% concordant with the classical

drug sensitivity results. WHO subsequently issued a statement supporting the use of MTBDR plus directly on smear-positive samples for rapid detection of drug resistance. Limitations of the assay include reduced sensitivity for detection of INH resistance compared with rifampicin resistance.[42,43]

In situ hybridisation is another method which has been used for molecular diagnosis for a number of years. It is called fluorescence *in situ* hybridisation (FISH), if fluorescence is used as detector molecule, and chromogenic *in situ* hybridisation (CISH), if detection of the probe is performed with a secondary reaction and colour.[23] DNA chain analysis is accepted as the gold standard and the most reliable method in the identification of mycobacteria, which can be used to detect species of mycobacteria and genetic mutations responsible for drug resistance. With the commercially available MicroSeq® (Applied Biosystems, Inc (ABI), Foster City, CA), DNA chain setting of a 500 bp part of the 16S rRNA gene is possible. This test is mostly used for research rather than for routine laboratory examinations because it is cheap but difficult and needs experience to be performed. DNA microarray is used for the identification of *Mycobacterium* species and the detection of mutations related to antibiotic resistance; because it is very expensive, it is a molecular method mostly used in research.

Some genotyping methods used for diagnosis and typing of mycobacteria like spoligotyping, MİRU-VNTR (Variable Number Tandem Repeats of mycobacterial interspersed Repetitive Units) are primarily used in research. Molecular methods should especially be used in smear-negative patients with clinically suspected tuberculosis and especially to examine airway specimens. To come to a conclusion for diagnosis in a short time, specimen AFB positive and NAA test positive patients should be accepted as TB. For extra-lung specimens more examinations are needed.

Serology

These are methods based on detection of antibodies produced against MTB antigens like ES-31, ES-43, EST-6 antigen 5, antigen 60, P32 and lipoarabinomannan by ELISA. No serological first-line methods are currently used for TB, particularly due to variability in results and cross-reactivity with environmental mycobacteria, which leads to false-positive results.[30] The sensitivity of these tests is high in patients with smear-positive disease, but much lower in children, patients with extra-pulmonary disease, HIV infection or smear-negative cases. Moreover, these tests cannot reliably distinguish latent infection from active disease or different species of mycobacteria.[30] LAM-ELISA may be a suitable option for the diagnosis of human immunodeficiency virus (HIV)-associated TB in urine specimens from patients with low CD4 cell counts.[44] MTB antigen detection provides direct evidence of TB; LAM, 65 Kd, 14 Kd antigens were widely used; it is very quick and easy to perform, but the main limitation is low sensitivity.[45]

In addition to these methods, detection of enzymes (adenosine, deaminases, lysosomes) excreted from various cells are also used in the diagnosis of MTB. But these methods could not replace classical culture.[23]

CONCLUSION

Culture is still the gold standard in the diagnosis of TB, even molecular and non-molecular tests cannot replace it. When using molecular tests for diagnosis, it should be kept in mind that there may be many problems arising because of user applications, and quality control standards should be applied exactly. Results of these tests should be evaluated together with culture, microscopy and clinical findings. It should be kept in mind that inappropriate specimens or a single finding may not mean anything. There should be good communication between the physician and the laboratory, and clinical findings should be followed closely. This not only enables a quick diagnosis and treatment, but also a control on infection. Using these tests in a country with limited economical resources, cost/effectiveness analysis should be made carefully.

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AIRWAY CLEARANCE IN THE INTENSIVE CARE UNIT

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Disclosure: No potential conflict of interest.
Received: 23.04.13 **Accepted:** 14.08.13
Citation: EMJ Respir. 2013;1:135-139.

ABSTRACT

Mechanically-ventilated patients in the intensive care unit (ICU) may suffer from retained secretions from several causes. Airway clearance techniques have the potential to improve mucociliary clearance by reducing mucus plugging and enhancing the removal of secretions, including inflammatory cells and bacteria. This short review describes recent progress in airway clearance management in ICU patients.

Keywords: Mechanical ventilation, airway clearance, chest physiotherapy, intrapulmonary percussive ventilation, in-exsufflation.

INTRODUCTION

Mechanically-ventilated patients in the intensive care unit (ICU) may suffer with retained secretions from several causes. Endotracheal intubation reduces the mucociliary clearance, increasing infectious risks by increasing mucus volume and consistency. Prolonged immobility can result in atelectasis, impairment of cough, and secretion retention. Expiratory muscle weakness, by reducing the expulsive force needed to perform cough and fluid restriction, may also contribute to secretion retention.[1-3]

Airway clearance techniques (ACTs) have the potential to improve mucociliary clearance by reducing mucus plugging and enhancing the removal of secretions, including inflammatory cells and bacteria. These techniques may result in improved ventilation, a reduction of airway obstruction and atelectasis, an improved ventilation–perfusion mismatch, and a decrease in proteolytic activity in the airways.[4-6] Nevertheless, the role of ACTs is poorly defined, and there is a paucity of supporting evidence in the ICU,[7,8] especially due to the difficulties in assessing the effectiveness of ACT. Among these are the choice of outcomes to evaluate therapeutic effects; either physiological outcomes, such as mucus transport or change in pulmonary function, or clinical outcomes, such as days spent in the hospital or quality of life.[9,10]

PERCUSSION AND VIBRATION

Manual percussion of the chest wall and vibrating the chest during expiration, in patients under mechanical ventilation (MV) with retained secretions, are useful in order to move secretions from the peripheral towards the central airways[11-13] (Figure 1). Increase in mucus clearance was described by Stiller[14] in critically ventilated patients with normal cough competence without a significant change in blood gases and lung compliance. However, there are some negative effects of this modality, such as pain, anxiety, atelectasis, and increase of oxygen consumption.[15]

