EMJ EUROPEAN MEDICAL JOURNAL

RESPIRATORY

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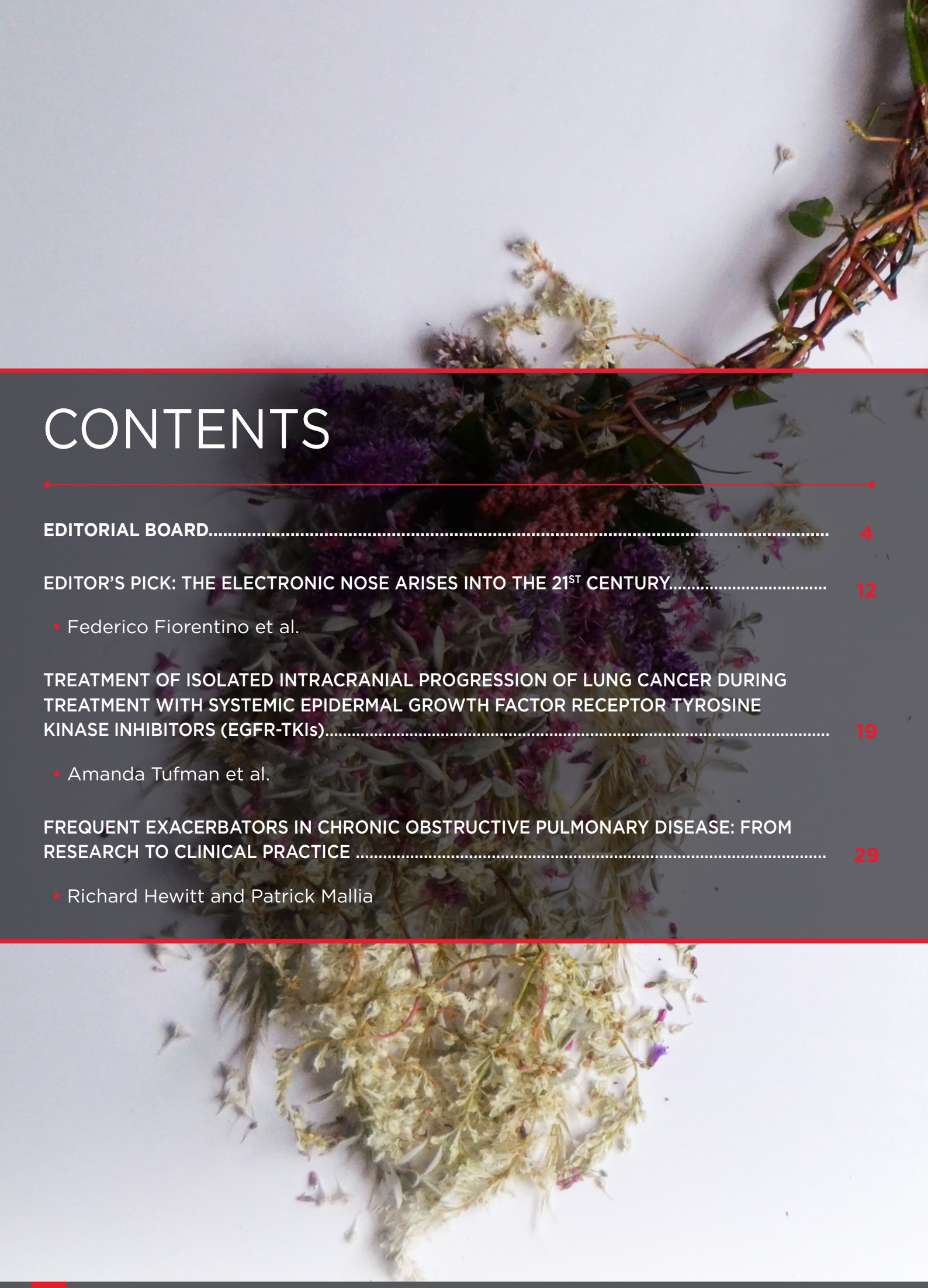

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EMJ GORELY NEW MEDIA

European Medical Journal
EMJ Respiratory Vol 3.1
May 2015

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Welcome

Hello and a warm welcome to the latest edition of *European Medical Journal Respiratory,* which features a whole host of high-quality articles written by distinguished professionals in respiratory medicine. Indeed, some of the most important current issues and discoveries in this pivotal field will be covered in this journal, which will provide you with the critical information you require as the year nears its half-way point.

In their paper *'Deep neck abscesses complicating acute fusobacterial tonsillitis',* Ling Lee and Manson analyse a case study of an adult patient with deep neck suppurative inflammation - the major life-threatening complication following throat infection – due to infection with *Fusobacterium necrophorum.* The clinical manifestations, diagnostic methods, therapy, and outcome are all highlighted in this absorbing report, which is sure to be of interest to many clinicians.

The response to the major global health challenge of HIV-associated tuberculosis (TB) is also featured strongly in this edition of *EMJ Respiratory,* particularly regarding the World Health Organization's target to reduce TB deaths by 95% and new cases by 90% by 2035. With this in mind, Gupta-Wright and Lawn in *'Advances in the diagnosis of HIV-associated tuberculosis'* discuss the recent advances relevant to this area, including new diagnostic technologies.

The treatment of pneumonia remains a pressing issue in this discipline. The article entitled *'Established statin use reduces mortality from community-acquired pneumonia: a systematic review and meta-analysis'* authored by Fitzgerald et al. reviews the association between established statin therapy and outcomes of patients with severe community-acquired pneumonia.

In addition, Hewitt and Mallia, in their article *'Frequent exacerbators in chronic obstructive pulmonary disease: from research to clinical practice',* discuss the mechanisms of frequent acute exacerbations in chronic obstructive pulmonary disease (COPD) in order to promote greater understanding of this important condition, and so that new therapies can be targeted to these high-risk patients. There is certainly a highly urgent need for new treatment options for COPD, with the disease being a major cause of morbidity and mortality.

Respiratory medicine is an incredibly fast-paced and varied field, and we hope that this edition of *EMJ Respiratory* will provide you with some valuable information to potentially assist you in improving your patients' outcomes. As such, we hope that you find the content highly informative and wish you all the best for the challenges that lie ahead this year.

Spencer Gore

Team Principal, European Medical Journal

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Foreword

Prof Nikolaos Siafakas

Professor Emeritus of Thoracic Medicine, University of Crete, Heraklion, Crete, Greece

Dear colleagues,

It is my pleasure to welcome you to the latest edition of *European Medical Journal Respiratory,* which we hope will provide you with summaries of the most important developments in the field. The previous year has certainly been a busy one for pulmonologists, with the publication of new clinical guidelines for the treatment of severe asthma, occupational asthma, bronchiolitis obliterans following transplantation, and lung cancer, as well as a technical report on the standards to be expected from walking tests used to assess the exercise capacity of patients in the clinic. Also, I would like to stress the importance of respiratory guidelines for the practicing physician as well as for the basic scientists working in the respiratory field. It is well known that the cardinal aim of all guidelines is to improve and maintain the level of healthcare for patients.

Some of the most compelling topics in this issue include: a look at deep neck abscesses due to *Fusobacterium necrophorum* infection, an examination of increased cough sensitivity in patients with idiopathic pulmonary fibrosis, and a comprehensive review of the association between statin therapy and outcomes of patients with community-acquired pneumonia that is severe enough to require hospitalisation. Some of the latest developments from the most topical areas in respiratory medicine will be covered inside, making it a truly interesting read.

“Also, I would like to stress the importance of respiratory guidelines for the practicing physician as well as for the basic scientists working in the respiratory field. It is well known that the cardinal aim of all guidelines is to improve and maintain the level of healthcare for patients.”

It is with much anticipation that we remind you of the 25th International Congress of the European Respiratory Society (ERS), which will be taking place in Amsterdam, the Netherlands, on 26th-30th September 2015, and its proceedings will be covered extensively in the next edition of *EMJ Respiratory,* to be published shortly after the conclusion of the meeting.

Finally, I would like to thank all of the contributing authors and my fellow editorial board members for the work they have put in to producing this eJournal. We very much hope that you find the articles informative and useful.

Yours sincerely,

Nikolaos Siafakas

Professor Emeritus of Thoracic Medicine, Department of Thoracic Medicine, University General Hospital, Medical School, University of Crete, Heraklion, Crete, Greece; Past President of the European Respiratory Society (2009-2010).

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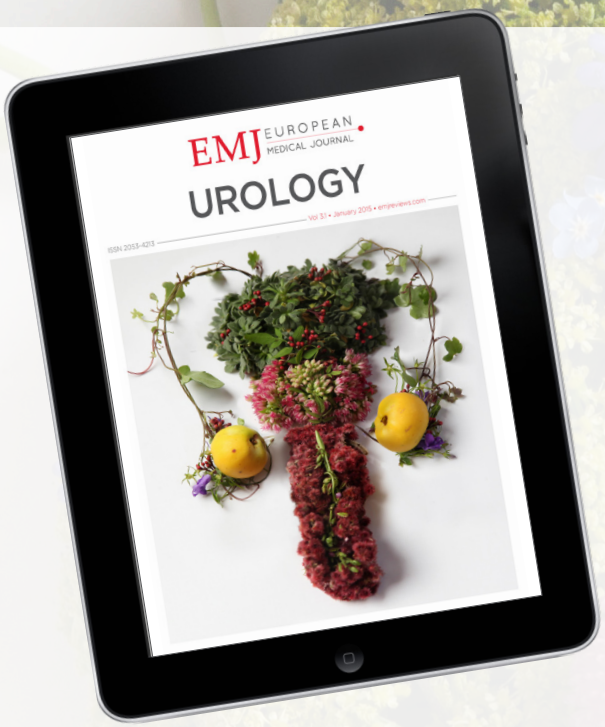

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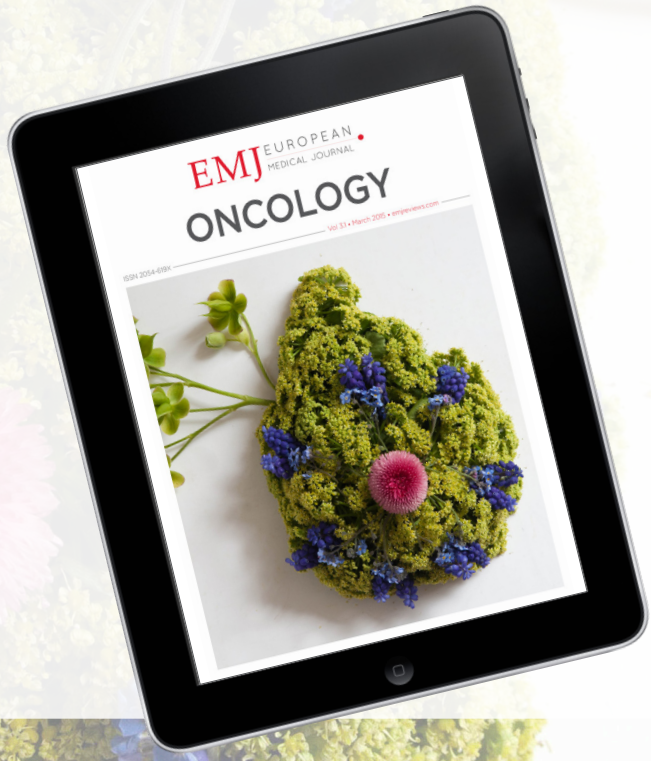

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EDITOR'S PICK

An electronic nose is a small, portable device that can detect, with reasonable accuracy, the breath 'fingerprints' of various respiratory diseases. It detects volatile organic compounds in exhaled air and can differentiate between common respiratory illnesses. However, larger studies are needed in order to evaluate and standardise the device.

Prof Nikolaos Siafakas

THE ELECTRONIC NOSE ARISES INTO THE 21st CENTURY

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ABSTRACT

Electronic noses (eNoses) are instruments designed to imitate the sense of smell. These devices are used to detect and precisely distinguish odours within complex samples at a relatively low cost, and these properties make them very useful in a diverse range of clinical scenarios. An eNose is typically composed of a multisensor array, an information-processing unit, and a pattern-recognition algorithm. The multisensor array combines to respond globally to a wide range of volatile organic compounds (VOCs) and the output provides a distinct digital response pattern. Clinical 'breathprints' or 'smellprints' contain VOCs and respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma, and lung cancer can be detected by this novel technique. Moreover, patients with exacerbated COPD and a positive microbiological culture can be differentiated from those with stable disease. The eNose displays high accuracy in detecting obstructive sleep apnoea syndrome, and common conditions in the intensive care unit such as acute respiratory distress syndrome and ventilator-associated pneumonia have also been studied in relation to the use of eNoses. Information contained within breathprints interpreted by eNoses may serve as non-invasive biomarkers in respiratory medicine and infectious diseases, as well as other branches of medicine.

Keywords: Electronic nose (eNose), volatile organic compounds, asthma, chronic obstructive pulmonary disease (COPD), lung cancer, obstructive sleep apnoea syndrome, ventilator-associated pneumonia, a cute respiratory distress syndrome.

INTRODUCTION

The widespread adoption of technology across almost all fields of medicine represents a key point in the evolution of many diagnostic procedures and therapeutic options. The electronic nose (eNose) is an example of cutting-edge technology adapted to provide a new medical tool. Exhaled breath from individual patients contains volatile organic compounds (VOCs) that can provide

important information. For example, 'smellprints' can be used to identify different conditions or processes occurring inside an organism. Smellprints are the result of analysis by a series of nanosensors contained within the eNose, these sensors are capable of detecting VOCs. The electrical resistance of these sensors changes specifically when exposed to volatile particles, which generates a signal that can be interpreted using various methods. Using mathematical algorithms, the eNose is trained to recognise smellprints through a process of comparison with previously recorded patterns. Data analysis includes a range of software, including pattern-recognition programs in MATLAB (v.R2012a) and SPSS, amongst others. Data obtained from pattern recognition can be analysed throughout cross validation, principal component analysis, and canonical discriminant analysis.

The basic setup for eNose sampling usually consists of a specific device containing the nanosensors, software for data interpretation, a collecting bag containing VOC-filtered ambient air that serves as a baseline, and a collecting bag containing the breath sample. The collecting bags are connected to the eNose through a purge inlet (baseline air) and a sample inlet (sample air) using a syringe as a connector between the bag and the eNose device. The eNose software allows several settings for sample processing. eNoses are handheld, portable devices that provide immediate results and, therefore, breathprints are rapid, non-invasive, cost-effective, and easy to perform compared with other methods currently available for analysing exhaled breath. These characteristics are important for the future clinical applicability of eNoses.

THE eNOSE IN OBSTRUCTIVE AIRWAY DISEASES

The most common obstructive lung diseases, chronic obstructive pulmonary disease (COPD) and asthma, share some common characteristics but differ in terms of treatment options and expected morbidity and mortality outcomes. Fens and colleagues[1] investigated whether breathprints from 30 patients with COPD, 20 asthmatic patients, 20 healthy smokers, and 20 healthy non-smokers could be differentiated using an eNose (Cyranose 320®; Smith Detections, Pasadena, CA, USA). Their results showed an accuracy of 96% ($p<0.001$) when discriminating between asthma and COPD samples, an accuracy of 95% ($p<0.001$) when discriminating between asthma and non-smoking control samples, and 92.5% ($p<0.001$) when discriminating between asthma and smoking control samples.

Asthma is characterised by airway inflammation; this pathophysiological base makes it an obvious condition in which to investigate potential non-invasive biomarkers, such as VOCs. A study including 10 young patients with mild asthma, 10 young controls, 10 older patients with severe asthma, and 10 older controls investigated whether an eNose (Cyranose 320) was able to differentiate between these groups.[2] The smellprints of patients with mild asthma could be fully differentiated from young controls (cross-validation value [CVV]: 100%), and patients with severe asthma could be distinguished from older controls (CVV: 90%). However, the accuracy of the eNose was lower when differentiating between patients with mild and severe asthma.

One of the many challenges in COPD is the assessment of eosinophilic inflammation used to identify subgroups of patients in whom the use of inhaled corticosteroids may be most effective. A sputum cell count is not always available; it is a time-consuming procedure and can be uncomfortable for the patient. In order to improve this situation, differential cell counts and airway inflammatory markers were obtained through induced sputum samples from 12 mild and 16 moderate COPD patients classified according to the former Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.[3] The eNose breathprints were highly associated with airway inflammation in the group of mild COPD patients, this association was not found in the moderate COPD group. Thus, breath analysis may be used for assessment and monitoring of airway inflammation in COPD.

A preliminary study by our group investigated the hypothesis that an eNose could identify infection-associated VOC patterns in breath samples from patients experiencing acute exacerbation of COPD (AECOPD), and differentiate these patterns from those obtained from COPD patients without exacerbation.[4] 53 patients with AECOPD, 18 with COPD with associated pneumonia, and 29 with stable COPD were enrolled in the study. Sputum samples for microbiological evaluation were obtained from all patients and exhaled air samples were processed by an eNose (Cyranose 320).

Breathprints from AECOPD patients were distinguishable from stable COPD in the presence of microorganisms (p=0.036; 84.4% sensitivity; 70.0% specificity), as well as in the absence of infection (p=0.014; 90.6% sensitivity; 80.0% specificity). However, breathprints from COPD patients with pneumonia failed to show a significant difference from AECOPD samples in the presence of microorganisms (p=0.097), but were distinguishable in the absence of infection (p=0.013; 87.5% sensitivity; 77.8% specificity) and from stable COPD (p<0.05; specificity 90.0%). Different breathprints related to positive infection can be detected by the eNose. In addition, breathprints obtained from AECOPD patients with or without pneumonia could be distinguished from those obtained from stable COPD patients, even in the absence of a positive microbiological culture.

In a proportion of patients with COPD, bacterial colonisation of the airways increases the rate of mortality due to augmented airway inflammation, and increases the frequency and severity of exacerbations. Sibila et al.[5] performed a study to evaluate the ability of an eNose (Cyranose 320) to discriminate between COPD patients with and without airway bacterial colonisation, determined by quantitative culture of specimen brush, the gold standard for the diagnosis of distal airway infection. 13 healthy controls and 37 clinically stable moderate-to-severe COPD patients (colonised versus non-colonised) were studied. Canonical discriminant analysis showed an accuracy of 89% (p<0.001) with a sensitivity of 89% and a specificity of 96% when discriminating between colonised and non-colonised COPD patients.

THE eNOSE IN CANCER

The eNose has been evaluated for the detection of smellprints from patients with COPD and lung cancer (LC). A cross-sectional study[6] included 10 patients with non-small-cell LC, 10 patients with COPD, and 10 healthy controls. Smellprints from LC patients were distinct from those of patients with COPD (CVV: 85%). In duplicate measurements, the eNose distinguished patients with LC from healthy controls (CVV: 90% and 80%, respectively). Machado et al.[7] performed a two-phase study in which the ability of an eNose to discriminate between samples from 14 patients with bronchogenic carcinoma and 45 healthy controls was demonstrated during the first phase. During the second phase of the study, a cancer prediction model was created and applied to a separate group of 14 patients with LC and 62 without. The results from the validation study showed that the eNose displayed a sensitivity of 71.4% and a specificity of 91.9% for the detection of LC.

Asbestos inhalation is associated with malignant mesothelioma and other respiratory diseases. A non-invasive screening tool for high-risk populations would be useful for detecting mesothelioma during the early stages of the disease. Using an eNose (Cyranose 320) and applying principal component analysis, two contemporary studies by Dragonieri et al.[8] and Chapman et al.[9] have reported almost identical results, with high levels of discrimination between samples from patients with malignant mesothelioma, patients with long-term asbestos exposure, and patients with other asbestos-related non-malignant diseases.

Another example of a rapid, diagnostic, clinically applicable, and non-invasive use of an eNose is in the detection of prostate cancer.[10] Urine headspace samples from pre-operative patients with prostate cancer have been compared with those from benign prostatic hyperplasia patients using an eNose (ChemPro® 100); the results using leave-one-out cross-validation reached a sensitivity of 78%, a specificity of 67%, and an area under the curve of 0.77. Head and neck squamous cell carcinoma (HNSCC) has also been evaluated using an eNose, with a sensitivity of 90% observed when discriminating between VOC patterns from 36 HNSCC patients and those from 23 controls with benign conditions.[11] Colorectal cancer (CRC) treatment and prognosis depends on the diagnostic stage of the disease. The VOC patterns obtained from the gas in stool samples collected from patients undergoing a colonoscopy have also been analysed using an eNose. A sensitivity of 85% and a specificity of 87% were observed when discriminating between smellprints obtained from 40 patients with confirmed CRC and those obtained from 60 patients with advanced adenomas as well as 57 healthy controls.[12]

SMELLING OTHER RESPIRATORY DISEASES

Differentiation of samples from healthy controls from those obtained from patients with obstructive

sleep apnoea syndrome (OSAS) has also been investigated using an eNose. Greulich et al.[13] analysed VOC patterns in this very common disease that is associated with an increased risk of cardiovascular events and metabolic disorders. The diagnosis of OSAS is currently dependent on expensive, not always available, and time-consuming techniques such as polysomnography or respiratory polygraphy. The study included 40 OSAS patients and 20 healthy controls, with asthma and COPD patients excluded. The results of the study showed a high sensitivity (93%) and specificity (70%), with the corresponding area under the receiver operating characteristics curve of 0.85 (95% confidence interval: 0.75–0.96). Moreover, changes in breathprints after 3 months of uncontrolled continuous positive airway pressure therapy confirmed a relationship between the OSAS disease process and the VOC patterns detected by the eNose. The high accuracy of this tool might suggest it represents a non-invasive, portable, and cheap diagnostic method suitable for the diagnosis of OSAS.

Breathonomics has also been used to assess the identification of pulmonary embolism (PE) in a proof-of-principle study[14] including 20 patients with confirmed PE and 20 patients in whom this diagnosis was excluded. Patients were categorised according to the presence or absence of comorbidities and exhaled breath samples were analysed using an eNose (Cyranose 320). For the non-comorbid group, PE and non-PE samples were differentiated with an accuracy of 85% (17/20 correctly classified, p=0.008), the positive predictive value was 0.86, and the negative predictive value was 0.83. However, the accuracy in the comorbid group was 65% (p=0.78), which demonstrates the confounding effect of comorbidities on the interpretation of breathprint data.

A strict follow-up on lung transplant recipients is necessary in order to prevent serious complications and invasive techniques are part of this process. Lung transplantation has also been a setting in which the eNose has been investigated. Invasive techniques are, at the moment at least, the only methods available to detect and monitor post-transplantation complications, such as organ rejection and infections, throughout the post-operative period; unfortunately, these invasive techniques are frequently associated with complications. Kovacs et al.[15] followed 16 patients receiving a lung transplant and compared them with healthy controls. When principal component analysis was applied, an eNose (Cyranose 320) was able to discriminate between the two patient groups with an accuracy of 73% (p<0.001), a sensitivity of 63%, and a specificity of 75%. These differences in the exhaled volatile compounds might be explained by the systemic or local airway changes ongoing in lung transplant receivers. An association between plasma levels of the immunosuppressant drug tacrolimus and the VOC patterns obtained from lung transplant recipients was also reported.

THE eNOSE AND INFECTIONS

Ventilator-associated pneumonia (VAP) is the most common infection in the intensive care unit that is associated with an increased risk of patient death. In a prospective comparative study, the ability of an eNose to identify VAP through analysis of bronchoalveolar lavage fluid was investigated in 44 VAP patients and 6 controls.[16] The eNose was able to correctly identify 77% of the VAP samples, with the accuracy being comparable with accepted microbiological techniques.

In an attempt to diagnose and classify acute respiratory distress syndrome (ARDS) based on pulmonary injury, inflammation, and bilateral rich pulmonary oedema, VOC patterns from mechanically ventilated intensive care patients were analysed within 24 hours of admission. Cases of ARDS were classified according to the Berlin definition as ‘mild’, ‘moderate’, or ‘severe’. A commercially available eNose (Cyranose 320) was trained using sparse-partial least square logistic regression with a 10,000-fold cross-validation to select variables and limit false positives. A sensitivity of 91% and a specificity of 62% were obtained when discriminating between moderate and severe ARDS, and the control group confirmed the results by temporal external validation. Modestly accurate diagnostic results were found when attempting to discriminate between patients with ARDS and patients with pneumonia and cardiac pulmonary oedema (CPO), although the breathprints of patients with pneumonia and CPO could be differentiated from those obtained from patients with moderate or severe ARDS with a greater degree of accuracy.[17]

A small number of patients experiencing prolonged chemotherapy-induced neutropaenia were assessed through the analysis of exhaled breath to determine invasive aspergillosis.

The sensitivity and specificity were 100% and 83.3%, respectively. The high mortality rate of pulmonary aspergillosis can be reduced by timely diagnosis. eNose technology (Cyranose 320) could enable the detection of invasive aspergillosis at an earlier stage compared with available diagnostic tools by principal component analysis. VOC patterns from patients with prolonged chemotherapy-induced neutropaenia are different and can be detected via eNose technology.[18]

Active tuberculosis remains a major global health problem and many diagnostic techniques are not available in rural areas. A study has been carried out to determine the diagnostic accuracy of an eNose (DiagNose, C-it BV) when used to identify tuberculosis infection using exhaled breath. The proof-of-principle study[19] involved 30 patients and reported a sensitivity of 95.9% and specificity of 98.5%, whereas a validation study including 194 participants reported a sensitivity of 93.5% and a specificity of 85.3% when discriminating tuberculosis patients from healthy controls.

Breathprints from 64 paediatric patients suffering from cystic fibrosis (CF) were analysed by an eNose (Cyranose 320) in order to investigate potential differences when compared with samples from 21 patients with primary ciliary dyskinesia and 21 healthy volunteers, with statistically significant results being reported. Moreover, VOC patterns from CF patients with chronic *Pseudomonas aeruginosa* infection differed significantly from those obtained from non-chronically infected CF patients.[20]

Faecal VOC patterns from paediatric patients were studied by an eNose in order to investigate the potential detection of inflammatory bowel disease (IBD). Samples from IBD patients during both active disease and remission were compared with samples obtained from a control group.[21] Patients with IBD were further categorised according to whether the patients received a diagnosis of ulcerative colitis (UC) or Crohn´s disease (CrD). Smellprints from patients with UC and CrD could be discriminated from each other as well as from controls. In addition, UC samples could be differentiated from CrD samples during both active disease (sensitivity of 97%, specificity of 92%) and during clinical remission.

Smellprints from tracheal aspirates (TAs) were obtained from mechanically ventilated pre-term neonates and analysed by an eNose (Cyranose 320) in order to distinguish acutely infected from non-infected patients, as well as identify the presence of bronchopulmonary dysplasia (BPD).[22] VOC patterns from patients with laboratory-confirmed bloodstream infections were different from those without infection regardless of a positive ($p<0.0001$) or negative microbiological culture from the TA sample ($p<0.0001$). Smellprints from patients who had BPD were different from those without BPD. The authors conclude that the simple and rapid eNose technique could be a useful tool to determine VOC patterns in TA samples as a diagnostic marker in pre-term neonates.

LIMITATIONS AND CONCLUSION

Great expectations surround the potential utility of the eNose for the diagnosis of various medical diseases. Being a simple, non-invasive, and transportable technique that offers quick access to results, the eNose represents a compelling piece of technology from a medical research point of view. Published research would suggest that, during the early stages of various diseases, VOC patterns may play a key role in the phenotyping of patients. In addition, the eNose may play a role in offering a more effective, personalised approach to therapy in the future.

One of the main limitations of the eNose technique is the absence of a standardisation related to the different methods used in the collection of VOC samples and the generation of smellprints. Indeed, expiratory flow rate, breath-holding capacity, and anatomic dead space were studied to determine if the method of collection affected results obtained from exhaled breath samples from LC patients and controls. Differences were found between the results obtained using the two methodologies, mainly in the control group where VOC patterns might be influenced by the technique applied for sample collection.[23]

The interpretation of VOC patterns requires various statistical analysis and software applications, which is an important barrier that must be overcome in order for the technology to be embraced in future daily clinical practice. Small groups of patients with a known diagnosis have been included in the published eNose studies, but large-scale studies are needed in order to validate the different commercial eNose devices available, the stability of the biological samples obtained, and the reproducibility of intra and inter-laboratory sample measurements. Prospective

studies including population-based screening for respiratory diseases should be a key point in determining the potential applicability of the eNose to daily clinical activity.

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TREATMENT OF ISOLATED INTRACRANIAL PROGRESSION OF LUNG CANCER DURING TREATMENT WITH SYSTEMIC EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS (EGFR-TKIs)

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ABSTRACT

Background: Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are an effective treatment for non-small-cell lung cancer (NSCLC) harbouring *EGFR* mutations. The development of isolated central nervous system (CNS) metastases is a relevant clinical problem in patients who respond well to EGFR-TKIs.
Methods: We present a patient with isolated progression of brain metastases during treatment of *EGFR*-mutated NSCLC with an EGFR-TKI and review the treatment options in this setting, including the evidence for and toxicity of treatment with high-dose TKIs.
Results: Oligometastatic CNS progression during TKI therapy may be treated locally. Both whole brain radiotherapy (WBRT) and stereotactic brain irradiation are well tolerated and effective in this setting. The use of high-dose pulsed TKIs is intended to increase the concentration of TKI in the brain and has been reported to be effective and without significant toxicity in case reports and small case series. These therapeutic options are illustrated in the case of a 44-year-old NSCLC patient who developed CNS progression after WBRT during second-line erlotinib and was treated locally with stereotactic radiosurgery (SRS) and, upon further CNS progression, with high-dose pulsed erlotinib. This resulted in intracerebral response; however, significant haemorrhage also occurred. Severe haemorrhage has not previously been described as a complication of high-dose pulsed erlotinib.
Conclusion: Possible explanations for isolated CNS progression during TKI treatment include inadequate dosing across the blood—brain barrier and longer survival on TKIs. The efficacy and tolerability of high-dose pulsed TKIs for CNS metastases has been previously reported. None of the cases reported showed the severe haemorrhage and cerebral oedema that developed in our patient. Simultaneous anticoagulation as well as previous SRS may have predisposed our patient to haemorrhage and may prove to be relative contraindications to high-dose pulsed erlotinib. Most centres only see a few patients in this clinical situation, and co-operative efforts are needed to collect and analyse similar cases and to develop appropriate treatment strategies.

Keywords: Lung cancer, targeted therapy, tyrosine kinase inhibitor, brain metastases.

BACKGROUND

Advanced-stage lung cancer carries a very limited prognosis. Although many patients benefit from palliative systemic chemotherapy, resistance to chemotherapy generally develops within several months. Tyrosine kinase inhibitors (TKIs) that target mutations of the gene encoding epidermal growth factor receptor (EGFR) have become an established treatment option and can improve both progression-free survival (PFS)[1] and overall survival (OS),[2] in particular in patients whose tumours harbour deletions in exon 19 of the *EGFR* gene.[3] Nevertheless, resistance to EGFR-TKIs continues to cause disease progression.[4] The approach to progression during EGFR-TKI treatment varies depending on the rate and clinical significance of the progression, as well as the number and localisation of active metastases.[5] For patients with oligometastatic progression, local treatments with continuation of EGFR-TKIs may lead to an additional progression-free interval.[6,7]

Isolated central nervous system (CNS) progression presents a particular challenge and is a relevant clinical problem in patients who respond well to EGFR-TKIs. However, it is unclear whether the *EGFR* mutation itself predisposes to CNS metastases, to what degree incomplete CNS penetration of EGFR-TKIs plays a role, or whether the longer survival of patients responding to EGFR-TKIs exposes late steps in the natural history of the disease. There is evidence that *EGFR* mutations may predispose patients to CNS metastases. A Japanese case series[8] suggested higher rates of *EGFR* mutation in patients with brain metastases than in unselected patients; and a study of Korean patients with resected non-small-cell lung cancer (NSCLC) found a trend of increased rates of brain relapse in patients with *EGFR* mutation.[9] An association between *EGFR* mutation and risk of brain metastases was also reported by Shin et al.[10] in a recent analysis of 314 Korean patients with lung adenocarcinoma. In addition, in 629 European patients with lung adenocarcinomas tested for *EGFR* mutation, there was a trend to more brain metastases at first diagnosis in those with *EGFR*-mutated compared with wild-type (WT) tumours (19% versus 13%, p=0.078).[11]

Patients treated with EGFR-TKIs seem to develop CNS metastases more often than otherwise seen in advanced lung cancer. Omuro and colleagues[12] reported that the CNS was the initial site of recurrence in 7 of 21 patients with adenocarcinoma of the lung who had responded to gefitinib. Four of these seven patients did not have systemic disease progression.[12] An analysis of 287 Korean patients treated with erlotinib or gefitinib showed that 16% had progression in the CNS, and 7% had isolated CNS progression. Those patients who had responded well to EGFR-TKIs were at higher risk of developing isolated CNS progression (26% versus 4%, p<0.001).[13] CNS metastases are an important cause of death in patients with *EGFR*-mutation-positive lung cancer. A retrospective analysis of Chinese patients tested for *EGFR* mutation and treated with first or second-line EGFR-TKIs showed that patients who died of CNS metastases had undergone EGFR-TKI treatment for longer than patients who died of other causes (median time on EGFR-TKIs: 8 versus 1.9 months; p<0.0003). More patients with EGFR mutations (44.8%) died of CNS metastases than did *EGFR* WT patients (8.3%) (p<0.001).[14] It has been suggested that some *EGFR*-mutated patients with asymptomatic brain metastases can be initially treated with a systemic TKI alone.[15]

Further strategies for the treatment of CNS metastases include whole brain radiotherapy (WBRT), stereotactic radiosurgery (SRS), conventional chemotherapy, and alternate dosing of EGFR-TKIs including high-dosepulsed administration. Here we present a patient with isolated intracranial progression of an *EGFR*-mutated lung adenocarcinoma who was treated with WBRT, three courses of SRS and, finally, high-dose pulsed erlotinib, and discuss the relevant evidence in this setting.

METHODS

We analysed the course of a patient treated at our centre who developed isolated intracranial progression of CNS metastases during treatment of *EGFR*-mutated NSCLC with an EGFR-TKI. We reviewed the treatment options in this setting, including the evidence for and toxicity of treatment with WBRT and SRS during EGFR-TKI treatment and the use of high-dose pulsed EGFR-TKIs.

RESULTS

Clinical Case Presentation Part 1

A 44-year-old Caucasian female never-smoker presented with left arm weakness. Computed tomography revealed several brain lesions and a mass in the right lung. Positron emission

tomography showed metastases in the liver and right kidney. Following bronchoscopic biopsy, adenocarcinoma of the lung in clinical Stage 4 was diagnosed. Whole brain irradiation (2.5 Gy on weekdays to 35 Gy) and the first three cycles of chemotherapy (cisplatin 75 mg/m^2 and pemetrexed 500 mg/m^2 1 day every 3 weeks) led to a partial response; however, during the fifth cycle of chemotherapy the patient presented with pulmonary embolus, which was treated with tinzaparin, and progression of both the intrathoracic primary tumour and the abdominal metastases. Molecular analyses showed an exon 19 deletion in *EGFR*, and second-line treatment with erlotinib at 150 mg/day was initiated. This led to regression of all tumour sites. After 13 months of erlotinib, magnetic resonance imaging (MRI) showed isolated progression of two cerebral metastases with continued response of the systemic disease.

Review of the Literature

EGFR-TKIs in patients with brain metastases

EGFR-TKIs are able to cross the blood—brain barrier to some degree and their activity in CNS metastases from lung cancer was reported more than ten years ago.[16] Since then TKIs have been used widely in this setting, and complete responses of CNS metastases under TKI treatment have been reported.[17] Leptomeningeal metastases may also be treated with EGFR-TKIs in patients with *EGFR* mutations.[18] A retrospective analysis of patients with brain metastases treated initially with either a TKI or chemotherapy showed lower risk of CNS progression in those treated with TKIs compared with chemotherapy.[19] Gefitinib, erlotinib, and afatinib have all been reported to have activity against CNS metastases. While there has not been a direct comparison of the efficacy of these agents in this setting, individual case reports and small case series have suggested that some patients experiencing progression under gefitinib or erlotinib may benefit from a switch to another TKI.[20-22]

A recently published analysis of patients with CNS metastases treated with afatinib during a pre-market compassionate use programme showed significant rates of response to treatment with afatinib.[23] Patients included in this analysis were heavily pre-treated, receiving afatinib as a salvage treatment following progression after at least one course of platinum-based chemotherapy and at least one TKI. 35% of patients had a cerebral response, and 66% had cerebral disease control on afatinib. Further studies describing the treatment of CNS metastases with EGFR-TKIs are summarised in recent reviews.[24-26] Based on the available evidence, patients with *EGFR*-mutated NSCLC and asymptomatic CNS metastases should be treated with an EGFR-TKI. Progressive or symptomatic CNS disease may require additional treatment. The efficacy of third-generation EGFR inhibitors, such as AZD929127 and CO-168628, in patients with brain metastases and evidence of resistance mutations has yet to be determined.

Safety and efficacy of whole brain radiotherapy during treatment with EGFR-TKIs

Several studies and case series have investigated EGFR-TKI treatment during WBRT. Ma and colleagues[29] administered WBRT during gefitinib treatment. There was no unexpected toxicity and quality of life was found to improve during the course of treatment. The safety and efficacy of gefitinib with WBRT was also reported by Gow and colleagues[30] who found a trend towards improved OS in patients treated with simultaneous TKI+WBRT (OS: 22.3 months) versus WBRT alone (OS: 11.7 months, p=0.121). The safety of erlotinib and WBRT was reported by Lind and colleagues[31] in a Phase I dose-finding study combining erlotinib 100 mg/day and erlotinib 150 mg/day with WBRT. Expected rates of systemic erlotinib side-effects were observed. In contrast to the relatively good tolerability described by the previous authors, Olmez and colleagues[32] describe a small case series in which 3 of 8 patients treated with WBRT and EGFR-TKIs experienced unexpected clinical deterioration including altered mental status, hyponatraemia, and liver toxicity. The authors hypothesise that this may have been due to the poor general state of health and high tumour burden of these patients.

Several medium-sized trials have investigated WBRT+EGFR-TKI in patients not selected for *EGFR*-mutation status in an attempt to exploit the radiosensitising effects of EGFR-TKIs. Welsch and colleagues treated 40 patients with WBRT during treatment with erlotinib 150 mg/day. EGFR status was tested in 17 patients and found to be positive in 9. The authors describe good tolerability, with no significant neurotoxicity and no Grade 4-5 toxicity. The overall response rate was 86%.[33] Lee et al.[34] conducted a similar randomised trial of WBRT +/- erlotinib in predominantly *EGFR* WT lung cancer patients with brain metastases.

A PFS or OS advantage for concurrent erlotinib during WBRT was not observed, and patients treated with erlotinib during WBRT had more rash and fatigue but no decrease in quality of life compared with those receiving placebo.[34]

WBRT plus sequential focal radiation boost with image-guided intensity-modulated radiotherapy has also been investigated as a treatment strategy for NSCLC patients with brain metastases. Zhou and colleagues[35] describe 29 patients treated in this setting and report a 1-year intracranial control rate of 63%. Patients previously treated with EGFR-TKIs (11 of the 29 patients studied) had better survival; however, these patients also showed a higher incidence of Grade 2-3 cognitive impairment and Grade 2 leukoencephalopathy on follow-up MRI.

Safety and efficacy of stereotactic radiosurgery during TKI treatment

The combination of SRS with erlotinib was investigated by the Radiation Therapy Oncology Group (RTOG). The RTOG 0320 trial compared WBRT and SRS alone with WBRT and SRS with temozolomide or erlotinib in NSCLC patients with 1-3 brain metastases,[36] using erlotinib as a radiosensitising agent independent of *EGFR* mutation status. A total of 126 patients were enrolled and 41 were randomised to erlotinib plus WBRT and SRS. In this predominantly *EGFR* WT population OS was numerically higher in patients who did not receive erlotinib or temozolomide (13.4 months versus 6.1 months), although without statistical significance. Also, more Grade 3-5 toxicity was seen in patients treated with either temozolomide (41%) or erlotinib (49%) during WBRT and SRS compared with WBRT and SRS alone (11%) ($p<0.001$). In the erlotinib plus WBRT and SRS arm one patient had Grade 4 brain necrosis and Grade 5 haemorrhagic stroke.[36] A recent analysis of 282 Chinese patients treated with WBRT, SRS, and/or surgery combined with EGFR-TKIs showed that the simultaneous administration of EGFR-TKIs during WBRT and SRS was associated with improved OS, freedom from intracranial disease progression, and freedom from extracranial disease progression. This population included a high proportion of *EGFR* mutations (55 of 109 tested). Toxicity was not explicitly reported; however, the investigators conclude that combining EGFR-TKIs with local therapies is beneficial to this patient group.[37]

In conclusion, EGFR-TKIs combined with WBRT or WBRT+SRS in patients without *EGFR* mutations are not beneficial to unselected patients and may in fact be harmful. The combination may be beneficial to patients with *EGFR*-mutated tumours. There is no evidence that EGFR-TKIs should be stopped during WBRT or SRS in patients with *EGFR*-mutation-positive NSCLC showing good clinical response to TKIs.

Clinical Case Presentation Part 2

Our patient had already received WBRT at the time of first diagnosis, and so the decision was made to perform SRS of the two brain metastases with 18 Gy. This was well tolerated without significant toxicity. Four months later one further brain metastasis appeared and was also irradiated with 18 Gy. Unfortunately, 3 months later multifocal CNS progression developed. A neurosurgical biopsy confirmed the presence of the same exon 19 deletion as in the primary tumour, with no evidence of known resistance mutations, in particular T790M.