INTRAPULMONARY PERCUSSIVE VENTILATION

Another technique used is intrapulmonary percussive ventilation (IPV), which improves mucus clearance through direct, high-frequency, oscillatory ventilation, helping alveolar recruitment.[16] With this technique, high-frequency ventilation is delivered into the lungs in the form of intrapulmonary percussions through a face mask, a mouthpiece, an endotracheal tube, or a tracheostomy.[17]

Dimassi et al.[18] studied patients at high risk for extubation failure who were receiving preventive non-invasive ventilation (NIV) after extubation. They concluded that both NIV and IPV reduced the respiratory rate and the work of breathing, but IPV was less effective in improving alveolar ventilation. The addition of IPV was associated with improvement of oxygenation, expiratory muscle performance and reduced risk of late onset pneumonia in tracheostomised patients.[19]

POSITIVE EXPIRATORY PRESSURE

Positive expiratory pressure (PEP) is defined as breathing with a positive expiratory pressure of 10-20 cmH_2O.[6] The system employs a mask, or a mouth-piece connected to a resistance nipple, to provide positive pressure during expiration, and the blow-bottle device in which the resistance consists of a water seal. The pressure achieved is dependent on the performance of the manoeuvre, the adjustable expiratory resistance, and the patients' active expiratory flow.[6,20]

Ingwersen and colleagues[21] conducted a prospective randomised trial in post-operative patients to compare continuous positive airway pressure (CPAP), PEP and several airway clearance techniques. There were comparable decreases in pulmonary function and arterial oxygen tension (PaO_2), and comparable rate of atelectasis in all the treatment groups. Authors concluded that PEP therapy was a preferable technique by the patient without any significant difference in outcomes.

Richter-Larsen and colleagues[22] studied post-operative patients treated with routine chest physiotherapy alone or supplied with either PEP or a device creating both inspiratory and expiratory

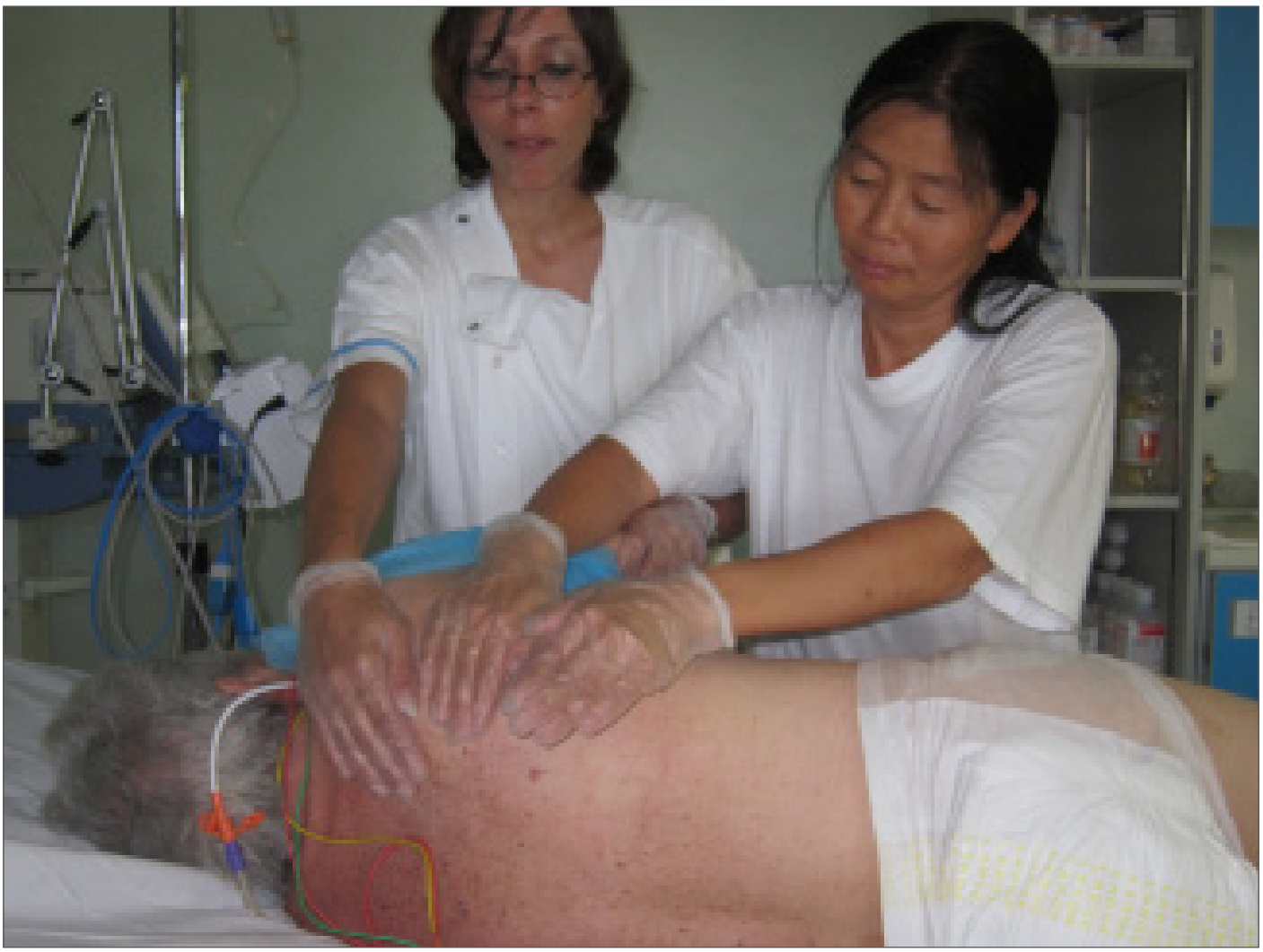

Figure 1. Nurse and Physical Therapist performing manual clapping of thorax in a mechanically ventilated patient.

resistance. PEP and postural drainage and percussion were preferred methods by the patient. This study also suggested that the patients using PEP and the device had tendency toward less risk of postoperative complications.