Review of the Literature

High-dose and pulsed EGFR-TKIs

Standard doses of erlotinib (150 mg/day) may result in clinically relevant cerebrospinal fluid (CSF) concentrations of both erlotinib and its active metabolite OSI-420, with a CSF penetration rate of approximately 5%.[38] Other authors have reported CSF penetration of approximately 1% (Jackman et al., 2006).[39] The pharmacokinetics and CSF concentration of EGFR-TKIs appear to be influenced by patient genetics. Fukudo and colleagues[40] measured the concentration of erlotinib in the serum and CSF of patients with leptomeningeal metastases and found the germline *ABCG2 421A* allele to be associated with increased CSF penetration of erlotinib and its active metabolite OSI-420. There is evidence that the administration of high-dose pulsed EGFR—TKIs results in improved penetration of the blood-brain barrier and increased concentrations of TKI in the CSF.

Increasing the dose of erlotinib from 75 mg/day to 150 mg/day results in an increase in serum concentration and a corresponding increase in CSF concentration.[41] Further increases in EGFR-TKI dose, as applied in pulsed high-dose TKI treatments, result in increases in both serum and CSF concentration.[39,42] These concentrations appear sufficient to treat CNS metastases

progressing under standard doses of EGFR-TKI. The safety of high-dose weekly erlotinib was reported by a Phase I/II study that treated NSCLC patients with chemotherapy resistance with 1,200 mg, 1,600 mg, or 2,000 mg erlotinib weekly.[43] The response rate was low, likely due to the inclusion of WT tumours; however, there was no Grade 4 or 5 toxicity and only one case each of Grade 3 fatigue, dehydration, and pneumonitis.

The use of high-dose pulsed EGFR-TKIs in *EGFR*-mutated tumours with CNS metastases has been reported in case studies and series. A case series published in 2011[44] reported on 9 patients with *EGFR*-mutant lung cancer treated with 1,500 mg erlotinib once a week for CNS metastases. There was a partial response in 4 of 9 patients (44%), disease stabilisation in 3 patients, and progressive disease in 2 patients with no Grade 3-5 toxicity. A second case series describes 10 patients with lung adenocarcinoma treated with 1,000-1,500 mg erlotinib once weekly.[45] The authors describe CNS response in 1 of 10 patients, with stabilisation of cerebral disease in a further 2 patients. These case series and case reports describing the use of high-dose and/or pulsed TKIs are summarised in Table 1.[45-49]

Clinical Case Presentation Part 3

Faced with multifocal CNS progression, our patient consented to a trial of high-dose pulsed erlotinib.

Table 1: Clinical response and toxicity during high-dose pulsed tyrosine kinase inhibitors: summary of case reports and case series.

Publication	n	*EGFR* mutation	Drug and dose	Disease control rate	Duration of treatment effect	Toxicity >Grade 3	Toxicity Grade 1-2	Haem-orrhage
Grommes et al.[44]	9	Exon 19 deletion (n=3), exon 19 insertion (n=1), exon 21 L858R substitution (n=4), exon 18 G719S/exon 21 L861Q substitutions (n=1)	Erlotinib 1,500 mg once weekly	7/9 (78%)	Mean time to CNS progression 2.7 months	None	Grade 1-2 rash, Grade 1 fatigue, diarrhoea, nausea, hair thinning, intratumoural CNS haemorrhage	Grade 1 in 3/9 (33%)
Jackman et al.[45]	10	Exon 19 deletion (n=6), exon 21 L858R substitution (n=3), exon 21 L858R substitution + *T790M* (n=1)	Erlotinib 1,000-1,500 mg once weekly	3/10 (30%)	Mean time to CNS progression 2.5 months	NR	NR	NR
Santhosh-Kumar et. al.[46]	1	Exon 19 deletion, no *T790M* mutation	Erlotinib 600-750 mg daily for 3 days on and 2 days off. Total dose 2,550 mg/week	1/1 (100%)	>12 months	None	Grade 1 rash and diarrhoea	None
Dhruva and Socinski[47]	1	NR	Erlotinib 600 mg every 4 days plus bevacizumab	1/1 (100%)	Treatment for 10 months	None	Skin and gastrointestinal toxicity	None
Clarke et al.[42]	1	*L858R* mutation and *T790M* mutation	1,000-1,500 mg/week	1/1 (100%)	Survival after CNS metastases 14 months	Hydrocephalus treated with VP shunt	NR	None
Hata et al.[48]	1	Exon 18 G719A	Erlotinib 300 mg/day on alternate days	1/1 (100%)	>6 months to CNS progression	None	Rash	None

Table 1 continued.

Publication	n	*EGFR* mutation	Drug and dose	Disease control rate	Duration of treatment effect	Toxicity >Grade 3	Toxicity Grade 1-2	Haem-orrhage
Yi et al.[49] Patient #1*	1	*EGFR*-mutation positive, details NR	Gefitinib 750 mg/day	1/1 (100%)	Survival after CNS metastases >18.6+ months	NR	NR	NR
Yi et al.[49] Patient #11*	1	EGFR-mutation positive, details NR	Gefitinib 500 mg/day	1/1 (100%)	Survival after CNS metasta-ses >8.6 months	NR	NR	NR
Jackman et al.[3]	1	Deletion exon 19	Gefitinib daily 500-1,000 mg	1/1 (100%)	Survival after high-dose gefitinib 4 months	Somno-lence, elevated hepatic transami-nases		None

* Yi et al.[49] describe various approaches to CNS metastases in 11 individual patients. Patient #1 and Patient #11 are presented separately within the paper and for that reason are presented as individual case reports in the table.
NR: not reported; EGFR: epidermal growth factor receptor; CNS: central nervous system.

Erlotinib pulses (600 mg every 4 days) were added to the daily treatment with erlotinib 150 mg. This resulted in a weekly erlotinib dose of 2,100 mg. After 2 weeks the patient developed increasing headache and arm weakness. MRI showed haemorrhage into three brain metastases and partial response of the other brain metastases (Figure 1). Erlotinib and anticoagulants were stopped, and due to a 6 mm midline shift the largest haematoma was evacuated surgically. After a period of post-operative recovery the patient resumed erlotinib pulses with a 25% dose reduction (450 mg every 4 days). There was no further haemorrhage; however, 24 days later cerebral oedema and subdural hygroma (Figure 2) developed and the patient died, 24 months after the initial diagnosis of brain metastasis.

DISCUSSION

We describe a patient with *EGFR*-mutation-positive lung adenocarcinoma and isolated intracranial progression during second-line treatment with erlotinib. If she had presented today, this patient would likely be treated with a first-line EGFR-TKI rather than chemotherapy. Large randomised Phase III trials of EGFR-TKIs have consistently shown higher response rates and longer PFS in *EGFR*-mutated tumours treated with TKIs compared with platinum-based chemotherapy.[1,4,50,51] An OS benefit for first-line EGFR-TKIs versus TKIs after progression on chemotherapy is less clear, as most clinical trials have addressed only the type and not the sequence of systemic therapy. It was recently shown that patients with exon 19 deletions show a clinically relevant survival benefit on first-line afatinib compared with first-line platinum-based chemotherapy.[2] Systemic therapy is the standard of care for most patients with Stage 4 NSCLC; however, patients presenting with oligometastatic disease, in particular with isolated CNS or adrenal metastases, may benefit from combined systemic and local therapies[53] including surgery[54] or stereotactic irradiation.[55]

While a neurologically asymptomatic patient may not have required specific treatment of the brain metastases during TKI therapy, our patient presented with arm weakness and would have been a candidate for WBRT during TKI treatment. The available literature shows the combination of WBRT and EGFR-TKI to be well tolerated. There is evidence that adding SRS to WBRT is beneficial to patients with 1-3 brain metastases,[56] and that SRS without WBRT may limit toxicity in these patients;[57] however, these studies

did not include patients treated concurrently with EGFR-TKIs.

Isolated progression of CNS metastases poses a challenge to the treating physician. Inadequate dosing of EGFR-TKIs across the blood—brain barrier is one explanation, and has led to attempts to increase the CNS dose of TKIs through the administration of high-dose pulsed TKIs.[52]

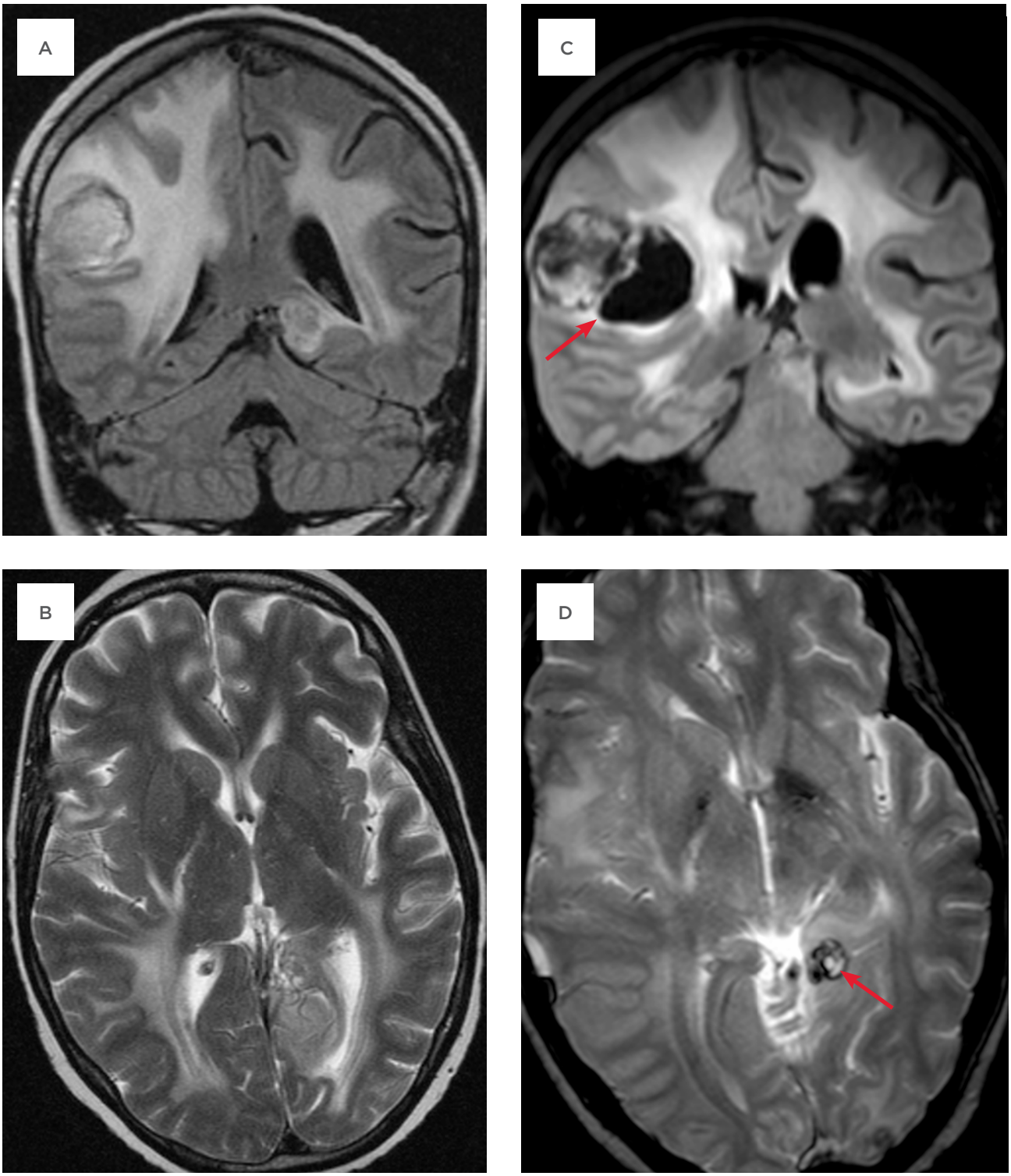

Figure 1: MRI scans showing brain metastases before and during treatment.
A: MRI image (FLAIR) before beginning high-dose pulsed erlotinib. B: MRI image (T2W) before beginning high-dose pulsed erlotinib. C: MRI image (FLAIR) showing haemorrhage (arrow) during high-dose pulsed erlotinib. D: MRI image (T2W) showing haemorrhage (arrow) during high-dose pulsed erlotinib. MRI: magnetic resonance imaging; FLAIR: fluid attenuation inversion recovery.

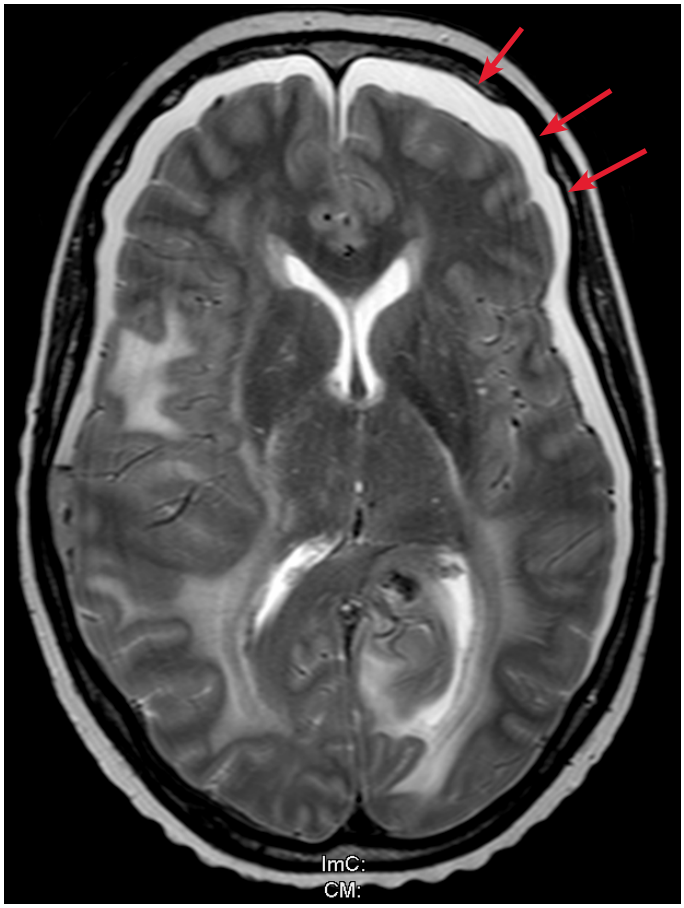

Figure 2: MRI image (T2W) showing hygroma development (arrows) following high-dose pulsed erlotinib.
MRI: magnetic resonance imaging.

This treatment strategy has not been evaluated in structured trials; however, evidence from published cases suggests that it may be effective and well tolerated. The absence of resistance mutations in biopsy material from our patient's brain metastases suggests that inadequate dosing, and not molecular resistance, may have been responsible for her disease progression. Had a *T790M* mutation been present our patient may have been a candidate for treatment with a third-generation TKI; however, to our knowledge none of the current trials of these drug candidates allow for the recruitment of patients with clinically unstable brain metastases.

Our patient responded to high-dose pulsed erlotinib but suffered significant haemorrhage. Simultaneous anticoagulation for pulmonary embolus as well as previous SRS may have predisposed her to haemorrhage, and may therefore be relative contraindications to high-dose pulsed EGFR-TKIs. Because most centres only see a few patients in this situation, co-operative efforts are needed to collect and analyse these cases, and to develop appropriate treatment strategies.

CONCLUSION

Patients with *EGFR* mutations presenting with asymptomatic brain metastases may be treated with a first-line EGFR-TKI. There has not yet been a direct comparison of the EGFR-TKIs in this setting; however, responses to one TKI after failure of another have been reported. Symptomatic brain metastases in patients with *EGFR* mutations may be treated with WBRT parallel to EGFR-TKI treatment. There is no significant evidence that the EGFR-TKI erlotinib has clinical utility as a radiosensitising agent during WBRT or SRS in patients with WT tumours. SRS combined with EGFR-TKIs and high-dose pulsed EGFR-TKIs may be considered in individual patients with isolated CNS progression; however, these treatments have yet to be adequately studied in clinical trials, and severe toxicity including haemorrhage is possible. Efforts should be made to include patients in

clinical trials. If clinical trials are not available, cases should be collected in regional registries in order to allow for a better understanding of toxicities and outcomes and to avoid the publication bias inherent with the publication of single cases of good treatment effect and low toxicity.

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FREQUENT EXACERBATORS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: FROM RESEARCH TO CLINICAL PRACTICE

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ABSTRACT

Acute exacerbations of chronic obstructive pulmonary disease (COPD) are major causes of morbidity and mortality and contribute to disease progression. The frequency with which COPD patients experience exacerbations can differ markedly between patients, even those with a similar severity of airflow obstruction. This has led to the concept of 'frequent exacerbators' that represent a unique phenotype of COPD patients who experience frequent exacerbations and have poorer outcomes compared with patients with infrequent exacerbations. However, the mechanisms whereby some COPD patients experience frequent exacerbations remain undetermined. Understanding the mechanisms of frequent exacerbations will lead to the development of new therapies that can be targeted to these high-risk patients, thereby reducing exacerbations and improving outcomes.

Keywords: Chronic obstructive pulmonary disease (COPD), exacerbations, frequent exacerbators, biomarkers.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and is the fourth leading cause of mortality worldwide.[1] The clinical course of COPD is characterised by a progressive decline in lung function and worsening health status that is punctuated by periods of increased symptoms that are termed 'acute exacerbations'.[2] Acute exacerbations are the major contributors to morbidity, mortality, and excess healthcare costs in COPD, and often result in unscheduled healthcare visits, excess medication use, and hospitalisations. Exacerbations also have long-term effects beyond the acute episode as they are associated with faster decline in lung function, airway and systemic inflammation, and impaired quality of life.[3,5] Therefore, prevention of exacerbations is a major therapeutic goal in COPD. Although acute exacerbations are common not all COPD patients have the same frequency of exacerbations, with some experiencing a higher than average number of exacerbations. This brief review analyses the evidence for a frequent exacerbator phenotype and reviews the recent studies that have investigated the effects of treatments in this group of COPD patients.

DEFINING FREQUENT EXACERBATORS

The term 'frequent exacerbators' was first used in 1998 in a study from the East London COPD cohort examining the effects of exacerbations on quality of life (QoL) in COPD patients.[5] The median number of exacerbations was 3 per patient per year, and this was taken as a cut-off point to divide the patients into infrequent exacerbators (≤2 exacerbations per year) and frequent exacerbators (≥3 exacerbations per year) groups. Using the St George Respiratory Questionnaire (SGRQ) patients with frequent exacerbations were found to have worse QoL compared with patients with infrequent exacerbations. The study also reported that frequent exacerbations in the

previous year and daily respiratory symptoms were predictive of frequent exacerbators. Since this initial publication there have been a number of studies examining the effects of frequent exacerbations, although not all these studies have used the same definition of frequent exacerbators.[4,6-8] The definition of frequent exacerbators has varied between studies as the definition of exacerbations themselves remains problematic. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines an acute exacerbation as an acute event characterised by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication.[9] This definition is adequate for clinical purposes but for epidemiological and research purposes it lacks precision. The assessment that a patient's symptoms are worse than usual fluctuations remains a subjective judgement both on the part of the patient and the healthcare provider. In addition, other medical conditions that commonly co-exist in COPD patients, such as congestive cardiac failure, pneumonia, and pulmonary embolism, also result in increased symptoms and therefore would be encompassed by such a definition.

Another major area of contention is the requirement for a change in medication. Some studies only include exacerbations that require treatment with antibiotics and/or oral corticosteroids, whereas others include all events experienced by patients irrespective of whether they require systemic treatment. In a cohort of COPD patients keeping daily diary cards of respiratory symptoms the rate of reported exacerbations was 0.8 per person per year, but the rate of exacerbations identified from the diaries was 2.7 per person per year.[10] Other studies have confirmed high rates of exacerbations that are experienced by patients but not reported to healthcare providers and are therefore not treated.[10,11] As a consequence, the rate of exacerbations in COPD patients will differ markedly depending on how exacerbations are defined and this in turn will influence which patients are defined as frequent exacerbators. Therefore, it is not surprising that there are discrepancies between studies on the prevalence and characteristics of frequent exacerbators that are highlighted in this review, when the definition of exacerbations is so variable. Symptom questionnaires such as the EXAcerbations of Chronic pulmonary disease Tool (EXACT)[12] and the COPD Assessment Tool (CAT)[13] are currently being validated as tools to provide more objective measures with which to define exacerbations. Future studies using such patient-reported outcome tools may lead to more objective definitions of exacerbations, further refinement of the definition of frequent exacerbators, and standardisation of definitions between different studies.