IN-EXSUFFLATION

The Cough Assist In-Exsufflator has proven to be a useful adjunct for airway clearance in patients with ineffective cough, and may result in benefit for intubated and tracheotomised patients.[23] Mechanical insufflation-exsufflation (in-exsufflation) consists of insufflation of the lungs with positive pressure, followed by an active negative-pressure exsufflation that creates a peak and sustained flow high enough to provide adequate shear velocity to loosen and move secretions toward the mouth for suctioning or expectoration[1,24,25] (Figure 2).

MANUAL HYPERINFLATION

Manual hyperinflation (MH) is frequently used in critically ill intubated and mechanically-ventilated patients.[26] The effectiveness of MH depends on higher expiratory flow and movement of sputum from distal to more proximal areas.[27,28] A study by Paulus et al.,[29] concluded that the rate of haemodynamic and respiratory adverse effects with MH is low when performed by experienced and trained nurses in stable, critically ill patients. Blattner et al.[30] conducted a randomised controlled trial to compare MH and standard care in post cardiac surgery patients. The result was an improvement in pulmonary compliance and PaO_2 and a reduction of MV duration. Improvement in pulmonary compliance with MH compared to standard care was also reported by several studies evaluating unselected ICU patients.[31,32] A summary of safety, effectiveness, and pros and cons of described techniques is shown in Table 1.

HUMIDIFICATION

Adequate humidification is important for airway clearance[33] since heating and humidifying the inspired gas is an established standard of care during MV,[7] although the contribution to temperature regulation appears small.[34] Appropriate heating and humidifying inspiratory gas are necessary to prevent complications associated with the drying of the respiratory mucosa, such as mucus plugging and endotracheal tube occlusion,[35] if impaired mucociliary clearance and cough have been

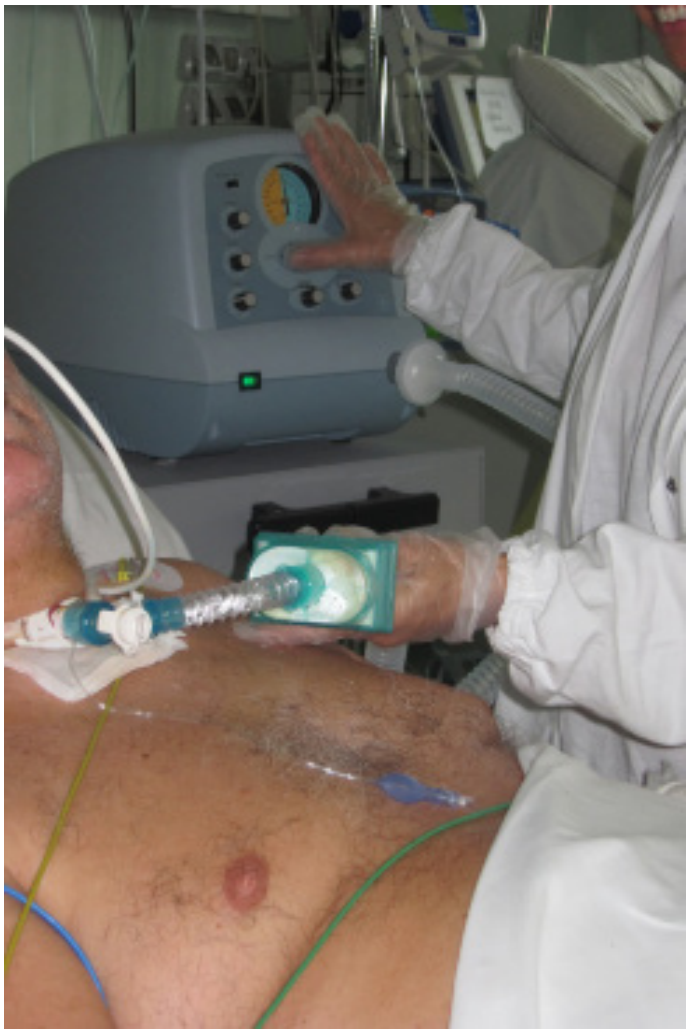

Figure 2. In-exsufflation device is applied through the tracheostomy tube and manually operated.

observed.[35-38] Solomita et al.[39] compared non-heated to heated-wire humidification over a wide range of minute ventilation values and concluded that at the same Y-piece temperature, heated-wire humidification may provide significantly less humidification than physiologic levels.

TRACHEA SUCTIONING

Routine suctioning via endotracheal tubes in intubated patients facilitates the removal of airway secretions, maintains airway patency and prevents pulmonary infection. Normal saline is frequently instilled into the trachea before suctioning as it may help to dislodge secretions and facilitate airway clearance.[40] However, tracheal suctioning is associated to mucosal injury,[41] and other adverse side-effects including decreased arterial oxygen tension.[42] Closed (in-line) endotracheal suction eliminates the need for disconnection from MV

Table 1. Safety, effectiveness, and pros and cons of airway clearance techniques in the ICU.