BIOMARKERS

The ongoing issues with the current subjective definitions of exacerbations have led to considerable interest in the role and utility of biomarkers. Identification of objective markers that can stratify COPD patients into either frequent or infrequent exacerbators offers the prospect of removing the uncertainty that surrounds the correct definition of exacerbations. A number of studies have identified relationships between inflammatory markers and exacerbation frequency in COPD patients. Six inflammatory biomarkers (C-reactive protein [CRP], white blood cell [WBC] count, interleukin 6 [IL-6], IL-8, fibrinogen, and tumour necrosis factor alpha) were analysed over a 3-year period in 1,755 COPD patients.[14] A subgroup of COPD patients (16%) demonstrated persistent systemic inflammation with ≥2 biomarkers (WBC count, CRP, IL-6, or fibrinogen) in the upper quartile distribution after 1-year follow-up, which was associated with an increased exacerbation frequency (1.5 versus 0.9 per year, $p<0.001$) and increased all-cause mortality (13% versus 2%, $p<0.001$). In a cohort of 6,574 COPD patients there was a stepwise increase in the risk of exacerbation from no baseline elevated biomarkers through to three high baseline inflammatory biomarkers: CRP (>3 mg/l), fibrinogen (>14 µmol/l), and WBC count (>9 x 10^9/l). The odds ratio (OR) for having frequent exacerbations in the first follow-up year was 3.7 (95% confidence interval: 1.9-7.4; 81 events/1,000 person-years) for individuals with elevated levels of all three biomarkers, compared with no elevated biomarkers (9 events/1,000 person-years).[15] Moreover, these markers identified an increased risk of exacerbation even in patients with no previous exacerbations. Frequent exacerbations are also associated with a greater increase in inflammatory markers over time. In a cohort of 148 patients with moderate to severe COPD, patients classified as frequent exacerbators (≥2.52 exacerbations per year) demonstrated a faster rise over time in baseline plasma fibrinogen and sputum IL-6.[3] Future studies will aim to determine the best

biomarker or combination of biomarkers, and how these can be best combined with clinical parameters to identify exacerbation risk in individual COPD patients.

EVALUATION OF COPD LONGITUDINALLY TO IDENTIFY PREDICTIVE SURROGATE ENDPOINTS

The largest and most comprehensive study of exacerbation frequency was from the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) cohort that enrolled 2,138 COPD patients and followed them over a period of 3 years.[16] Exacerbations were defined as events that led a healthcare provider to prescribe antibiotics or corticosteroids (or both), or that resulted in hospitalisation. Frequent exacerbators were defined as patients with ≥2 exacerbations per year, and infrequent exacerbators as those with <2 exacerbations per year. A relationship between exacerbation frequency and airflow obstruction was identified, with 22% of GOLD Stage II (moderate) patients experiencing frequent exacerbations compared with 47% of GOLD Stage IV (very severe) patients. However, disease severity is not the sole determining factor of exacerbation frequency, as up to a one-fifth of patients with moderate COPD experience frequent exacerbations and less than half of patients with very severe COPD are frequent exacerbators. The study also examined whether the occurrence of exacerbations remained stable over the 3-year study period. Of the 1,187 infrequent exacerbators during Year 1, 987 had <2 exacerbations in Year 2 (negative predictive value: 83%). Among the 492 patients with frequent exacerbations in Year 1, 296 had ≥2 exacerbations in Year 2 (positive predictive value: 60%). Thus, exacerbation frequency in the first year had a sensitivity of 60% and a specificity of 83% for the frequency in the second year. Over the whole 3-year study period 296 patients had frequent exacerbations in both Year 1 and Year 2, and of these 210 (71%) had frequent exacerbations in Year 3. There were 521 patients who had no exacerbation in both Year 1 and Year 2, and 388 (74%) of these also had no exacerbation in Year 3. Therefore, the authors suggest that the relative stability of exacerbation status over time warrants the description of a phenotype within COPD. The study also analysed the factors associated with frequent exacerbations and found that reduced lung function, poorer health status, a history of gastro-oesophageal reflux disease (GORD), and an increased WBC count were independently associated with frequent exacerbations.

OTHER STUDIES

Since the publication of the ECLIPSE study other studies have investigated the characteristics and prevalence of frequent exacerbators among COPD patients. Wan et al.[7] investigated 345 subjects with COPD GOLD Stages III and IV and defined frequent exacerbations as ≥2 exacerbations in the last year, or ≥4 exacerbations over the preceding 24 months with at least one exacerbation in the last year.[17] Significant clinical predictors of frequent exacerbator status were physician-diagnosed asthma, Medical Research Council dyspnoea score, and forced expiratory flow 25-75% predicted, but there was no relationship with current tobacco use, forced expiratory volume (FEV_1) percentage predicted, or previous exacerbation history. Another study of 121 patients with moderate to severe COPD defined frequent exacerbations as ≥2 exacerbations per year.[18] SGRQ total score and a course of oral corticosteroid within 3 months prior to the study together predicted best whether patients would be infrequent or frequent exacerbators over the course of the next year, but previous exacerbation history was not associated with exacerbation status. Unlike the ECLIPSE cohort there did not appear to be stability of exacerbator status as 58% of frequent exacerbators had <2 exacerbations in the previous year. A Chinese study of 227 patients identified lung function, comorbidities, and acute respiratory failure as predictors of frequent exacerbators.[19] A post-hoc analysis of the POET study[20] (Prevention of Exacerbations with Tiotropium in COPD) analysed data from 7,376 patients enrolled in a clinical trial of tiotropium. Patients were classified as non-exacerbators (63.5%), infrequent exacerbators (1 exacerbation, 22.9%), and frequent exacerbators (≥2 exacerbations, 13.6%). Similar to the ECLIPSE cohort, patients with frequent exacerbations tended to have more severe disease and treatment with ≥2 antibiotic or corticosteroid courses in the previous year. Other factors associated with frequent exacerbations were more pulmonary medication at baseline, female sex, and ex-smokers. Compared with ECLIPSE the proportion of patients classed as frequent exacerbators was considerably less (11.6% in GOLD Stage II patients and 15.6% in GOLD Stages III/IV). A database study of COPD patients in primary care in the UK reported that

20% of patients with FEV_1 >50% predicted and 30% of patients with FEV_1 <50% predicted had ≥2 exacerbations in the previous year.[21] By contrast, in a COPD cohort from Denmark, <2% of patients with FEV_1 >50% predicted and <10% of patients with FEV_1 <50% predicted had ≥2 exacerbations in the previous year.[22] An analysis of the patients in the ECLIPSE cohort who had different exacerbation frequencies from one year to the next failed to identify factors that could predict a change in exacerbator status.[23]

Therefore, different studies have had varying results with regards to the factors associated with frequent exacerbations, the stability of exacerbator phenotype over time, and the prevalence of frequent exacerbators in COPD populations. This is likely to be related to different definitions of frequent exacerbators, variations in study design and analysis, and different study populations. Despite these discrepancies the latest GOLD consensus report proposed a new staging system for COPD that incorporates exacerbation frequency as a component.[9] Whereas previously severity staging was based on lung function only, the new system characterises patients as high or low risk based on spirometry and exacerbation frequency. The high-risk patients (groups C and D) are defined as those with FEV_1 <50% predicted or ≥2 exacerbations in the previous year. It appears that this new staging system is not superior to the previous spirometry-based system in predicting hospitalisations and mortality,[24] one study has reported it to be inferior,[25] and therefore debate continues as to its validity.[26]

CONSEQUENCES OF FREQUENT EXACERBATIONS

Studies have identified associations between frequent exacerbations and a number of important outcomes in COPD, including faster decline in lung function,[4,27,28] impaired health status and depression,[5,7] reduced activity,[29] bacterial colonisation,[30] and systemic[3] and airway inflammation.[3,31] In the POET study frequent exacerbators constituted 13.6% of the population but accounted for 56.6% of exacerbation-related hospitalisations.[20] Thus, these studies highlight associations between frequent exacerbations and poor outcomes but they are unable to identify the direction of the relationship. It is not clear whether frequent exacerbations cause these outcomes, whether these factors contribute to the occurrence of frequent exacerbations, or whether they are markers of a COPD phenotype with no causal relationship. This has important clinical implications: if exacerbations cause poorer outcomes, then targeting these patients to reduce exacerbations will have therapeutic benefits.

THERAPEUTIC IMPLICATIONS

It is well established that inhaled therapies for the management of COPD, including inhaled corticosteroids (ICS), long-acting beta-2 agonists, and long-acting muscarinic antagonists (LAMAs) reduce the frequency of acute exacerbations.[32,33] Most of these studies were published prior to the description of frequent exacerbators and therefore it is not known whether the effects of treatments differ between patients with different exacerbation frequencies. Recent post-hoc analyses of previously published studies and new research have specifically examined the effects of treatments in subgroups of COPD patients according to exacerbation frequency.

ROFLUMILAST

Phosphodiesterase-4 (PDE-4) is an enzyme expressed in a range of inflammatory cells that catalyses the breakdown of cyclic adenosine monophosphate (cAMP), a second messenger molecule with immunomodulatory activity.[34] Inhibition of PDE-4 results in higher levels of cAMP, which acts to suppress the activity of immune and inflammatory cells. In a small, crossover, placebo-controlled study the PDE-4 inhibitor roflumilast resulted in a 35-50% reduction in the number of inflammatory cells in sputum of COPD patients.[35]

A post-hoc analysis of pooled data from two placebo-controlled, double-blind, multi-centre studies has shown that roflumilast can shift patients from a frequent to an infrequent exacerbator phenotype.[36] Symptomatic subjects with GOLD Stage III or IV COPD were randomly assigned to receive roflumilast or placebo for 1 year in studies M2-124 and M2-125.[37] Participants did not use ICS or LAMAs during the study period but continued all other standard therapies and the annual rates of exacerbations were assessed at study entry and after 1 year of treatment. In total 3,091 patients were analysed of which 830 (27%) were frequent exacerbators (≥2 exacerbations in the previous year). In the frequent exacerbators treated with roflumilast, 32% continued to have frequent

exacerbations, compared with 40.8% of those taking placebo at the end of 1 year. The risk of remaining in the frequent exacerbator group when treated with roflumilast was reduced by 20% compared with placebo, a risk ratio of 0.799. This effect on exacerbations was most evident for those with GOLD Stage III COPD, although numbers were smaller in the GOLD IV group. An interesting observation from this study was that 59.2% of the frequent exacerbators who received placebo did not have frequent exacerbations over the year of the study, suggesting that exacerbation frequency can be influenced by non-pharmacological interventions such as frequent patient monitoring that was part of the study protocol. The GOLD guidelines recommend the use of roflumilast to reduce exacerbations in patients with chronic bronchitis and FEV_1 <50% predicted.[9]

AZITHROMYCIN

Azithromycin is a macrolide antibiotic that possesses anti-inflammatory and immunomodulatory properties in addition to its antibacterial activity. A randomised, placebo-controlled trial has evaluated the use of azithromycin in reducing frequency of exacerbations in COPD patients.[38] The investigators randomised 1,142 subjects to receive a daily 250 mg dose of azithromycin or placebo for 1 year in addition to their standard treatment. The median time to first exacerbation was greater (266 days versus 174 days, p<0.001) and the frequency of acute exacerbations was lower (1.48 versus 1.83 exacerbations per patient-year, p<0.001 in those receiving azithromycin compared with placebo. A Cochrane systematic review of seven randomised controlled trials published between 2001 and 2011 concluded that continuous use of prophylactic macrolide antibiotics resulted in a clinically significant reduction in exacerbation rates in patients with at least moderate COPD.[39]

The COLUMBUS trial[40] was a randomised, double-blind, placebo-controlled study from the Netherlands that examined the effect of macrolide treatment in COPD patients with frequent exacerbations (≥3 exacerbations treated in the previous year). A total of 92 patients were randomly assigned to receive 500 mg azithromycin or placebo three times per week for 1 year. There were 84 exacerbations in the azithromycin group compared with 129 in the placebo group. After adjustment for covariates, the exacerbation rate ratio of azithromycin compared with placebo was 0.58 (p=0.001). The median time with first exacerbation was also greater in the azithromycin group compared with placebo (130 days versus 59 days, p=0.001). Concerns remain regarding the emergence of antibiotic resistance with long-term antibiotic use and current international guidelines do not recommend use of prophylactic azithromycin in COPD.[9]

N-ACETYLCYSTEINE

Exogenous and endogenous oxidative stress plays a central role in the pathogenesis of COPD, and increased oxidative stress and oxidant—antioxidant imbalance has been demonstrated in COPD patients.[41] Reduced levels of the antioxidant glutathione have been associated with increased risk of COPD exacerbations.[42] N-acetylcysteine (NAC) is a mucolytic agent that also has antioxidant effects by acting as a precursor of the antioxidant glutathione and a free radical scavenger.[43] NAC has been shown to reduce markers of oxidative stress in COPD[44] and a number of studies have evaluated its effect on clinical outcomes in COPD. The Bronchitis Randomised on NAC Study (BRONCUS) was a 3-year, double-blind, randomised, placebo-controlled trial of low-dose NAC (600 mg once daily) in COPD subjects with ≥2 exacerbations in the last year. NAC had no effect on the annual rate of decline in FEV^1 or the number of exacerbations, but subgroup analysis suggested that the exacerbation rate was reduced in patients not treated with ICS.[45] A study of higher-dose NAC (600 mg twice daily) carried out in Chinese patients did report a reduction in exacerbations with NAC.[46] A subsequent post-hoc subgroup analysis analysed the effects of NAC separately in high-risk (GOLD categories C and D) versus low-risk patients (GOLD categories A and B).[47] There were 89 patients classified as high risk of which 83.1% had suffered ≥2 exacerbations in the past year. In the high-risk group, the cumulative exacerbation frequency at 1 year was 1.08 with NAC, compared with 2.22 with placebo (p=0.04). Furthermore, mean time to first exacerbation was longer with NAC compared with placebo (258.2 days versus 203.6 days, p=0.02), and the proportion of exacerbation-free patients at 1 year was 51.3% with NAC compared with 24.4% with placebo (p=0.013). The effects of NAC on exacerbations remained significant if patients were stratified on the basis of frequent exacerbator

phenotype alone. The beneficial clinical effects of NAC were not seen in those patients classified as low risk.

ANTI-REFLUX TREATMENT

Symptoms of gastro-oesophageal reflux are common in COPD patients and associated with exacerbations;[48] the ECLIPSE study identified GORD as a risk factor for frequent exacerbations in COPD patients.[16] This association was also identified in a subsequent independent cohort of COPD patients.[49] The association would suggest that anti-reflux therapy may be beneficial in COPD patients if the relationship between GORD and exacerbations is causal, but to date there has only been a single study of anti-reflux therapy in COPD. In a randomised, observer-blind trial over 1 year 100 Japanese patients with COPD were randomly assigned to conventional COPD therapy or conventional therapy plus the proton pump inhibitor lansoprazole 15 mg daily.[50] The number of exacerbations was lower in those receiving lansoprazole compared with controls (0.34 versus 1.18, $p<0.001$) and the OR of having ≥1 exacerbation per year with lansoprazole compared with controls was 0.23 ($p=0.004$). However, there were imbalances in baseline characteristics between the subjects assigned to the two groups. Patients assigned to the placebo group had significantly more exacerbations per year (1.18±1.4 versus 0.34±0.72, $p<0.001$) and there were more patients with ≥1 exacerbations per year (52% versus 24%, $p=0.004$) compared with the treatment group. Therefore, the placebo group were at higher risk of exacerbation and the results may be attributed to this rather than a true therapeutic effect of lansoprazole. The lack of patient blinding also raises the possibility of a placebo effect. Larger double-blind studies with equal exacerbation risk between treatment arms are required to elucidate the role of anti-reflux therapy as an intervention to reduce exacerbations.

NON-PHARMACOLOGICAL THERAPIES

Non-pharmacological interventions such as pulmonary rehabilitation have beneficial effects on a number of outcomes in COPD including exacerbations, although this has not been reported in all studies. A Dutch group studied the effects of a comprehensive pulmonary rehabilitation programme on exacerbation frequency in 343 COPD patients with a high self-reported exacerbation rate (>3 exacerbations in the previous year).[51] The mean number of exacerbations decreased from 4.56±3.26 in the year prior to the pulmonary rehabilitation to 3.18±2.53 in the year following, with a significant decrease in hospitalisations. 69% of the participants had ≥2 exacerbations in the previous year, whereas during the year following pulmonary rehabilitation 54% of patients experienced frequent exacerbations. This was not a randomised trial and comparison was made with historical controls, thus it cannot prove conclusively that pulmonary rehabilitation reduces exacerbation frequency. Further randomised, prospective studies of non-pharmacological interventions in frequent exacerbators are warranted.

FUTURE DEVELOPMENTS AND RESEARCH

The description of a subset of COPD patients who experience frequent exacerbations has been widely accepted and incorporated into the updated GOLD system for COPD assessment. The description of this patient group as a phenotype implies the existence of an underlying mechanism (or mechanisms) determining susceptibility to exacerbations. The mechanism(s) of frequent exacerbations remain undetermined but possible candidates include susceptibility to respiratory infections, an altered respiratory microbiome, gastro-oesophageal reflux, psychosocial factors including symptom perception, depression and anxiety, and social support and medication adherence.[52] Although a number of studies have described the frequent exacerbator phenotype, no progress has been made in elucidating the underlying mechanisms. Such diverse potential mechanisms require radically different treatment approaches; therefore, the rational development of new therapies targeted at frequent exacerbators can only occur with an improved understanding of the underlying mechanisms. It is likely that there is not a single mechanism but that multiple mechanisms exist and that in the future the frequent exacerbator phenotype will be further refined according to underlying cause. Future research will need to examine each of these specific hypotheses to determine the key mechanisms of frequent exacerbations so that novel treatments targeting these pathways can be developed in order to reduce the burden of disease associated with COPD exacerbations.

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NERVES, COUGH, AND IDIOPATHIC PULMONARY FIBROSIS

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is the most common of the idiopathic interstitial pneumonias. It has a poor prognosis with a median survival of approximately 3 years, and whilst new therapies are finally beginning to offer hope of improved survival, most patients will require palliation of symptoms as their disease progresses. Whilst all patients with IPF complain of breathlessness, up to 80% develop a distressing cough, which is detrimental to their quality of life and difficult to treat. This article examines the possible causes of cough in the wider context of current theories of the pathogenesis of IPF and its associated comorbidities, which may also cause or exacerbate cough. We examine the evidence for increased cough sensitivity in patients with IPF and neuroplasticity in animal models of lung pathology. Finally, we discuss new therapies that are becoming available to treat cough in IPF and their possible mechanisms of action, and which highlight the need for further, appropriately powered studies that include objective measures of cough as an outcome.

Keywords: Idiopathic pulmonary fibrosis (IPF), pathogenesis, cough, therapy.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and invariably fatal lung disease and the commonest of the idiopathic interstitial pneumonias (IIPs). In the UK, the annual incidence is approximately 4.6 cases per 10,000 individuals and is increasing by 5% per annum,[1] a trend mirrored by hospital admission rates.[2] Patients who develop IPF usually present with symptoms of progressive breathlessness. Interestingly, up to 80% also complain of cough, which is either dry or productive of scanty amounts of clear sputum.[3] Cough as a symptom is most commonly associated with inflammatory conditions such as asthma, bronchitis, and viral infections affecting the airways where sensory innervation is extensive. However, cough is also prevalent in a number of IIPs. In some, such as hypersensitivity pneumonitis and sarcoidosis, it is likely to result from granulomatous inflammation of the airways, although sarcoidosis may also involve the upper respiratory tract, which has exquisitely sensitive innervation.[4,5] Superficially, it seems anomalous that a symptom such as cough should be associated with IPF - a disease process predominantly affecting the lung parenchyma where sensory innervation is relatively sparse.[6] In this regard, an analogy may be drawn with asbestosis, a fibrotic lung disease with a defined cause that resembles IPF in its clinical presentation and in which cough is also a common symptom.[7]

The histological pattern observed in IPF is termed usual interstitial pneumonia and is characterised by excess collagen and other molecules of the extracellular matrix (ECM) within the alveolar interstitium, together with a modest inflammatory cell infiltrate. These changes typically demonstrate spatial and temporal heterogeneity, giving the impression that the lung has been subject to recurrent micro-injuries.[8] Thus, areas of relatively normal lung are interspersed with areas of mild fibrosis, yet other areas show extensive fibrosis with subpleural, 3-10 mm mucin-filled cysts lined with bronchiolar epithelium, termed honeycombing.

The latter, which can be demonstrated using computed tomography (CT) scanning of the thorax, is typically worse at the posterior lung bases and spreads medially and cephalad as the disease progresses (Figure 1). Extensive disease is also characterised by subepithelial knots of mesenchymal cells and matrix situated at the interface between normal and fibrotic lung termed 'fibroblastic foci'.[8] This article will concentrate on what is known about cough in the context of current theories on the pathogenesis of IPF.

THEORIES ON THE PATHOGENESIS OF IPF

The precise pathogenesis of IPF remains unclear but an increasing body of evidence suggests that it arises as a result of recurrent micro-injury to alveolar epithelial cells (AECs). It is known that injured AECs release mediators including platelet-derived growth factor and transforming growth factor-beta (TGF-β), which stimulate interstitial fibroblasts to proliferate into myofibroblasts and secrete ECM. Furthermore, these myofibroblasts themselves secrete TGF-β, which has a number of effects. Firstly, it causes apoptotic cell death of AECs, thereby perpetuating a process of aberrant wound healing. In addition, it can initiate epithelial—mesenchymal cell transition (EMT), whereby epithelial cells de-differentiate and take on characteristics of mesenchymal cells.

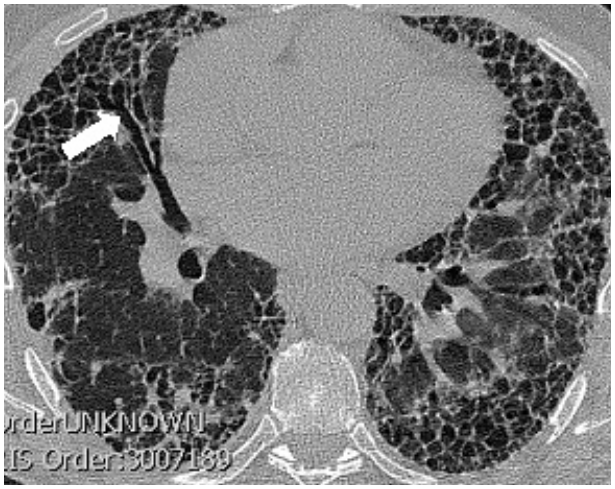

Figure 1: Computed tomography scan of the thorax of a patient with idiopathic pulmonary fibrosis. There is extensive, sub-pleural honeycomb shadowing at both lung bases and traction bronchiectasis (arrow).

Myofibroblasts are also capable of secreting tissue inhibitors of metalloproteases, which have the profibrotic effect of impairing matrix degradation. Thus, TGF-β is a key mediator in the crosstalk between epithelial cells and the mesenchymal cells responsible for matrix deposition and fibrosis of alveolar walls. For a more detailed review of these mechanisms see publications by Kage and Borok,[9] and Fernandez and Eickelberg.[10]

Genetic susceptibility is also thought to play a role in the pathogenesis of IPF, and mutations in genes encoding the surfactant proteins C and A2 as well as those maintaining telomere length are now known to be associated with IPF.[11-13] Familial IPF, defined as disease occurring in two or more family members, is estimated to account for 3.5% of cases.[14] More recently, a common polymorphism in the promoter for the gene *MUC5B,* encoding the mucin 5B which is important in innate immune defence, has been noted to have a high prevalence in familial (34%) and sporadic (38%) IPF.[15] This is one of very few examples of a common variant with a very large genetic effect, since each copy of the mutant allele confers a 1.6 to 8.3-fold increase in the risk of developing IPF. Interestingly, the presence of this polymorphism, whilst associated with increased risk of developing the disease in a dose-dependent manner,[11] also confers improved survival.[16] Genetic variants in the *TOLLIP* gene, a regulator of the pattern recognition Toll-like receptors and the TGF-β Type 1 receptor, located on the same genetic locus as the *MUC5B* gene, may also be associated with IPF.[17]

The increasing evidence that susceptibility to IPF involves genes important in innate immunity has given new momentum to the theory that infectious agents in the lower respiratory tract (LRT) may contribute to the initiation or progression of IPF. Recently, the polymerase chain reaction has been applied to define the respiratory microbial flora and their genes, the 'microbiome' of IPF, and differences in the quantity and relative abundance of bacteria, specifically *Haemophilus, Neisseria, Streptococcus,* and *Veillonella,* have been observed.[18] There is also evidence that this greater bacterial burden is associated with rapidly progressive disease.[19]

A completely different hypothesis that attempts to link the histopathological features of IPF with recurrent stretch injury, caused by tractional forces on the lung parenchyma exerted by the pressure changes during breathing, was recently suggested

by Leslie.[20] It is known that these forces are greatest at the pleural surface of the lung bases where the lung is more susceptible to alveolar collapse, a location where IPF is first evident radiologically and usually most severe (Figure 1). Leslie argues that the combination of mechanical force and alveolar collapse may cause lines of sheer stress, which fracture the epithelial—mesenchymal interface. Such injuries would then lead to fibroblast activation and healing in a 'reticulum of repair', thus explaining why fibroblastic foci are not discrete entities but part of a three-dimensional meshwork within the peripheral lung.[21] Other factors contributing to such injury might include inherited surfactant deficiencies and an ageing lung modified by environmental agents such as cigarette smoke.

PATHOPHYSIOLOGY OF COUGH

The cough reflex plays a vital role protecting the respiratory tract from inhaled noxious agents. The sensory fibres forming the afferent limb of this reflex are located in the vagus nerve, which innervates the larynx, trachea, and branching points of proximal airways. The afferent part of the reflex involves rapidly adapting receptors (RARs) and slowly adapting, or unmyelinated, C-fibres. RARs are myelinated Aδ nerve fibres that have their cell bodies in the nodose ganglion and are exquisitely sensitive to changes in pH, osmolality, and mechanical stimulation. However, they lack transient receptor potential vallinoid 1 (TRPV-1) receptors and are thus not chemosensitive.[22] Stimulation of these fibres in anaesthetised animals evokes a violent cough response, illustrating the involitional nature of this primarily protective reflex.[23] Unmyelinated C-fibres have their cell bodies in the jugular ganglion and unlike RARs are responsive to chemicals including the TRPV-1 agonists capsaicin and bradykinin. Inhalation of these substances causes an unpleasant, itchy 'urge to cough' experienced in many disease states.

The afferent fibres emerge from the nodose and jugular ganglia to converge on sites in the nucleus tractus solitarius in the brainstem. From here they connect to neurons in the central respiratory generator, which co-ordinates the efferent motor response in the larynx, diaphragm, and intercostal muscles. However, neurons in the cerebral cortex can also influence cough that can, in part, be voluntarily induced or suppressed. These higher cortical sites can be demonstrated using functional magnetic resonance imaging following inhalation of capsaicin.[24] It is increasingly recognised that neuroplasticity, whereby nerves switch phenotype, can occur in a number of pathological processes. Animal models have demonstrated altered tachykinin expression in tracheal Aδ fibres following viral infection,[25] and allergic inflammation can induce a switch with respect to the TRPV-1 receptor.[26] How such neuroplasticity may have relevance to cough in IPF will be discussed in the following section.

COUGH IN IPF

When asked to describe their cough, patients with IPF report having a 'nagging desire to cough constantly' and 'never being relieved after coughing'.[27] A recent study using a validated cough counter showed that patients with IPF cough as frequently as patients with cough hypersensitivity syndrome and significantly more than asthmatics.[28] Interestingly, cough in IPF is more prevalent in never-smokers and patients with advanced disease, and there is evidence that it is an independent predictor of disease progression and may predict time to death or transplantation.[29] The cough of IPF is also known to be associated with a variety of adverse physical and social sequelae, which have a profound and deleterious effect on quality of life.[30] A major obstacle when studying the pathogenesis of cough in IPF is the frequency of confounding comorbidities, which can both independently cause cough and/or have been proposed as potential pathogenic mechanisms that may initiate or lead to the progression of IPF.[31] These are listed in Table 1 and discussed below.

Table 1: Common comorbidities in idiopathic pulmonary fibrosis.

Gastro-oesophageal reflux	Obstructive sleep apnoea
Sinusitis/upper airway cough syndrome	Emphysema
Angiotensin converting enzyme inhibitor therapy	

COMORBIDITIES

Gastro-Oesophageal Reflux Disease

The first objective evidence that gastro-oesophageal reflux disease (GORD) is associated with IPF came from a study that used oesophageal pH monitors to demonstrate that acid reflux into the distal oesophagus was greater in patients with IPF than controls.[32] A number of subsequent studies have confirmed this observation and it is now accepted that up to 80% of patients have acid reflux, which may be asymptomatic.[33]

It is increasingly appreciated that gastric refluxate may be gaseous as well as liquid and also contains pepsin, duodenal enzymes, and bile.[34] There is preliminary evidence that exposure of airway epithelium to pepsin can result in EMT as discussed earlier,[35] whilst in the presence of unconjugated bile salts, AECs have been shown to produce TGF-β.[36] A recent study found detectable pepsin in the bronchoalveolar lavage (BAL) fluid of 54 patients with IPF, but there was no difference in the mean BAL pepsin levels between clinically stable patients and those undergoing an acute exacerbation.[37] However, a subgroup undergoing an acute exacerbation had markedly elevated levels of pepsin suggesting that aspiration may be contributory in some cases. It is also noteworthy that in rodent models, chronic aspiration of pH-neutralised gastric fluid produced a similar pattern of injury to acidic gastric fluid, whilst aspiration of acidic solution (hydrochloric acid) did not, suggesting that if reflux does play a role, it is not the acidity of the aspirate that is important.[38] This is supported by data from a recent, interventional study in which high-dose acid suppression using proton pump inhibitors (PPIs) reduced acid reflux in IPF patients but paradoxically increased non-acid reflux and had no impact on cough counts.[39]

Obstructive Sleep Apnoea

A number of recent observations have suggested that IPF may be associated with sleep-disordered breathing. A single-centre study of 50 patients with IPF who had a high mean body mass index of 32.3 found that 68% had moderate or severe obstructive sleep apnoea (OSA).[40] A further study observed that one-third of patients with OSA reported symptoms of chronic cough.[41] Interestingly, GORD is common in OSA and proportional to its severity.[42] There is also strong epidemiological evidence linking these two conditions.[43] It is therefore possible that IPF, GORD, and OSA are associated in what has been termed a 'vicious triad' provoking cough.[5]

However, not all evidence supports this notion. A single-centre study of 54 patients with fibrosing interstitial lung disease, many of whom had IPF, used polysomnography and oesophageal probes to demonstrate that reflux was no more prevalent or severe in subjects with OSA when compared with subjects without OSA.[44] Thus, current evidence suggests that confounding comorbidities alone are insufficient to explain cough in all patients with IPF and other possible mechanisms should be considered.

INCREASED COUGH SENSITIVITY IN IPF

It is known that patients with IPF have enhanced cough reflex sensitivity to inhaled capsaicin and that induced sputum from patients with IPF contains higher levels of the neurotrophins nerve growth factor and brain-derived neurotrophic factor (BDNF) than controls.[45,46] Furthermore, immunohistochemical studies have demonstrated that bronchial epithelial cells, subepithelial mesenchymal cells, and alveolar macrophages in normal human lung stain for neurotrophins and their receptors. In IIPs, this expression is enhanced particularly in IPF (Figure 2), where fibroblastic foci express high levels of BDNF and its receptor.[47-49]

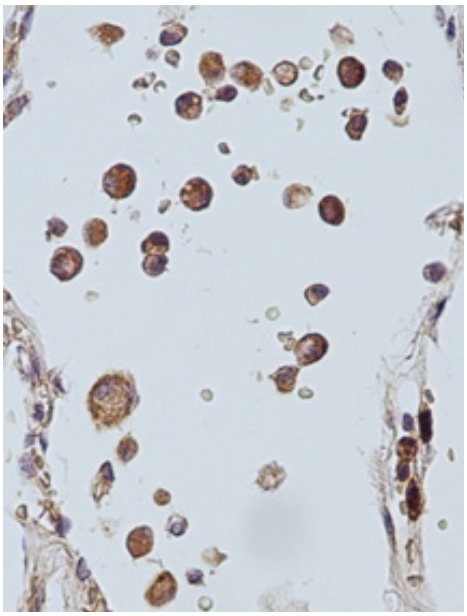

Figure 2: Alveolar macrophages in a lung biopsy from a patient with idiopathic pulmonary fibrosis show strong immunostain for nerve growth factor.

It has been proposed that these neurotrophins may induce the previously mentioned neuroplasticity by stimulating proliferation or differentiation of more proximal sensory nerves, resulting in increased cough reflex sensitivity.[5] Interestingly, a preliminary study of alveolar macrophages obtained from patients with IPF by BAL showed enhanced expression of messenger RNA for nerve growth factor when cultured *in vitro* with lipopolysaccharide compared with macrophages in culture medium alone (Figure 3).[49] These data, taken together with the aforementioned abnormalities in the microbiome of patients with IPF, provide a possible mechanism by which the LRT provides an environment that enhances upregulation of neurotrophins, neuroplasticity, and a lower cough threshold.

BIOMECHANICAL FACTORS AND COUGH IN IPF

It has long been recognised that histological abnormalities of IPF extend beyond the alveoli to include peribronchiolar fibrosis with resultant distortion of distal airway anatomy.[50] This is evident as traction bronchiectasis, which can be easily demonstrated on CT scanning (Figure 1). Commensurate with this observation and the previously discussed tractional injury hypothesis is the notion that mechanical distortion of the lung in IPF might directly alter sensory innervation either by increasing the number or sensitivity of mechanosensitive RARs, or conceivably, by destruction of nerves that inhibit the cough reflex. A recent study provided evidence to support this hypothesis by demonstrating that mechanical stimulation of the chest wall, particularly over the lung base where fibrosis is extensive, could induce a 'vibration cough' in patients with IPF but little or none in controls.[51] If this theory is correct, it would explain why patients with IPF cough when talking, as the enhanced transmission of vibration caused by phonation (as with tactile vocal fremitus) would increase mechanical stimulation of pulmonary sensory receptors. Indeed, vibration produced by the sound of cough might itself perpetuate further cough by a positive feedback mechanism.[5]

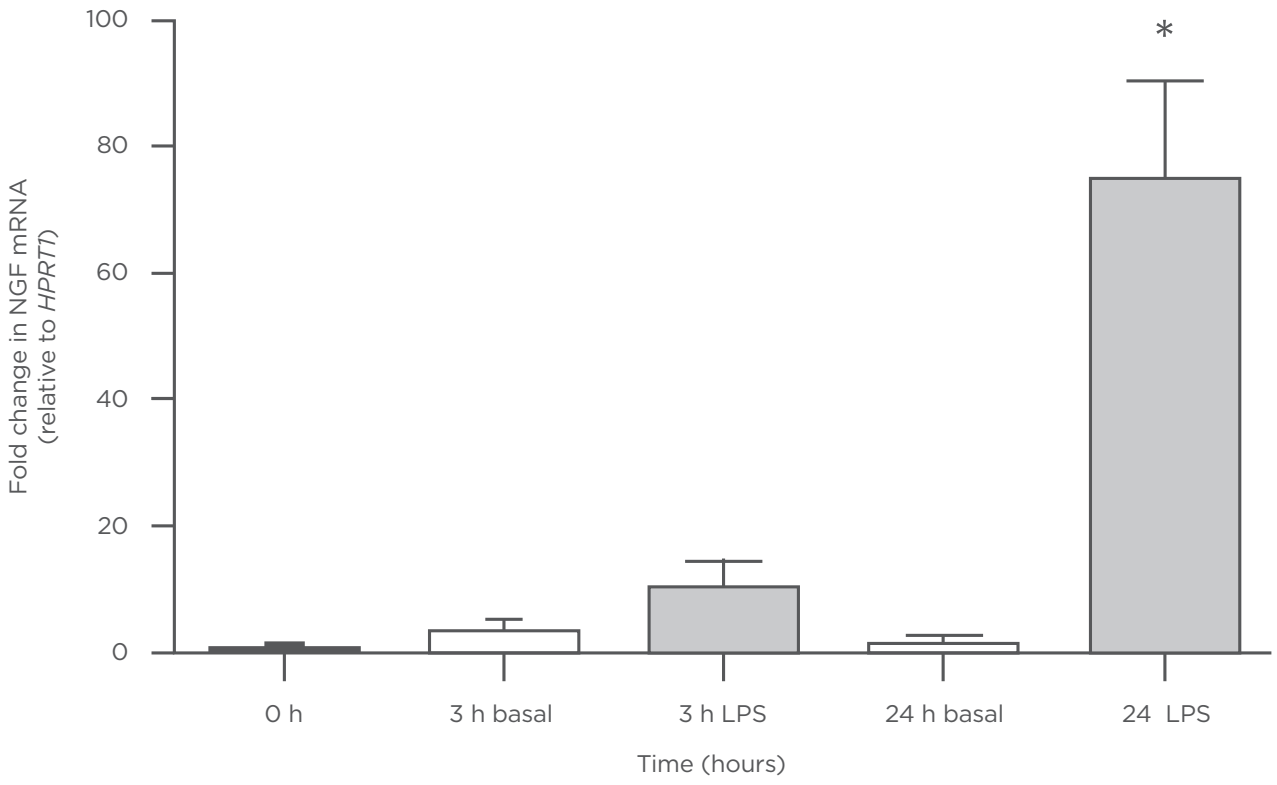

Figure 3: Alveolar macrophages from a patient with idiopathic pulmonary fibrosis show a time-dependant upregulation of messenger RNA (mRNA) for nerve growth factor (NGF) after stimulation with lipopolysaccharide (LPS).

*p<0.001.

Courtesy of Dr Mat Jones and Jones RM.[49]

TREATMENT OF COUGH

Treatment of IPF-associated cough is often unrewarding for the patient and clinician.[3] With regard to specific antitussive therapy, national guidelines recommend the use of opiate-based pharmacological agents, although these are often of only modest benefit and have unwelcome side-effects.[52] However, in patients with severe fibrosis and distressing cough, morphine can be an important part of palliative care. Clearly, it is important to address any of the possible comorbidities outlined in Table 1 that might be amenable to therapeutic intervention. In particular, if patients have symptoms of GORD, treatment with optimal doses of PPIs should be offered and supplemented if necessary with other antacid therapies and prokinetic agents. Interestingly, retrospective studies have shown prolonged survival in patients given antacid therapy; a recent study that analysed change in forced vital capacity (FVC) in patients assigned to placebo arms of three large randomised controlled trials found that patients taking antacid therapy at baseline had a smaller decrease in FVC at 30 weeks, compared with those not taking antacid therapy.[53]

It is also important to recognise that cough is a symptom that is notoriously susceptible to improvement with placebo, hence results of inadequately controlled clinical trials should be interpreted with caution.[54] A recent 24-week, double-blind, two-treatment, two-period crossover study showed a benefit of thalidomide as determined by visual analogue score, a cough specific questionnaire, and the St George's Respiratory Questionnaire.[55] These results are encouraging and it is interesting to speculate on the possible mechanisms by which thalidomide might exert its action. Whilst it is known that thalidomide has anti-inflammatory and anti-angiogenic effects, its side-effects include dizziness, peripheral neuropathy, and anti-cholinergic effects — actions which, taken with its apparent speed of onset, suggest that it could have a direct effect on pulmonary sensory nerves.

Until recently, there was no therapy proven to improve outcome in IPF. However, the anti-fibrotic agent pirfenidone and the tyrosine kinase inhibitor nintedanib have both been recently shown in randomised controlled trials to slow the rate of deterioration in lung function in patients with IPF, particularly those with relatively mild disease.[56-58] Whilst none of these studies included cough as an endpoint, one trial employed a simple, four-point cough severity score. When all patients were included, pirfenidone had no significant effect on cough. However, whilst post-hoc, subgroup analysis should be interpreted with caution, the authors identified a subgroup of patients whose cough appeared to benefit (i.e. deteriorate less) from high-dose pirfenidone.[59] Interestingly, pirfenidone has been shown to reduce cough in a guinea pig model of cough induced by ovalbumin sensitisation and capsaicin challenge. This effect was associated with a reduction in BAL prostaglandin E_2, leukotriene B_4, and substance P, all molecules thought to enhance cough sensitivity in the airways.[60] Less is known about the effect of nintedanib on cough. However, on comparing cough as a self-reported adverse event in the IMPULSIS trial, there was no statistical difference between those receiving nintedanib and those receiving placebo.

CONCLUSION

Whilst our understanding of the pathophysiology of IPF has improved greatly in the last decade, the precise mechanisms remain elusive. IPF likely involves a complex interplay between genetic factors, environmental insults, EMT, and crosstalk with mediators such as TGF-β. The contributory weighting of each component may vary with individual patients, but combined, these factors culminate in the distinctive, common pathological changes of usual interstitial pneumonia. Similarly, the distressing cough patients suffer may be a cumulative manifestation of a multitude of disease-related mechanical, biochemical, and neurophysiological changes within the lung, perhaps in some cases exacerbated by associated comorbidities. Recent trials of promising, disease-modifying drugs are now providing new hope for patients with IPF. Rigorous study of cough in IPF may help achieve more targeted and effective symptomatic therapies and provide further insight into its pathogenesis. Given that cough is such a ubiquitous and disabling symptom in this disease, it is essential that validated, objective measures of cough are included as outcome measures in future clinical trials.