Technique	Safety	Effectiveness	Pros	Cons
PERCUSSION AND VIBRATION	+++	++	Improved ventilation, reduction of airway obstruction and atelectasis, correction of ventilation–perfusion mismatch	Pain, anxiety, atelectasis, and increase of oxygen consumption
INTRAPULMONARY PERCUSSIVE VENTILATION	++	+++	Improvement of oxygenation, expiratory muscle performance and reduced risk of late onset pneumonia in tracheostomised patients	Expensive
POSITIVE EXPIRATORY PRESSURE	++++	++++	Low cost	------
IN-EXSUFFLATION	++	++++	Better airway clearance in neuromuscular patients	Expensive
MANUAL HYPERINFLATION	++++	++	Low rate of haemodynamic and respiratory adverse effects. Low cost	Experienced and trained nurses needed

and does not require single-hand sterile technique like open suction methods. Although data are not evidence-based since a metanalysis showed no difference in mortality or VAP rates between open and closed suction systems, [43] closed systems came with higher costs; these devices may be preferable because of their efficiency and smaller number of suction-induced complications.[44] The other risk of frequent suctioning is loss of PEEP and potential derecruitment. This can be potentially serious in a patient with high PEEP levels and severe hypoxaemia.

CONCLUSION

Patients in the ICU may suffer from retained secretions. Secretion clearance is therefore an integral component of disease management in these critically ill patients. In mechanically-ventilated patients, appropriate heating and humidifying inspiratory gas is necessary to prevent and remove complications associated with the drying of the respiratory mucosa, such as mucus plugging and endotracheal tube occlusion. Routine suctioning facilitates the removal of airway secretions, maintains airway patency and prevents pulmonary infection. Manually clapping the chest wall and vibrating the chest during expiration are useful to move secretions from the peripheral towards the central airways. Intrapulmonary percussive ventilation improves mucus clearance through direct high-frequency oscillatory ventilation, helping the alveolar recruitment. Positive expiratory pressure can be easily applied and is low-cost. The Cough Assist In-Exsufflator has proven to be a useful adjunct for airway clearance in patients with ineffective cough, especially due to expiratory muscle weakness. Manual hyperinflation is frequently used in critically ill intubated and mechanically-ventilated patients.

There is still limited evidence to support the use of any secretion clearance techniques such as a comprehensive approach in intensive care patients. Therefore randomised studies, with solid clinical short and long-term outcome measures, are needed. In the meantime, to choose a technique, care givers should consider the pathophysiologic rationale for the therapeutic use, the equipment cost, the adverse effect of the therapy, and patient preference.

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'Man's best friend' detects lung cancer

"In the breath of patients with lung cancer, there are likely to be different chemicals to normal breath samples, and the dogs' keen sense of smell can detect this difference at an early stage of the disease."

Professor Thorsten Walles, Schillerhoehe Hospital, Germany

SNIFFER dogs can successfully detect lung cancer in its early stages, a new study conducted in Germany has shown.

Detecting lung cancer early is a very rare occurrence as there are hardly any symptoms, and the current methods for detection are deemed by experts as unreliable.

The study, conducted by researchers from Schillerhoehe Hospital, Germany, consisted of 220 volunteers. Some were healthy, while others had lung cancer or chronic obstructive pulmonary disease (COPD).

The primary outcome of the study was to assess if sniffer dogs could identify volatile organic compounds (VOCs), a cancer which can be detected in the breath of patients. Professor Thorsten Walles, the author of the study said: "In the breath of patients with lung cancer, there are likely to be different chemicals to normal breath samples, and the dogs' keen sense of smell can detect this difference at an early stage of the disease."

The dogs identified 71 samples with lung cancer out of a possible 100, and also detected 372 samples out of 400 who did not have lung cancer. The highly-promising results proved that there was a stable marker for the detection of lung cancer.

It is thought further tests need to be conducted in even bigger study groups to enable practitioners to make a decision as to when this technique could be implemented, and replace current detection methods.

New technologies needed to treat lung injuries in premature babies

NON-INVASIVE techniques may not be as effective in treating extremely underweight infants with bronchopulmonary dysplasia (BPD) as once thought. These techniques are used to reduce the incidence of severe lung injury in very premature infants, the most common technique used being non-invasive nasal ventilation.

However, a multinational, randomised trial run by Dr Haresh Kirpalani, a neonatologist at the Children's Hospital of Philadelphia, USA, enrolled 1,009 infants from 34 neonatal intensive care units in 10 countries, with a birth weight under 1,000 grams (2.2 lbs) and a gestational age under 30 weeks.

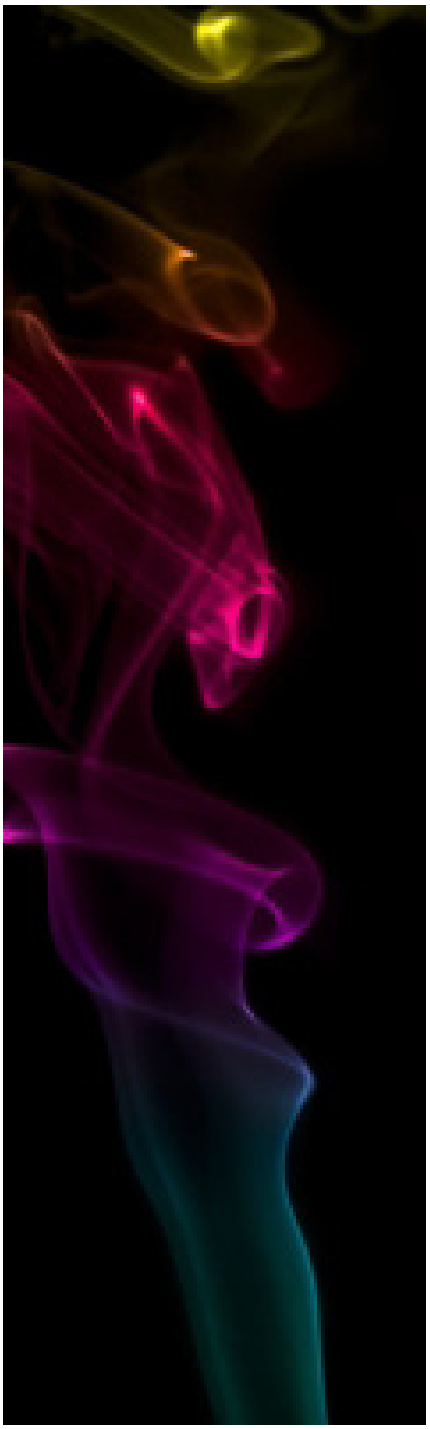
The trial compared two techniques, which are used to make breathing easier for the infant and to stop the lungs from collapsing. After randomly assigning the infants to either nasal continuous positive airway pressure (CPAP)