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ESTABLISHED STATIN USE REDUCES MORTALITY FROM COMMUNITY-ACQUIRED PNEUMONIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Background: Statin therapy (ST) has been associated with improved outcomes from sepsis. Our objective was to systematically review the association between established ST and outcomes of patients with community-acquired pneumonia (CAP) that is severe enough to require hospitalisation.
Methods: Two meta-analyses were conducted following a search of articles published before 31st January 2013. After exclusions, seven studies were included to assess the effects of statins on 30-day mortality from CAP, and eight studies were included to assess the effects of statins on the development of CAP. Endpoints were a reduction in the risk of 30-day mortality or risk of developing CAP.
Results: A reduction in the risk of 30-day mortality from CAP was identified in patients established on ST (pooled odds ratio [OR]: 0.70, 95% confidence interval [CI]: 0.65-0.76; adjusted OR: 0.58, 95% CI: 0.47-0.69). The pooled OR for risk of developing CAP in patients with and without established ST was 1.01 (95% CI: 0.98-1.04).
Conclusion: There appears to be weak evidence to suggest a potential benefit of established ST. It is associated with a reduced risk of 30-day mortality in patients subsequently hospitalised with CAP. Further evidence is required, but ST could be considered as a means of reducing the risk of mortality from pneumonia.

Keywords: Statin, pneumonia, respiratory tract infection, bacterial infection, bacterial pneumonia.

BACKGROUND

Community-acquired pneumonia (CAP) is the most common infectious disease requiring hospitalisation in developed countries. CAP is associated with an in-hospital mortality of approximately 10% and a significant risk of intensive care unit (ICU) admission (estimated to be 5.9%, with a subsequent in-hospital mortality of approximately 50%).[1] It represents a major public health threat in the developed and developing world.[2] Therefore, any beneficial adjuncts to traditional anti-microbiological therapy would be of great value in reducing its impact on both patient mortality and healthcare resources.

There has been much scientific interest in novel adjunctive therapy in the prevention and treatment of sepsis. Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) have been postulated to have pleiotropic effects that can modulate immune function.[3-5] Since their introduction and initial approval for lowering cholesterol in 1987,[6] the beneficial effects of statins on cholesterol have been demonstrated in many high-quality, landmark studies[7-9] and they are now an integral part of many international guidelines for prevention and treatment of cardiovascular disease.[10] If statins can be shown to be a useful adjunctive therapy in CAP, their introduction would hold significant benefits for drug development, as compared with the introduction of novel therapies.

Statins are already a widely used group of drugs, with over 60 million prescriptions each year in the UK alone.[11] They are inexpensive, readily available, easy to administer, display a well-established safety profile, and confer additional health benefits.

The mechanism(s) by which statins exert their beneficial effects in sepsis is not understood, but animal and *in vitro* experiments have provided some evidence that they reduce the levels of inflammatory cytokines, deactivate immune cells, and cause a reduction in the production of nitric oxide by cells. Statin therapy (ST) has been shown to reduce high sensitivity C-reactive protein, a clinical marker of inflammation and an acute-phase reactant produced in response to pro-inflammatory cytokines. These potential, adjunctive benefits in sepsis have been suggested in both animal models[12,13] and humans.[14-18] There is some evidence to suggest that such effects are mediated by an inhibitory effect of statins on isoprenoid synthesis.[3] This may be relevant in sepsis, where anti-inflammatory actions could reduce tissue damage and organ failure, thereby leading to improved clinical outcomes. It has been proposed that statins affect immune function in many ways, rather than via a particular mediator.[4]

Another theory relates to the effect of statins on low-density lipoprotein (LDL) receptors. There are some data to suggest a lower incidence of pneumonia in obese patients who have reduced levels of circulating pro-inflammatory cytokines (e.g. interleukin-6), i.e. a suppressed inflammatory response.[19] It has been postulated that this is because lipopolysaccharides (endotoxin) may be cleared more effectively if there are more adipose tissue stores. Proprotein convertase subtilisin kexin 9 (PCSK9) inhibitors increase cell-surface LDL and very low-density lipoprotein (VLDL) receptors, thereby improving the clearance of LDL, VLDL, and endotoxin. Experiments using PCSK9 knockout mice or PCSK9 antibody have shown a reduced inflammatory response, reduced endotoxin levels, and a lower incidence of sepsis. Perhaps more importantly, it has been suggested that polymorphisms in the *PCSK9* gene in humans are associated with a mortality benefit in patients with septic shock.[20] This could mean that by increasing cell-surface LDL receptors, statins will reduce circulating LDL levels and consequently dampen the inflammatory response. This could explain why starting a statin in the acute phase is unhelpful, whereas established statin use could be beneficial.

The clinical studies of statins in patients with a broad diagnosis of sepsis are very heterogeneous, largely observational and retrospective, and use very different endpoints (e.g. mortality, development of sepsis) and patterns of statin use. Some studies show no benefit of adjunctive ST in certain cohorts of patients.[21-24] This review includes studies in a less heterogeneous group of patients (CAP requiring hospitalisation) in order to investigate the potential adjunctive effects of established ST on two different but important outcomes.

METHODS

Data Source and Study Selection

The database search was limited to English language articles and those including human adults. Both MEDLINE and EMBASE databases were searched (before January 31st 2013) using the following terms:

- (ANTICHOLESTERAEMIC AGENTS) or LOVASTATIN or (HYDROXY-METHYLGLUTARYL-CO A REDUCTASE INHIBITORS) and each of:
- (PNEUMONIA: BACTERIAL or PNEUMOCOCCAL or RESPIRATORY TRACT INFECTIONS);
- (BACTERIAL INFECTIONS or BACTERAEMIA/ VIRUS DISEASES);
- (SEPSIS; SYSTEMIC INFLAMMATORY RESPONSE SYNDROME);
- (RESPIRATORY TRACT [DISEASES OR INFECTIONS] or RESPIRATORY SYSTEM)

Pertinent references from identified articles were retrieved.

Exclusion Criteria

Studies primarily including patients with:

i) Immunocompromise (e.g. transplant recipients) due to the potential influence on the proposed immunomodulatory effects of statins and a higher risk of atypical infections

ii) Post-operative infections

iii) Chronic obstructive pulmonary disease due to potential immunomodulatory effects

iv) Multi-organ failure and acute lung injury due to non-infectious causes

v) Sepsis with no respiratory cause

Inclusion Criteria

i) Studies in which ST was established prior to hospital admission (within 90 days)

ii) Primary studies with an endpoint of 30-day mortality from CAP or development of CAP requiring hospitalisation

Statistical Methods

The data from all of the eligible studies were pooled and used in two meta-analyses:

i) Established ST and 30-day mortality from CAP

Only studies with 30-day mortality data were included. Many studies used different endpoints that could not be pooled, e.g. 90-day or in-hospital mortality. Where possible the 30-day data were obtained from authors. The pooled data were analysed using a nonlinear mixed model (SAS Proc NLMIXED) to obtain an unadjusted and an adjusted odds ratio (OR), so as to account for any confounding variables. This models the log odds of death comparing statin and non-statin patients reported in the studies using 30-day mortality as an endpoint. It has been reported to be superior to a DerSimonian and Laird model as it specifically models the inter-study heterogeneity.[25] Each study was treated as a random variable. The overall adjusted OR included age, sex, and smoking status.

ii) Established ST and development of CAP

Data from eligible studies were pooled (number of patients hospitalised with CAP and prior established statin use). The statistical analysis used a random-effects meta-regression model (SAS Proc NLMIXED) to assess the effects of study-level covariates on the overall OR. Models not improved with the addition of covariates were estimated without covariates using a more simplified random effects model (DerSimonian and Laird).

We used the mixed model estimates of inter-study variance in order to assess study heterogeneity. The grades of recommendation, assessment, development, and evaluation (GRADE) system was used to establish the quality of the individual studies. This is important since it influences the interpretation of the results of the meta-analysis. The GRADE system helps to guide recommendation for clinical application of the use of statins in the circumstances studied.[26]

RESULTS

A total of 146 articles were identified using our search criteria, of which 116 were selected for further review. After excluding reviews, abstracts, comments, and letters, 23 studies were specific to pneumonia.[27-40] Of these, seven relevant studies reported the effects of statins on 30-day mortality from pneumonia[27-31,39,40] and eight studies reported the effects of statins on development of CAP.[32-38] These studies were included in the meta-analyses.

It is important to note that many of these studies did not provide data about the severity of CAP (e.g. those requiring ICU admission), the pneumonia itself (e.g. lobar, bilateral), or the micro-organism(s) responsible. The diagnosis of CAP in the individual studies was largely obtained from coding and the comorbidity scores varied between studies. A number of other studies were excluded from the meta-analysis because, despite their methodologies being similar, many lacked sufficient data for univariate and multivariate analyses.[39-42] Some studies lacked the correct endpoint (they used 90-day or in-hospital mortality), others lacked any breakdown of comorbidities or used very different and incomparable methodology. For some of these studies additional unpublished data were provided by the authors, which enabled these studies to be included.[28-31,39,40]

Established ST and 30-Day Mortality from CAP

Eleven observational studies were identified (Table 1) but, even after seeking additional data from authors, only five were suitable for inclusion in the meta-analysis to give an adjusted OR,[27-31] and a further two were suitable to contribute to an unadjusted OR. These two studies contained insufficient data to calculate an adjusted OR, the published adjusted OR used a different combination of covariates.[39,40] The remaining studies were excluded either because of insufficient published data,[41] a different mortality endpoint,[42,43] or an unusual study design.[44] The total number of patients included was 87,909 in the pooled adjusted OR and 126,461 in the unadjusted OR. The total number of patients who died was 19,885 (14,379 in the five studies included in the adjusted OR).

The unadjusted OR from the seven studies was 0.70 (95% confidence interval [CI]: 0.65-0.76) (Figure 1A). Nine different covariates were

considered in the adjusted analysis (age, sex, smoking status, ischaemic heart disease [IHD], heart failure [HF], hypertension, dementia, malignancy), but the overall adjusted OR only included age and sex. Of note, IHD was associated with a higher risk of mortality (OR: 2.5, 95% CI: 0.7-4.3) while diabetes and HF were associated with a lower risk of mortality (OR: 0.31, 95% CI: 0.03-0.59; and OR: 0.31, 95% CI: 0.12-0.49, respectively). The meta-analysis shows that in the five included studies the adjusted OR was 0.58 (95% CI: 0.47-0.69) (Figure 1B). The difference between the ORs in Table 2 and Figure 1 can be accounted for by the weighting of studies.

Table 1: Studies showing the effect of statin therapy on mortality due to pneumonia.

Study	Population studied	Design	n	Primary outcome	Results	30-day mortality rate	Evidence GRADE	Reason for exclusion from adjusted analysis
Included in adjusted and unadjusted meta-analysis:								
Mortensen et al.[27]	USA 1999-2002 Adults with CAP	Mc R C	787	30-day mortality	AOR=0.36 (0.14-0.92)	Statin 4.5% No statin 10.3% Overall 9.5%	Low	-
Majumdar et al.[28]	Canada 2000-2002 Adults hospitalised with CAP	Mc P C	3,415	In-hospital mortality (30-day data obtained)	AOR=1.10 (0.76-1.60)	Statin 8.6% No statin 10.0% Overall 9.9%	Very low	-
Myles et al.[29]	UK 2001-2002 Adults with discharge diagnosis of pneumonia	R C	3,709	30-day mortality	OR=0.25 (0.14-0.44) AOR=0.33 (0.19-0.58)	Statin 9.9% No statin 26.6% Overall 25.3%	Low	-
Douglas et al.[30]	UK 1995-2006 Adults in contact with GP in last 6 months	R C	9,073	6-month mortality (30-day data obtained)	OR=0.62 (0.47-0.81) AOR=0.67 (0.49-0.91)	Statin 5.9% No statin 10.0% Overall 9.1%	Low	-
Nielsen et al.[31]	Denmark 1997-2009 Adults hospitalised with CAP	R C CC	71,746	30-day mortality	AOR=0.73 (0.67-0.79)	Statin 11.3% No statin 13.3% Overall 13.1%	Low	-
Included in unadjusted meta-analysis:								
Thomsen et al.[39]	Denmark 1997-2004 Adults hospitalised with CAP	R C CC	C: 29,900 CC: 2,692	30-day and 90-day mortality	Cohort: OR=0.63 (0.54-0.75) Case control: OR=0.63 (0.51-0.78) AOR=0.64 (0.52-0.8)	Statin 10.3% No statin 15.7% Overall 15.5%	Low	Insufficient data available to use covariants
Mortensen et al.[40]	USA 1999-2000 Adults hospitalised with CAP/influenza	R C	8,652	30-day mortality	OR=0.54 (0.42-0.7) AOR=0.57 (0.45-0.73)	Statin 5.0% No statin 11.0% Overall 9.9%	Very low	Insufficient data available to use covariants
Excluded studies								
Chalmers et al.[41]	UK 2005-2007 Adults hospitalised with CAP	P C	1,007	30-day mortality	AOR=0.46 (0.25-0.85)	-	Low	Insufficient data available

Table 1 Continued.

Study	Population studied	Design	n	Primary outcome	Results	30-day mortality rate	Evidence GRADE	Reason for exclusion from adjusted analysis
Yende et al.[42]	USA 2001-2003 Adults hospitalised with CAP	Mt P C	1,895	90-day mortality/risk of severe sepsis	AOR=0.74 (0.48-1.24)	-	Low	Endpoint is not at 30 days
Rothberg et al.[43]	USA 2003-2005 Adult. Discharge diagnosis of pneumonia	R C	121,254	In-hospital mortality	AOR=0.86 (0.79-93)	-	Low	Endpoint is not at 30 days
Sever et al.[44]	UK cohort of ASCOT RCT Adults assigned statin after closure of ASCOT-LLA	R (RCT data)	2,434	Mortality due to infection or respiratory illness	RRR=36%; p=0.04	-	Very low	Cross over design; endpoint is not at 30 days

Mc: multi-centred; R: retrospective; P: prospective; CC: case control; C: cohort; OR: odds ratio; AOR: adjusted odds ratio; RRR: relative risk reduction; RCT: randomised controlled trials; CAP: community-acquired pneumonia; LLA: lipid-lowering arm; GP: general practitioner.

Table 2: Studies showing the effect of statin therapy on development of community-acquired pneumonia.

Study	Population studied	Design	Primary outcome	n	Cases	Results	Evidence GRADE
van de Garde et al.[32]	UK 1987-2001 Adult diabetics. Controls and CAP	R CC	Development of CAP	142,175	4,719	OR=0.51 (0.37-0.68) AOR=0.49 (0.35-0.69)	Very low
Schlienger et al.[33]	UK 1995-2002 Adults: Controls and CAP	R CC	Development of fatal pneumonia	134,262	1,253	AOR=0.71 (0.56-0.89)	Low
Smeeth et al.[34]	USA 1995-2006 Adults started on statins	P CC	Development of CAP	600,241	8,837	AOR=0.84 (0.74-0.95)	Low
Dublin et al.[35]	USA 2000-2002 Adults: Controls and CAP	R CC	Development of CAP	46,824	1,125	AOR=1.26 (1.01-1.56)	Low
Fleming et al.[36]	UK 1998-2006 Adults: Controls and CAP	R CC	Occurrence of acute respiratory infection	329,881	707	AOR=0.91 (0.73-1.13)	Low
Vinogradova et al.[37]	UK 1996-2005 Adults: Controls and CAP	R CC	Development of CAP	98,239	17,755	OR=0.78 (0.74-0.87)	Low
Nielsen et al.[31]	Denmark 1997-2009 Adults hospitalised with CAP	R CC	Development of CAP	780,054	70,914	AOR=0.80 (0.77-0.83)	Low
Novack et al.[38]	International 2003-2006 Healthy Adults	P RCT	Incidence of infections (pneumonia)	17,802	8,901	OR=0.80 (0.67-0.97)	Low

CAP: community-acquired pneumonia; R: retrospective; P: prospective; CC: case control; RCT: randomised controlled trial; OR: odds ratio; AOR: adjusted odds ratio.

Established ST and Development of CAP

Eight studies were identified (Table 2), all of which were included in the analysis.[31-38] These represent 114,211 cases of pneumonia in 2,149,478 patients. Models were not improved with the addition of covariates and so the final overall OR was estimated without covariates. The overall OR was 1.01 (95% CI: 0.98-1.04) (Figure 1C). Inter-study variance was estimated for all final models and in all cases estimates were not statistically significant (p>0.09).

Figure 1A: Effect of established statin therapy on risk of 30-day mortality from community-acquired pneumonia (unadjusted OR, 95% CI).

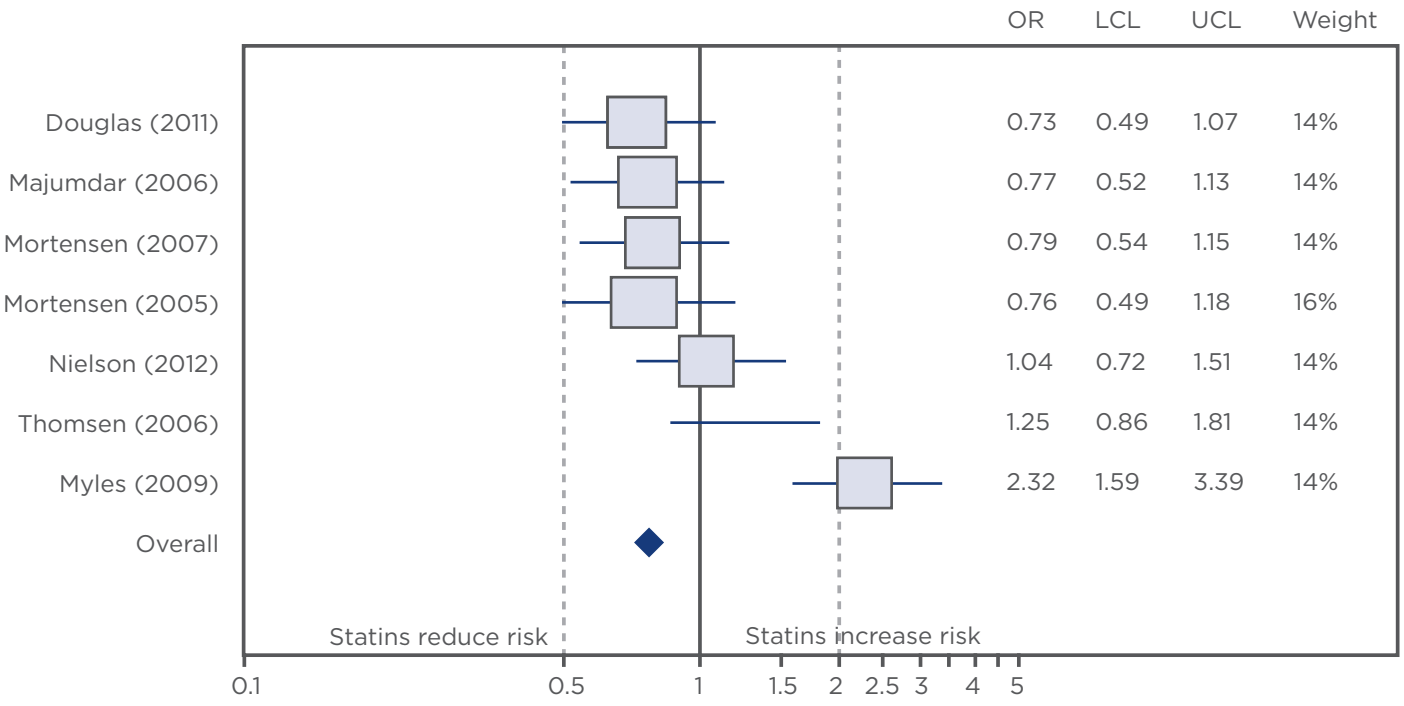

Figure 1B: Effect of established statin therapy on risk of 30-day mortality from community-acquired pneumonia (adjusted [sex and age] OR, 95% CI).