or intermittent positive-pressure ventilation (IPPV), the researchers hypothesised that extra pressure delivered through nasal IPPV would be more beneficial in helping infants inhale and prevent BPD, compared to nasal CPAP, which provides positive pressure when the infant inhales.

However, there was no significant difference in the primary outcome of either death or survival with BPD, as Dr Kirpalani explained: "The research is significant as it refutes the common assumption that non-invasive therapies being used are reducing severe lung injury in these tiny babies."

Dr Kirpalani also attested the need to develop new therapies for babies to avoid lung injury and BPD.

Incense smoke becomes major cause of concern



BURNING incense could possibly lead to an inflammation in human lung cells, according to researchers from the Gillings School of Global Public Health at the University of North Carolina, USA.

The study found that incense smoke can emit a significant amount of harmful particles such as carbon monoxide, formaldehyde, and oxides of nitrogen, all of which result in cellular inflammatory response.

For the World Health Organization (WHO), indoor air pollution has become a major concern. 94% of the population living in the United Arab Emirates (UAE) burn incense weekly in their homes, where they also spend 90% of their time. A high amount of exposure to these gasses can lead to chronic respiratory disease (COPD), leading to an estimated one million deaths every year.

As previous studies have highlighted the health risks associated with incense smoke, the current study aimed to identify the number of particles and gasses emitted from both Oudh and Bakhoor, two popular types of incense used.

In order to test the impact, human lung cells were placed in a chamber and exposed to the smoke over 3 hours. They were then incubated for 24 hours to allow the particles to settle and the cells to respond.

The researchers found that this type of exposure was similar to being exposed to cigarette smoke; they both have inflammatory reactions which can cause asthma and other respiratory problems.

The authors of the study have recommended that when incense is used, better ventilation, such as opening a window or a door, should also be implemented. Furthermore, they have recommended that future studies should also measure the additional compounds caused by incense smoke, and further analyse the inflammatory markers.

Body-oxygenating particles breakthrough

"It sounds like magic, but it was just the start of what, after years of investigation, became this real life-giving liquid in a bottle."

Dr John Khier, Boston Children's Hospital, USA

MICROPARTICLES that can be injected into the bloodstream to quickly oxygenate the body, even if the patient has stopped breathing, have been developed at Boston Children's Hospital, USA. This breakthrough in medical technology could sustain a patient for around 15 to 30 potentially life-saving minutes.

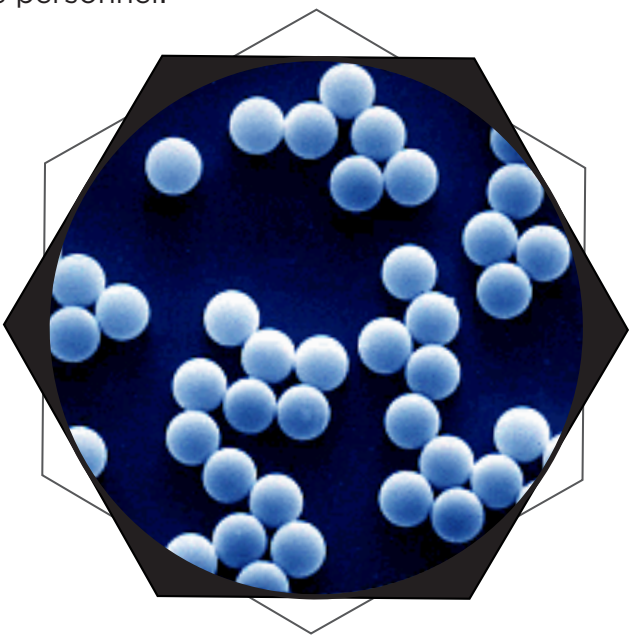
In 2006, Dr John Khier at the Department of Cardiology, Boston Children's Hospital, treated a young girl with severe pneumonia. Though she later died from severe brain injuries, the operating team only needed a few extra minutes to save the girls life. It was this event which inspired Dr Khier to develop these particles which could now save millions of lives every year.

Dr Khier grouped together chemical engineers, particle scientists, and medical doctors to make this idea a reality. The early results, Dr Khier points out, were very promising: "We drew each other's blood, mixed it in a test tube with the microparticles, and watched blue blood turn immediately red, right before our eyes."

The particles are made up of oxygen gas and are packed in a layer of lipids, a natural molecule which stores energy or acts as a component to cell membranes. When injected into animals under critical lung failure, oxygen was restored, which in turn gave them additional life-saving minutes.

Dr Khier added: "It sounds like magic, but it was just the start of what, after years of investigation, became this real life-giving liquid in a bottle."

As the particles are 2 to 4 μm they can be easily suspended in a liquid solution, thus they are easily transportable and can be carried by paramedics, emergency crews, and intensive care personnel.



Bigger lungs better for saving lives?

SARAH Murnaghan, a 10-year-old who was diagnosed with end-stage cystic fibrosis, was the first patient under the age of 12 to receive an adult lung since 2007. This operation, combined with the results from a new study, has shown larger lungs could mean a better survival rate.

Children's organs rarely become available for transplantation, and as Sarah's condition had deteriorated, something had to be done. Her parents petitioned for a change in the policy which prohibited patients under the age of 12 from receiving adult lungs.

Dr Ashish Shah, Surgical Director of Lung Transplantation at the Johns Hopkins Hospital, USA, said: "You have to look at each patient very carefully and determine what their lung capacity is. There may be children who could take adult lungs that would be oversized for them with a good result. And there may be small adults who would do well with paediatric lungs."

It is the hope of many doctors at the Johns Hopkins Hospital that their new research, which suggests bigger lungs can increase survival, will remove restrictions when allocating lungs for transplantation. Moreover, it will help doctors make a more personalised decision based solely on individual cases and the size of the organs available.

unfortunately, are not as good as with other solid organ transplants like liver, kidney and heart. But our study tells us that if we were to routinely transplant larger lungs into patients, we could potentially make a real impact on survival. And that's the goal of research."

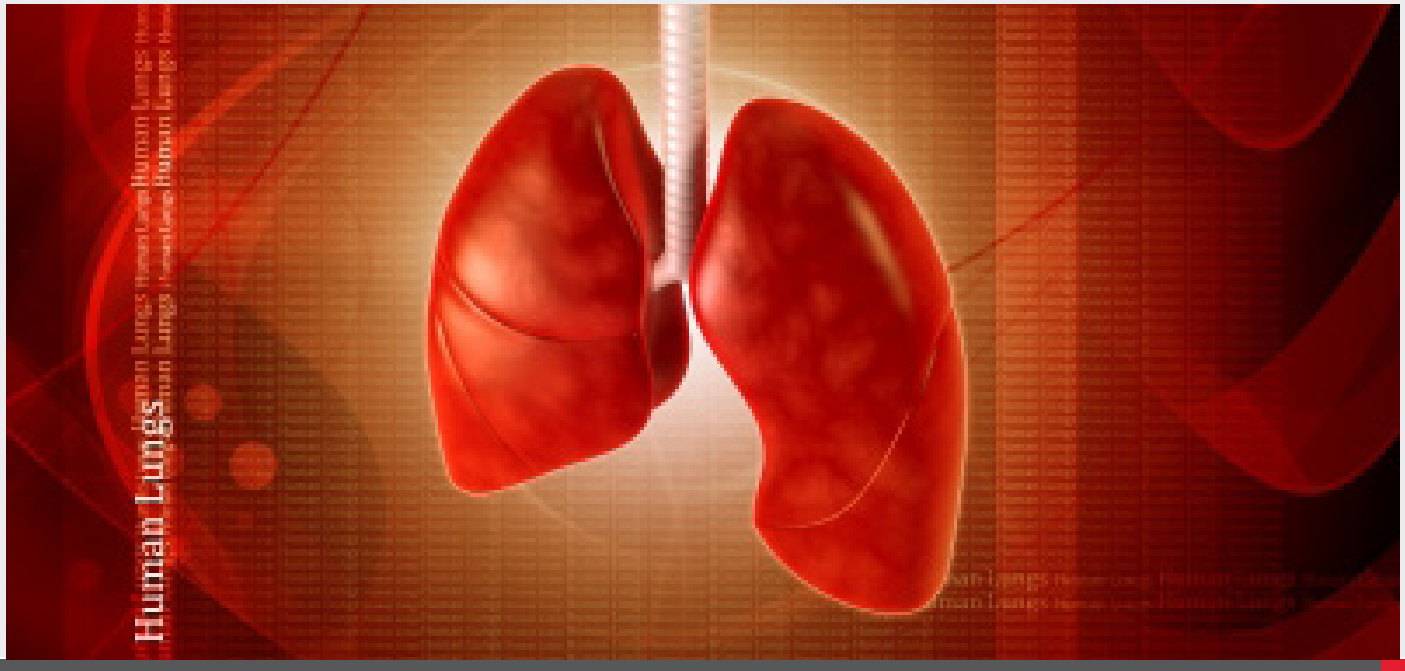
Though researchers have warned that lungs can still be too large for transplantation, leading to various complications, Dr Shah added that this does not mean smaller lungs should be rejected, arguing that they would be better than no transplant at all.

Sarah received transplanted adult lungs on the 12th June 2013 and is living healthily with no further complications or health complaints. Her surgery has not only highlighted that adult

"You have to look at each patient very carefully and determine what their lung capacity is. There may be children who could take adult lungs that would be oversized for them with a good result. And there may be small adults who would do well with paediatric lungs."

Dr Ashish Shah, Surgical Director, Lung Transplantation, Johns Hopkins Hospital, USA

Dr Christian A. Merlo of the Johns Hopkins University School of Medicine said: "Despite what we thought, bigger lungs turn out to be better. The survival rates for lung transplant, lungs can be suitable enough for children, but it has also thrust the issue into the national spotlight, an issue which could possibly change medical legislation.



Heart problems: what the deodorant can doesn't tell you

PARENTS and doctors have warned against the potentially fatal associations of spray deodorants and other aerosol products. Though fairly commonplace within day-to-day life, headaches, eczema, asthma, or even fatal heart problems, can result through overuse.

With 50% of children now using deodorant by the age of 11, it is believed self-consciousness concerning body odour can lead to spraying in excess.

Despite two cases of aerosol-related deaths in the UK alone, the British Aerosol Manufacturers Association (BAMA) maintains that propellants in household aerosol products have been used for 40 years safely, with 600 million aerosols used annually in the country.