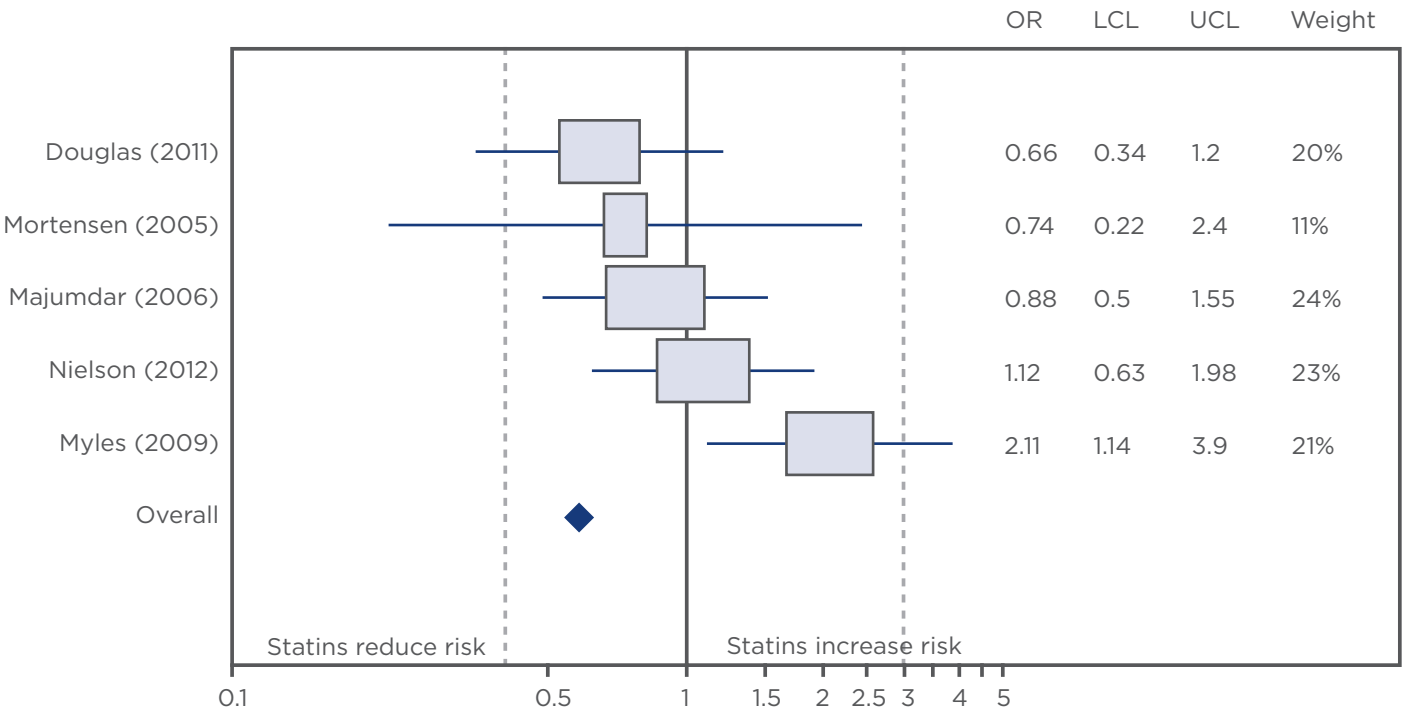

Figure 1C: Effect of established statin therapy on risk of developing community-acquired pneumonia (unadjusted OR, 95% CI).

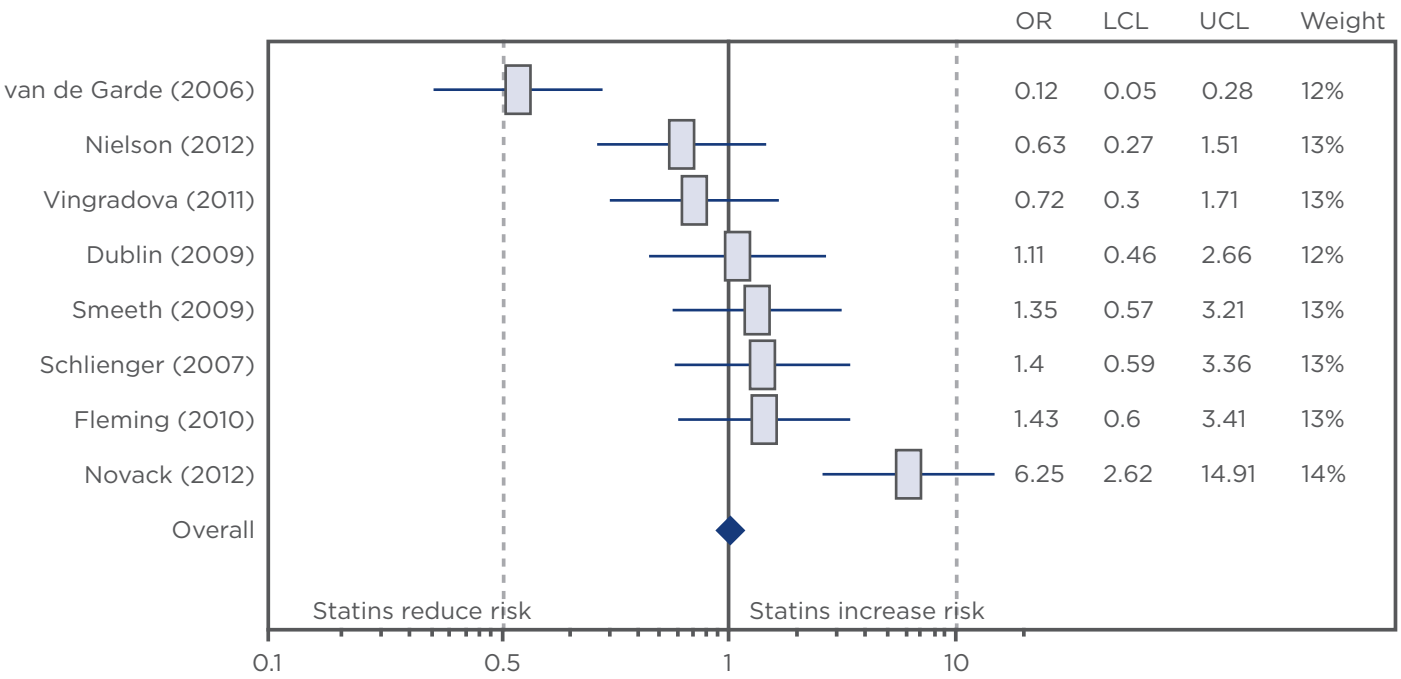

Figure 1: Forest plots to illustrate pooled odds ratios.
CI: confidence interval; LCL: lower control limit; OR: odds ratio; UCL: upper control limit.

DISCUSSION

There is an association between established ST and lower risk of 30-day mortality from CAP. The overall GRADE recommendation is weak because the quality of evidence is low or very low. However, the intervention (ST) does not influence or prevent the use of other treatments (except that certain drug–drug interactions may need to be avoided, e.g. clarithromycin). There is also an apparent lack of undesirable effects with the suggestion of some beneficial effects, i.e. there is at least reasonable scientific evidence to suggest that the benefits outweigh any potential risks. The usefulness of a meta-analysis of these heterogeneous studies is arguably questionable, although it does provide an estimate that is more precise due to its large number of patients, but it is prone to bias due to underlying differences in the constituent studies. The evidence from the individual studies does, however, support the trend that the meta-analysis suggests (Figure 1).

The studies excluded from the meta-analysis[41-44] report results are consistent with a mortality benefit from established statin use in patients hospitalised with CAP (Table 1). Only one study gave an adjusted OR, but this was of a similar magnitude to that obtained from our meta-analysis.[41] The two studies that used in-hospital mortality as an endpoint showed a lower adjusted OR, but this may have included patients with a variable length of stay.[42,43] The interpretation of the randomised controlled trials (RCTs) is very difficult due to large differences in study design and the lack of a pre-determined endpoint.[44]

Paradoxically, the OR is elevated (non-significantly) when IHD is included, which perhaps is in keeping with the idea that statins provide beneficial effects through a mechanism other than an effect on the cardiovascular system. The OR is reduced when HF is included, but with very wide confidence intervals around the ORs, suggesting that these could be confounding variables that lead to model instability. Since our aim is not to evaluate the individual predictors of the OR but instead to improve the overall predictive power of the model, we chose a simplified model including only age and sex as covariates, which were both important on their own and make sense from a biological perspective.

With regards to the second hypothesis, there is no evidence to suggest that established statin use is associated with a reduction in the risk of developing CAP that requires hospitalisation. The

pooled analysis shows that this is not significant and the quality of evidence was generally poor. There is no evidence from this analysis to suggest a recommendation for the use of statins as an adjunct for reducing the risk of developing CAP that requires hospitalisation.

There is little evidence available that addresses the concept of initiating ST in an already unwell patient. Critically ill patients with sepsis were randomised to receive either atorvastatin or placebo in a recent RCT.[45] The research group concluded that there was no statistically significant reduction in mortality with initiation of *de novo* statin treatment, but that continued use of atorvastatin therapy in established users was associated with a reduction in 28-day mortality. This is consistent with the results that we have found and could indicate that statins need time in order to exert any beneficial effects, and that established therapy utilises a mechanism of action that *de novo* therapy does not.

The concept of harm from statin use was not specifically addressed by any of the trials identified in this search. There was no declaration of adverse effects (AEs) secondary to statin use in any of the studies. This would be consistent with the known, very well-established side-effect profile that comes from their widespread use, and the fact that they are generally well tolerated. Their serious AEs appear to be minimal.

The studies exhibit some selection bias. Some include low numbers of patients with liver disease, a low rate of statin use in older patients, and a higher number of comorbidities in statin users.[28-30] The 'healthy user effect' is a potential source of bias and has been used as a reason to explain the positive impact of statins in studies that dispute these effects.[28] Some studies suggest that the universal access to healthcare and/or low-to-no cost prescriptions removed this effect.[39-41] However, if this were true, one would expect to see a similar decrease in mortality with other prescription drugs, but this is not consistently seen.

Importance of Statin Types

One would expect lipophilic statins (e.g. simvastatin, atorvastatin) to penetrate cell membranes more readily than hydrophilic statins (e.g. rosuvastatin) and consequently to elicit more pleiotropic effects,[3] thus supporting a theory that some of the cholesterol-independent effects of statins may be mediated by a reduction in circulating isoprenoid levels. The type of statin used varied between and within studies. One study claimed that simvastatin lowered mortality more than other statins,[39] while another did not disclose the drug used.[33] Doses were often not disclosed and when they were, they varied enormously.

LIMITATIONS

This review does have some limitations despite looking at a less heterogeneous patient population. The studies are largely observational, mostly retrospective, and have inherent weaknesses relating to data collection and identification of associations and not causality. There are some important inter-study variations that must be considered, e.g. exclusion criteria and country of study, which varied immensely (and have potential for huge variations in practice and criteria for prescription of statins).

There were also variations in data sources and quality and standards of care (e.g. in one study, only 50% of patients received antibiotics within 8 hours of arrival in hospital).[27] Many studies used general practice (GP) databases but even these varied, with some data originating from GPs completing once-weekly returns.[36] Two studies used the same data sources.[28,32]

The diagnostic criteria for CAP were similar, with many identifying their participants using coding data rather than a bacteriological diagnosis, potentially allowing misclassification. This is demonstrated by an abstract (excluded) that showed, on reviewing the data, only 108 of 200 had the correct diagnosis.[46] Escalation of care to ICU is not mentioned by the majority of studies and is therefore a major limitation in comparing mortality outcomes. Duration of pre-admission statin treatment was also extremely variable. This analysis has tried to compensate for this by defining statin use as within the previous 90 days.

Evidence from RCTs is lacking in this subject area. The JUPITER trial[38] looked at the effects of ST in previously well adults and suggested a benefit by reducing the risk of developing pneumonia. However, it was not designed to study this and relies on reports of respiratory infections by trial investigators. These may be incomplete, and therefore are graded as low quality. There have only been four other RCTs conducted, none of which look specifically at pneumonia.[47-50] Historically, it has been difficult

to recruit to RCTs, many have terminated early and consequently there are only a few registered - these are mostly inactive.

CONCLUSION

The evidence available seems to suggest that established statin use is beneficial as an adjunctive therapy to reduce the risk of 30-day mortality from CAP (weak recommendation), but that established statin use does not reduce the risk of developing CAP requiring hospitalisation. It would seem from the current evidence that long-term statin use could be beneficial. The questions that we really wish to know the answers to are yet to be accurately addressed in the literature: i) Are statins useful generic adjuvants in pneumonia, and ii) will giving statins to patients not ordinarily requiring them improve their chances of avoiding or surviving pneumonia?

One could propose that a long-term, placebo-controlled, prospective trial should be conducted that includes patients not previously prescribed statins and looks specifically at development of CAP and outcomes from CAP (e.g. hospitalisation, critical-care admissions, and mortality). However, a study would need to be very large to gain adequate power, as the incidence of CAP is approximately 5 cases per 1,000 individuals per year and the estimated reduction in incidence from observational studies is only 13%. Overall, there is potential for a reduction in risk of mortality from CAP in established statin users, but this is not consistently demonstrated in the studies. Ideally, more evidence is required, but it is difficult to conduct studies when there are so many confounding variables.

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DEEP NECK ABSCESSES COMPLICATING ACUTE FUSOBACTERIAL TONSILLITIS

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ABSTRACT

A case of deep neck abscesses due to *Fusobacterium necrophorum* in a young adult patient after therapy with phenethicillin is reported. The clinical manifestations, diagnostic methods, therapy, and outcome are described.

Keywords: Deep neck abscesses, *Fusobacterium necrophorum,* phenethicillin, amoxicillin/clavulanic acid.

INTRODUCTION

Deep neck suppurative inflammation is rarely encountered in the post-antibiotic era, but is the major life-threatening complication after throat infection.[1] It is characterised by a sore throat with fever, and painful swelling in the neck region caused by bacterial infection, most commonly by the commensal oropharyngeal flora. A multidisciplinary approach including surgical drainage in addition to intravenous antibiotic treatment is required. The prognosis is usually favourable, provided that the condition is recognised early. This report presents a case of an adult patient with peritonsillar abscess involvement of the parapharyngeal, submandibular, and retromandibular regions due to infection with *Fusobacterium necrophorum* after phenethicillin (phenoxyethylpenicillin) therapy.

CASE REPORT

A 23-year-old male presented to the emergency department with a sore throat and a painful swelling in his neck. For 5 days prior to admission the patient had experienced increasing swelling in the left side of his neck and pain when swallowing. He also had a fever, as well as difficulty in eating and drinking, as it was difficult for him to open his mouth. He had been receiving phenethicillin potassium 500 mg four times a day for 1 week, but the symptoms continued to worsen. Previous medical history recorded various interventions in the ear, nose, and throat area: a median palatoschisis and a Langenbeck palatorrhaphy in 1983, bilateral grommets insertion in 1990, and an adenoidectomy and pharyngoplasty in 1998. He had no prior history of tonsillitis.

Upon physical examination, the patient was found to have a blood pressure of 110/70 mmHg, a pulse rate of 80 beats/min, a temperature of 38.8°C, and no chills. There was a swelling in the neck measuring 4 x 5 cm in region II left. On palpation this was found to be a painful, large, fixed, elastic mass without fluctuations. Trismus with a mouth opening of 2 cm was noted. There were normal heart and breath sounds without adventitious sounds during auscultation. The abdomen was supple and without liver and spleen enlargement.

Oropharyngeal examination revealed bilateral tonsillar swelling with whitish yellow exudate. The left tonsil was larger than the right one. Evidence of a pharyngoplasty was visible in the throat. Flexible endoscopy revealed symmetrical movements of both halves of the larynx, and the lumen was not constricted. Laboratory findings showed a high white cell count of 21.1 x 10^9/l and a high C-reactive protein concentration of 343 mg/l. Levels of haemoglobin, electrolytes, liver enzymes, blood urea nitrogen, and creatinine were

within normal ranges. The monosticon test for the detection of heterophile antibodies was negative.

Given the elevated inflammatory parameters, together with the clinical picture, a parapharyngeal abscess was considered to be the most likely diagnosis. A chest radiograph was performed and the result showed clear lung fields. A neck computed tomography (CT) scan was carried out and showed an extensive abscess collection in the left submandibular and retromandibular spaces, extending from the tonsil. The left internal jugular vein was not thrombosed. As a final, supplementary examination, a panoramic radiograph (OPG) was taken in an effort to trace the source of the abscess. No dentogenic focus was documented. A left tonsillectomy was carried out together with drainage of the parapharyngeal abscess. The surgery went without complications. Phenethicillin was replaced by a combination of amoxicillin 1,000 mg and clavulanic acid 200 mg intravenously every 8 hours. The remaining clinical course was characterised by improvement.

The excised tonsil was sent for histological examination and the result was acute tonsillitis with no distinguishing characteristics. The pus was sent for culture and the Gram stain preparation was found to contain large numbers of leukocytes, moderately Gram-positive rods, and weakly Gram-negative rods. Colonies were grown on Brucella blood agar (Oxoid) and after 48 hours of incubation indicated anaerobic Gram-negative rods, with *Fusobacterium* spp. as the suspected agents. Attempts at phenotypic identification were unable to distinguish between the various species of the *Fusobacterium* genus and therefore a 16S ribosomal RNA (rRNA) sequence test was carried out. The isolate was confirmed as *F. necrophorum.* In addition, antimicrobial susceptibility testing was conducted according to guidelines established in 2014 by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[2] and confirmed that this microorganism was susceptible to penicillin.

DISCUSSION

F. necrophorum is an obligate anaerobic Gram-negative rod, and a microorganism resident in the human oral cavity. It was previously known as *Bacillus funduliformis, Bacteroides funduliformis, Sphaerophorus necrophorus,* and *Bacteroides necrophorus.*[3] It has been usually associated with Lemierre's syndrome (LS), septic internal jugular vein thrombosis, and disseminated metastatic abscesses in various organ systems, all preceded by a throat infection.[4] After Group A streptococci, *F. necrophorum* is the most common causative agent of peritonsillar abscess and recurrent throat infection.[5] In the current case, the patient had no past medical history of recurrent tonsillitis, but he had various interventions in the oropharyngeal area, which could be a predisposing factor for *F. necrophorum* infection. These procedures, usually performed during infancy, could impact negatively even after so many years and may imply an important role for innate immunity, although further studies are needed to confirm this.

Given the nature of *F. necrophorum,* the laboratory diagnosis of infection with this microorganism can be challenging. Analysis of the 16S rRNA sequence is a 'gold standard' molecular technique for identifying this species. However, matrix-assisted laser desorption ionisation time-of-flight mass spectrometry is now available, which makes it easier and cheaper to identify this species.[6]

The Dutch College of General Practitioners[7] and the Dutch Working Party on Antibiotic Policy[8] recommend that phenethicillin be administered as a first-line antibiotic for tonsillitis. Phenethicillin is the first semisynthetic penicillin with a narrow spectrum of antibacterial activity similar to that of penicillin G.[9] In an *in vitro* test, penicillin G had lower inhibitory activity than amoxicillin against clinical isolates of *F. necrophorum.*[10] Given the increasing prevalence of beta-lactamase-producing *F. necrophorum,* however, amoxicillin is no longer effective. In this case, preference is given to amoxicillin/clavulanic acid treatment. In the current case, the pathogen appeared sensitive to penicillin *in vitro,* yet an abscess still developed. The microbiological basis for phenethicillin treatment failure is not known. It seems to be more likely to occur in the setting of high bacterial burdens and low tissue permeability, leading to abscess formation.

In patients who are allergic to penicillin, other antimicrobial agents (e.g. clindamycin, azithromycin, metronidazole, and tetracycline) may serve as an alternative.[11,12] However, resistance to these regimens among *F. necrophorum* has been reported.[11] Notably, among tetracyclines, tigecycline seems to possess the greatest activity against *Fusobacterium* spp.[12]

The differential diagnosis of a sore throat with fever and swelling in the neck space may indicate

LS and Epstein–Barr virus (EBV) infection, especially in young adults. In this patient, a neck CT scan revealed no thrombophlebitis of the internal jugular vein, thus ruling out LS. Infectious mononucleosis could be ruled out too with a normal liver-function test and negative monosticon result. This test is less sensitive than the currently available serological test for antibodies specific for EBV antigens. However, the enzyme-linked immunoassay method is not available in all laboratories. Lastly, the possibility of Ludwig's angina should be considered, but this almost never occurs in combination with pharyngitis, in which a purulent inflammation fills various spaces in the submandibular region, caused by dental abscesses.[13] No dental focus was observed in the OPG, which meant that the possibility of Ludwig's angina could be eliminated.

In conclusion, an individual who has undergone various interventions in the oropharyngeal area may be more likely to suffer *F. necrophorum* throat infection. However, a further study is required to confirm this hypothesis. Clinicians prescribing phenethicillin to treat throat infection should be aware of the possibility of deep neck abscesses, and a change in therapy is indicated if there is no evidence of clinical improvement. A combination of amoxicillin and clavulanic acid should be considered when choosing initial empiric therapy for this group of patients.

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ADVANCES IN THE DIAGNOSIS OF HIV-ASSOCIATED TUBERCULOSIS

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ABSTRACT

HIV-associated tuberculosis (HIV-TB) remains a global public health challenge, with the major burden being borne by countries in low-resource settings. If World Health Organization targets to reduce TB deaths by 95% and new cases by 90% are to be met by 2035, major improvements in diagnostic strategies are among the most pressing needs. HIV coinfection presents particular challenges in the diagnosis of TB due, for example, to the relatively low mycobacterial burden in sputum specimens and rapid dissemination beyond the lungs. Low and middle-income countries still typically rely on traditional diagnostics such as chest radiology and sputum microscopy, which lack sufficient accuracy. Desired characteristics for an HIV-TB diagnostic test are well described and include the ability to test a wide variety of clinical samples, diagnose extra-pulmonary TB, have good accuracy to detect low mycobacterial burden disease, and be deployable at the peripheries of healthcare systems. Following a long period of under-investment in TB research, development of TB diagnostics has progressed rapidly over the past decade and the technology landscape looks much more promising. This article will summarise advances in diagnostics that are particularly relevant to HIV-TB. The Xpert® MTB/RIF and Determine™ TB LAM assays have the most evidence assessing their use in HIV-TB. In addition to nucleic-acid amplification tests and antigen detection we will review new diagnostic technologies. Finally, we discuss whether use of empirical TB treatment offsets the potential impact and reduces the need for new diagnostics.