Although there are warnings on the can not to spray in confined spaces, or for prolonged periods of time, Dr Peter Dingle, Environmental Scientist and Consultant Toxicologist, argues: "It is not enough for the authorities to say 'It's okay, there's a warning on the back of the can'. If two children have died by spraying deodorant in a confined space, there will be countless more spraying the same way. Do we have to have another death before the authorities act?"

One tragic example of deodorant-related death is Jonathon Capewell, who died of a heart attack in his bedroom. According to the 1998 inquest, the 16-year-old had 10 times the lethal amount of butane and propane in his blood, as gasses used in aerosol propellants had built up in his body over a number of months.

"He was 16 and his body was changing," Jonathon's father, Keith, said. "He was starting to sweat more and worry about how he smelled. It wasn't unusual for him to have two or three showers a day. Afterwards he would spray deodorant all over his body, even in his hair, and the bathroom was the smallest in the house."

Around one in three adults in the UK have some form of allergic disease – asthma, rhinitis, or eczema – and their symptoms are easily aggravated by perfumed products and exacerbated by aerosol chemicals.

Jonathan Clague, a consultant heart specialist at the Royal Brompton Hospital said: "The main cause of death is usually suffocation, known as hypoxia. If oxygen is not being breathed in and something else is inhaled, such as chemicals, then suffocation occurs and the heart stops."

"If two children have died by spraying deodorant in a confined space, there will be countless more spraying the same way. Do we have to have another death before the authorities act?"

Dr Peter Dingle,
Environmental Scientist and Consultant Toxicologist

Experimental ovarian cancer drug may be useful in treating lung cancer

THE EXPERIMENTAL drug PARP (Poly ADP ribose polymerase) inhibitors, used to treat ovarian cancer, may also be administered in cases of non-small-cell lung cancer (NSCLC), Cancer Research-funded scientists reported.

NSCLC is the leading cause of cancer deaths worldwide, there are however, very few treatment options for patients with the condition. Available treatments have a very limited effect, therefore, it is deemed crucial that there are developments within this area.

The researchers used an experimental technique to 'switch off' a molecule called CDK1 – another part of the cell division 'engine' – in combination with PARP inhibitors. Given together, the two effectively killed cancer cells growing in the lab and shrunk lung tumours in mice.

Chief Executive of Cancer Research UK, Dr Harpal Kumar, said: "[Lung cancer] is proven to be one of the hardest cancers to study and survival rates remain poor. We're making substantial investments in lung cancer research to discover better ways to diagnose and treat the disease."

PARP inhibitors were found to be useful in 50% of NSCLC tumours. The PARP inhibitors are successful as they act as a double blow; they eradicate lung cancer cells while keeping the healthy cells intact.

> "We now need to build on this promising early research by testing PARP inhibitors against lung cancer in clinical trials to confirm whether they can benefit patients."
>
> *Dr Chris Lord, Cancer scientist, The Institute of Cancer Research*

The study author Dr Chris Lord, a Cancer Research-funded scientist at The Institute of Cancer Research (ICR), commented: "Our research opens up an exciting new route, by showing how we could repurpose drugs originally designed for use against other forms of cancer."

As the testing in the lab displayed promising results, the next step is to conduct clinical trials in selected patients. Dr Lord added: "We now need to build on this promising early research by testing PARP inhibitors against lung cancer in clinical trials to confirm whether they can benefit patients."

Asthma in children may lead to allergies and ADHD

BOYS with a history of allergies and asthma have an increased risk of suffering from attention deficit hyperactivity disorder (ADHD).

Scientists in both the Netherlands and Boston, USA, have studied 884 boys with ADHD and 3,536 boys without the disorder. They found that there was an increased risk of ADHD in boys with a history of asthma and an even stronger risk associated with milk intolerance, as 34% of the children suffering from ADHD had asthma, and 35% had an allergic disorder.

According to the American College of Allergy, Asthma and Immunology, a child has a 75% chance of being allergic if both parents have an allergy. It is estimated that 60-80% of children with asthma also have an allergy. These findings highlighted that the medications that are being used to treat these conditions may be associated with an increased risk of ADHD.

"Medications for these conditions far outweigh the risks, and can be life-saving in some conditions. Treatment should not be stopped, unless advised by a board-certified allergist," emphasised Dr Gailen Marshall, Editor-in-Chief of Annals of Allergy, Asthma and Immunology.

The cause of ADHD is unknown, but it is thought that the disorder is genetic. Dr Marshall advised that further research is needed to understand why there appears to be an increased risk of developing ADHD in children with allergy and asthma.

Gene variations: The new indicator to treatment response

"Using a personalised medicine approach to match the best treatment option to a patient based on his or her genetics will lead to better outcomes."

Dr Matthew Schabath, H. Lee Moffitt Cancer Center & Research Institute

FOUR recently-discovered inherited genetic variants in non-small cell lung cancer (NSCLC) patients may help to predict survival and treatment response, personalise treatment and improve outcomes.

NSCLC is the most common type of lung cancer and represents more than 80% of lung cancer diagnosis. Lead study author Dr Matthew Schabath, Assistant Member of the Cancer Epidemiology Program at H. Lee Moffitt Cancer Center & Research Institute, explained that NSCLC has a very poor 5-year survival rate with only 16% of all patients surviving for 5 years, and only 4% of patients suffering from the later stages of the disease live longer than 5 years.

The researchers analysed 651 DNA samples from patients with NSCLC. They found that on one inflammation-related gene, named TNFRSF10B, there were four survival-related genetic variations which increased the risk of death by 41%. The risk of death increases further if the patient's treatment plans just included chemotherapy, opposed to both surgery and chemotherapy.

"Part of the difficulty in treating lung cancer is the genetic diversity of patients and their tumours," Dr Schabath said. "Using a personalised medicine approach to match the best treatment option to a patient based on his or her genetics will lead to better outcomes."

Previous studies have highlighted associations with other variants in the same gene family as TNFRSF10B, but there has been no published data examining these four specific variants concerning cancer risk or outcome.

"Having a validated genetic biomarker based on inherited differences in our genes may allow physicians to determine the best treatments for an individual patient based on their unique genetics," Dr Schabath added.

"Having a validated genetic biomarker based on inherited differences in our genes may allow physicians to determine the best treatments for an individual patient based on their unique genetics."

Dr Matthew Schabath, H. Lee Moffitt Cancer Center & Research Institute

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One of the world's top pharmaceutical companies, Teva's vision is to be the most indispensable medicines company for the world, executing on their obligation to patients, customers, and shareholders, employing 46,000 people in 60 different countries.

Dedicated to progress in the respiratory field through investing time and money in R&D to bring innovative products to the field, they strive to make life easier for patients and healthcare professionals alike by finding simple solutions to complicated problems.

ResMed is a leading developer, manufacturer and marketer of medical equipment for diagnosis and treatment of sleep-disordered breathing. ResMed is committed to education and awareness by supporting physicians and researchers exploring the link between SDB and life-threatening disease worldwide.

ResMed employs about 2,700 employees worldwide, has direct operations in 18 countries, and distribution in more than 50 countries.

They also consider the OSA market as underpenetrated, and recently joined a partnership with LifeScan, a Johnson & Johnson company, to increase awareness for OSA amongst type 2 diabetes patients.

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1st International Workshop on Lung Health, COPD: The New Paradigms

12th-14th December 2013

Monte Carlo, Monaco

This Conference is dedicated to Chronic Obstructive Pulmonary Disease (COPD) and the impact that it has on lung health. This meeting will focus on the physiological effects of COPD, the updates within the discipline, as well as novel therapeutic agents. It has been recognised that there is a need for a deep discussion and understanding within this area; this conference offers an ideal platform to discuss and share experiences of these issues with top experts and practitioners.

Medical Conference: Practical Management of Tuberculosis (TB) – Diagnosis, Inpatient and Outpatient Care

21st January 2014

London, United Kingdom

The conference hopes to leave the delegates with a deeper understanding of the use of treatment and its potential adverse effects. This one-day conference will review various clinical presentations and also the use of various diagnostic tools within the clinical setting.

Lung Science Conference 2014: Lung Inflammation and Immunity

21st-23rd March 2014

Estoril, Portugal

The lung science conference promises to be both beneficial and informative to all those who attend. Over the course of three days the sessions will address the effects and mechanisms of allergies, immune responses in chronic lung disease and also present new trends in immunology and asthma. The presentation of work will be based on oral presentations of submitted abstracts.

ELCC 2014: The 4th European Lung Cancer Conference

26th-29th March 2014

Geneva, Switzerland

This multidisciplinary conference will address the scientific and educational needs of medical and radiation oncologists, thoracic surgeons, pathologists, pneumonologists, radiologists, research physicians, and various other health professionals. The Conference will address the variety of new developments within treatment, and also present new findings in clinical trials.

WCA 2014: The 12th World Congress of Asthma

29th March-1st April 2014

Mexico City, Mexico

INTERASMA is now approaching its 60th anniversary, and is one of the most senior international asthma and respiratory disease organisations in the world. This year's scientific programme focuses on new insights in epigenetics, proteomics, mechanism, rick factors, and new diagnostic and therapeutic tools. The 2014 hopes to attract paediatricians, primary care clinicians, and asthma specialists.

European Respiratory Society Research Seminar: The Many Faces of Stem Cells in Respiratory Disease

10th-12th April 2014

Barcelona, Spain

This research seminar will explore the many clinical implications of stem cell research. It hopes to group together the varied opinions concerning the use of stem cells by gathering together experts and young scientists to explore the obstacles within stem cell research in an open environment.

European Cystic Fibrosis Society 37th European CF Conference

11th-14th June 2014

Gothenburg, Sweden

The European Cystic Fibrosis Society is continually growing in size with over 2,000 delegates expected to attend this year; their involvement representing a wide range of CF professional disciplines. The conference hopes to cover the entire spectrum of CF-related issues through their discussions, workshops, plenary sessions, symposia, and special interest groups.

ESTI Annual Scientific Meeting 2014

12th-14th June 2014

Amsterdam, The Netherlands

Through a range of case-based discussions and interactive sessions, this meeting is dedicated to showcasing the most recent developments and challenges in thoracic imaging as well as in education and training, while also recommending guidelines for appropriate practices in chest imagine. To mirror the importance of interdisciplinary cooperation, topics will be discussed from a clinical and radiological point of view.

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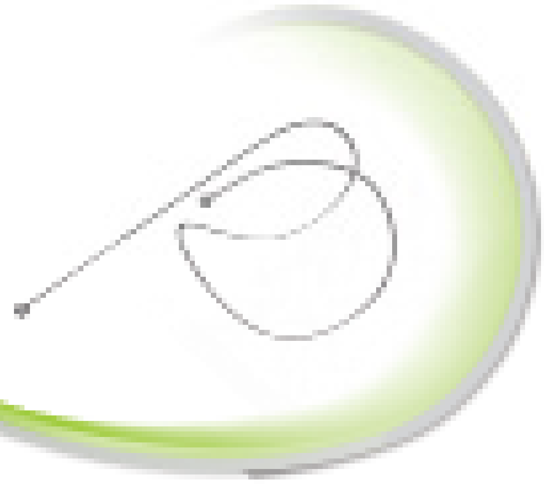

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