Keywords: Tuberculosis, HIV, AIDS, *Mycobacterium tuberculosis,* diagnosis, Xpert MTB/RIF, nucleic acid amplification test, lipoarabinomannan, Determine TB LAM, antigen detection, urine.

INTRODUCTION

HIV-associated tuberculosis (HIV-TB) remains a major global public health challenge and is the leading cause of HIV-related mortality with approximately 1.1 million cases and 360,000 deaths in 2013.[1] Although Sub-Saharan Africa accounts for the majority of the global caseload (85%), focussed epidemics also occur elsewhere, for example, among intravenous drug users and prisoners in Eastern Europe, where there is a major overlap with high rates of drug-resistant TB and high mortality attributable to TB.[1-4] In patients living with HIV infection, the relatively low mycobacterial burden in sputum specimens and rapid dissemination beyond the lungs render diagnosis challenging.[5-9] Low and middle-income countries still typically rely on traditional diagnostic tests such as chest radiology and sputum microscopy, which lack sufficient accuracy.[10,11] Turnaround times for liquid culture (the most sensitive diagnostic test) are often too long to be clinically useful and the required laboratory infrastructure is very often not available in the highest-burden settings. Clinical suspicion and empirical treatment are widely used but can lead to under or over-treatment.[12,13] Data from hospital post-mortem studies show that up to half of HIV-TB remains undiagnosed at the time of death.[14-16]

Addressing the scale of the TB epidemic and accelerating progress towards elimination is the focus of an ambitious post-2015 Global Health Strategy announced by the World Health Organization (WHO) and partners.[17] If targets to reduce TB deaths by 95% and new cases by 90% by 2035 are to be met, major improvements in diagnostic strategies are among the most pressing needs.[17–19] WHO have also recognised the public health importance of HIV-TB coinfection and have recommended a series of 12 collaborative interventions to reduce the burden of HIV-TB and strengthen delivery of care;[20] better diagnostics are central to many of these interventions.

Desired characteristics for an HIV-TB diagnostic test are well described,[21] and include the ability to test a wide variety of clinical samples to detect extra-pulmonary TB (EPTB) in addition to pulmonary TB (PTB). Diagnostics need to be suitable for use at peripheral health units to screen those most at risk and intervene early in the disease. Point-of-care (PoC) assays are needed to enable diagnosis and treatment initiation in the same clinical encounter. Assays need to be amenable for use not only in adults but also in children, who are even less likely to be able to produce sputum and to test positive using smear microscopy.[22] Diagnostic tests also need to differentiate latent from active TB (due to differences in their subsequent management) but still be able to detect low mycobacterial burden disease. Obtaining and testing samples should be safe for patients and healthcare workers, even in resource-limited settings where biosafety equipment is often unavailable. Multi-drug resistant TB (MDR-TB), which is a leading challenge for TB control and for which HIV is a risk factor,[23,24] remains mostly undiagnosed and inadequately treated due to lack of access to drug-susceptibility testing. Therefore, new diagnostics should ideally be able to screen simultaneously for drug resistance using the initial diagnostic sample.

Following a long period of under-investment in TB research, development of laboratory diagnostics has progressed rapidly over the past decade and the technology landscape looks more promising (Figure 1). Advances include improvements in old diagnostic technologies such as sputum-smear microscopy and culture-based systems.[25,26] However, much progress has also been made with respect to development of new technologies, including rapid molecular tests.[27] This article will summarise advances in laboratory diagnostics that are particularly relevant to HIV-TB, focussing especially on recently developed technologies, but excluding radiological imaging techniques and advances in mycobacterial culture-based diagnostics. The enormous diagnostic challenges of TB drug resistance and TB diagnosis in HIV-infected children are important, but are beyond the scope of the present article. Since the vast majority of the HIV-TB burden is in resource-limited settings where TB diagnostic capacity is weakest, we provide considerable focus on solutions for these settings. Finally, we discuss whether use of empirical TB treatment offsets the potential impact and reduces the need for new diagnostics.

NUCLEIC ACID AMPLIFICATION TESTS

Nucleic acid amplification tests (NAATs) rely on the amplification and detection of nucleic acid sequences specific to *Mycobacterium tuberculosis* (MTB) complex. Early NAATs were developed for TB over 20 years ago, although limited test performance meant that these were not widely implemented. Development of newer NAATs, however, makes them one of the leading commercially available TB diagnostic technologies at present (Figure 1).

Line Probe Assays

Line probe assays (LPAs) use polymerase chain reaction (PCR) amplification of DNA for diagnosis directly from clinical samples or by testing culture isolates. Their use was endorsed by WHO in 2008, and they are capable of screening for genotypic drug resistance by detecting known genetic mutations.[29] A key disadvantage of LPAs is that they function best when testing samples with high bacillary load (smear-positive). The cost and complexity of LPAs also requires laboratory infrastructure and personnel capable of performing DNA extraction and PCR (rarely available in high-burden settings). Further advances in LPA technology now permit genotypic testing for resistance to some second-line TB drugs with moderate sensitivity and high specificity (GenoType MTBDRsl assay, Hain Lifescience, Germany).[30] Moreover, newer assays have lower limits of detection and can be used to directly test smear-negative sputum samples (MTBDR*plus* version 2.0, Hain Lifescience).[31–33] These have the potential to be used at reference laboratory level and screen for resistance to important second-line agents, especially in better-resourced settings and where drug resistance is more common.

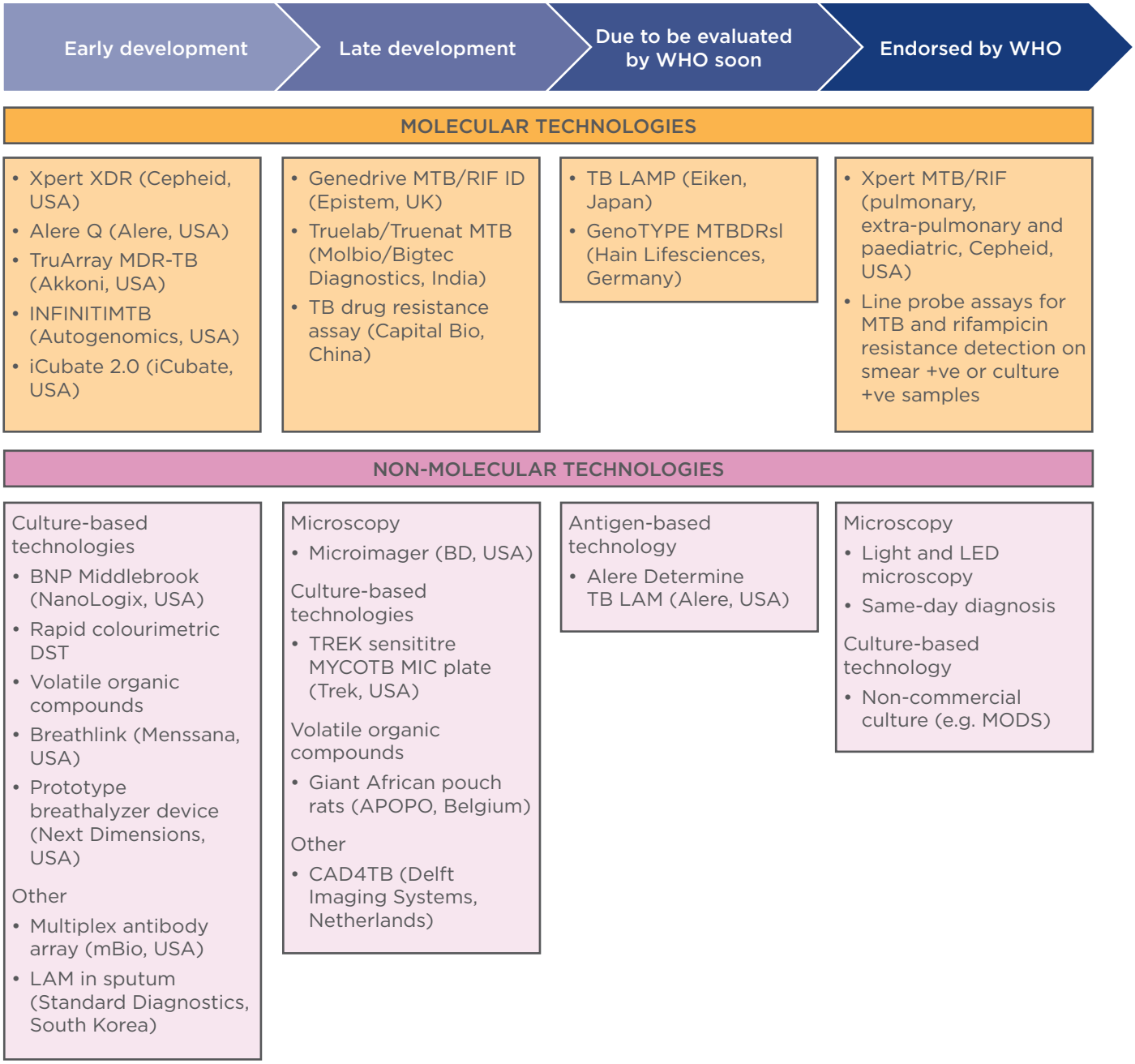

Figure 1: Current tuberculosis diagnostics pipeline listing examples of different types of diagnostics and their development phase (as of October 2014).

WHO: World Health Organization; LED: light-emitting diode; MODS: microscopic observation of drug susceptibility; LAM: lipoarabinomannan; MTB/RIF: mycobacterium tuberculosis/rifampicin; TB: tuberculosis.

Adapted from FIND, UNITAID,[27] and Geneva.[117]

Xpert MTB/RIF Assay

The next NAAT endorsed by WHO has been referred to as a 'game changer' in the field of TB diagnostics. The Xpert MTB/RIF assay (Xpert, Cepheid Inc., USA) is a self-contained, fully integrated, and semi-automated NAAT developed for relatively unskilled users.[34,35] Within a single-use cartridge, it uses hemi-nested real-time PCR and 'molecular beacon' technology to detect DNA sequences within MTB's RNA polymerase β subunit gene *(rpoB),* allowing diagnosis of MTB and genotypic detection of 95% of rifampicin (RIF)-resistant strains.[36–38] It was endorsed by the WHO in 2010 due to its diagnostic accuracy and several other positive attributes.[39] These included ease of use, a substantially reduced biosafety risk

compared with sputum-smear microscopy, and relatively short processing time (just over 2 h).[40,41] Initial recommendations were for Xpert's use as the primary diagnostic test for HIV-TB, or in settings where MDR-TB was suspected.[41]

The potential limitations of Xpert use in resource-constrained settings include the need for a stable electricity supply and operating temperatures, external quality assurance, regular calibration of instruments, and robust supply chains and storage facilities for Xpert cartridges.[42] On a health-systems level, challenges to implementation and scale-up include changes to diagnostic pathways, policies and notification systems, increased human resource needs, and increased capacity for investigating and treating suspected drug-resistant TB.[42] These logistical and health-system factors may mean that use of Xpert outside of the laboratory environment will be challenging, which may in turn reduce potential impacts of Xpert on patient outcomes.[43]

Several studies assessing the diagnostic accuracy of the Xpert for HIV-TB have been undertaken since the seminal multi-country assessment.[44] The pooled sensitivity of Xpert for diagnosis of PTB in HIV-infected individuals is 79% (95% confidence interval [CI]: 70-86%), and specificity is 99% (95% CI: 98-100%).[32] Sensitivity is 61% (95% CI: 40-81%) for sputum smear-negative TB, compared with 97% (95% CI: 90-99%) for sputum smear-positive disease.[32] Xpert's sensitivity is related to mycobacterial load, thus it is likely to be lower in patient populations with fewer symptoms and low rates of smear-positive disease, for example, screening asymptomatic HIV-infected patients.[42,45] This may explain the variability in sensitivity observed between studies.

Xpert MTB/RIF assay for non-respiratory samples

The high proportion of patients with HIV-TB who have EPTB provides another important diagnostic opportunity using this assay.[46] Systematic reviews have reported very high specificity of Xpert when testing a very wide variety of non-respiratory clinical samples,[47,48] despite the fact that culture is an imperfect reference standard for EPTB and may lead to underestimation of specificity.[49]

Overall sensitivity was high for smear-positive samples (97.4%, 95% CI: 95.5-99.3%)[47] and for all culture-positive samples tested (median: 83%, interquartile range [IQR]: 68-94%).[48] Sensitivity using a culture reference standard varied substantially between sample types, with good sensitivity observed in lymph node tissue (83.1%, 95% CI: 71.4-90.7%) and cerebrospinal fluid (80.5%, 95% CI: 59.0-92.2%), and poor sensitivity in pleural fluid (46.4%, 95% CI: 26.3-67.8%).[47] It is difficult to directly compare the diagnostic accuracy of Xpert for EPTB in HIV-infected and uninfected individuals due to paucity of data, but accuracy estimates did not differ substantially in studies with high proportions of HIV-infected patients.[47] WHO has now endorsed the use of Xpert for non-respiratory samples.[50]

Two studies have also assessed the Xpert's utility in diagnosing HIV-TB using urine samples. Urine was positive in 44.4% of outpatients with sputum culture-positive TB and CD4 count <50 cells/μl, and 47.8% of inpatients with culture-positive HIV-TB.[51,52] Diagnostic yield was higher among those with lower CD4 counts (<100 cells/μl) in both studies, suggesting positive urine samples represent disseminated TB in the sickest HIV-infected patients.

Xpert MTB/RIF assay in resource-rich settings

Few data exist on the use of Xpert in resource-rich settings, despite recommendations in guidelines and wide availability in European centres.[53,54] A study conducted in a university hospital TB clinic in Canada (low TB and HIV prevalence) reported only moderate sensitivity compared with liquid-culture reference standard (46% overall, 28% in smear-negative cases).[55] It was concluded that the potential for impact was minimal due to less extensive TB disease on admission and robust existing diagnostic algorithms. However, other researchers have suggested that using Xpert as the initial investigation for TB could reduce the use of empirical treatment, unnecessary contact investigations, in-hospital respiratory isolation, and length of hospital stay.[56–58] There is also potential impact when used as a 'rule-in' test,[59] for example in immunosuppressed HIV-infected patients, when TB is a common opportunistic infection and can be difficult to rapidly distinguish from non-tuberculous mycobacterial (NTM) disease and other bacterial infections. There is also potential benefit in settings where HIV-TB and MDR-TB overlap (for example prison populations and injecting drug users), as Xpert has demonstrated accurate detection of MTB and RIF resistance.[60] However, cost-effectiveness of the Xpert assay has not been proven in resource-rich settings, and this will depend on factors such as prevalence of HIV, MDR-TB, and existing laboratory infrastructure.[61]

Loop-Mediated Isothermal Amplification

The loop-mediated isothermal amplification (LAMP, Eiken Chemical Co, Japan) is another simplified laboratory-based NAAT that is under development for use in resource-limited settings. Up to 14 specimens can be batch-processed in 2 hours, and the assay detects MTB by rapidly amplifying DNA in sufficient quantities so that results can be read by simple visual determination of fluorescence (compared with positive and negative controls).[27,62] Although no specific large-scale evaluations have been undertaken for HIV-TB, sensitivity and specificity for PTB were 80% (95% CI: 86-93%) and 96% (95% CI: 95-97%), respectively, compared with culture reference standard.[63] Sensitivity is lower when testing smear-negative samples (53.8%), suggesting that it may be less useful in the context of HIV.[64] WHO endorsement was declined in 2013 due to 'inadequate evidence' and persisting concerns over sub-optimal specificity.[65] However, further evaluations are underway.

Emerging Nucleic Acid Amplification Test Technologies

Several 'fast follower' technologies to Xpert are emerging, although none have yet been endorsed by WHO (Figure 1).[27] These systems have several potential benefits over the Xpert. Many are smaller (often handheld), more robust, lower-cost, have lower power requirements due to isothermal amplification, and can operate from batteries. Genedrive (Epistem, United Kingdom) is a new molecular PoC PCR that can diagnose MTB and detect genotypic RIF resistance.[27] A single study reported sensitivity of 90.8% (95% CI: 81.0-96.5%) for MTB and 72.3% (95% CI: 59.8-82.7%) for *rpoB* mutations, although few smear-negative samples were assessed.[66] Truelab MTB (Mobilio, India) is a chip-based NAAT using a battery-powered device with sensitivity of 99.1% in smear-positive pulmonary TB, and 75.9% in smear-negative, culture-positive pulmonary TB, with 100% specificity.[27,67] If these PoC technologies demonstrate good sensitivity for specimens with low mycobacterial load then there is huge potential for their use in screening HIV-infected patients for TB, and large studies in high-burden settings will be warranted.

ANTIGEN DETECTION

Whereas immune-based assays of the host response to MTB are likely to be undermined in patients with HIV coinfection, direct detection of MTB antigens has long been recognised as having great diagnostic potential in such patients. Several different antigens have been detected in the urine of patients with pulmonary TB.[68,69] Urine has several advantages over sputum as a diagnostic medium, including relative ease of collection and lower biohazard risk during specimen handling. The most promising antigen to date is lipoarabinomannan (LAM), a major glycolipid component of the MTB cell wall, and commercial enzyme-linked immunosorbent assays detecting LAM have been available for a decade.[70,71] LAM is released from metabolically active MTB and is only detectable in those with active TB as opposed to latent infection. The mechanism by which LAM enters the urine of TB patients remains unproven, but possible mechanisms are free LAM entering the urine from the systemic circulation or direct infection of the renal tract with MTB.[72,73]

Urine Lipoarabinomannan Assays

Over the past 3 years, a simple and low-cost lateral-flow assay has become commercially available (Determine TB LAM Ag, Alere, USA). This test can be performed at the bedside by adding 60 µl of unprocessed urine to the test strip - the result is read after 25 minutes by comparing visible bands with the manufacturer's reference card (Figure 2). In contrast to other diagnostic assays for TB, urine-based LAM detection appears to have greater diagnostic accuracy in patients with HIV coinfection.[74] Sensitivity is <25% in HIV-uninfected populations, making it of little diagnostic use in this group.[74]

Several studies have assessed the diagnostic accuracy of urine-LAM testing for HIV-TB. Early studies reported a pooled sensitivity of 56% (95% CI: 40-71%) and specificity of 95% (95% CI: 77-99%).[74] A key observation is that diagnostic accuracy increases with more advanced degrees of immunosuppression.[72,75] This probably relates to higher mycobacterial load and more disseminated disease leading to greater probability of renal involvement.[15,76-78] Studies stratifying urine LAM by CD4 cell count have shown sensitivities of 56-85% among patients with CD4 <50 cells/µl.[79-85] Similarly, sensitivity has been found to be higher among hospital inpatients than among outpatients (58-67% versus 17-32%),[79-81,84-86] reflecting the strong relationship between higher sensitivity and greater disease severity.[87]

Some variation in specificity of urine-LAM assays has been reported between studies. Most report excellent specificity (96-100%), but some report lower specificities (86-89%).[75] The most likely reasons are: (i) inadequate reference standard using only sputum culture underestimates true prevalence of HIV-TB due to difficulty obtaining quality sputum samples and missing HIV-TB without pulmonary involvement;[88] (ii) using the 'Grade-1' cutoff for the TB LAM assay - a very faint band on the test strip that is difficult to read accurately.[75] Using 'Grade-2' rather than 'Grade-1' cutoffs for TB LAM improves specificity with only small decreases in sensitivity.[78,81] Consensus among investigators is that only 'Grade-2' cutoffs should be used,[89] and since January 2014 the manufacturer of TB LAM has removed the old 'Grade-1' cutoff from reference cards.[80] Disease due to NTM[90] or urine contamination with NTM could also theoretically cause false-positive results. The use of the urine LAM lateral-flow assay will be subject to a WHO expert review in 2015.

Urine Sampling May Increase Overall Diagnostic Yield

Urine-LAM testing identifies a different population of HIV-TB patients compared with sputum-based diagnostic assays, raising the possibility of combining them to screen for or diagnose HIV-TB. Studies using both urine and sputum-based diagnostics have demonstrated increased diagnostic yield compared to sputum alone, identifying up to 70-90% of diagnosable TB (Figure 3).[76,81,82,91] Similarly, urine LAM identified a different population to those treated empirically for HIV-TB.[52] In addition to increasing diagnostic yield, urine LAM appears to identify those patients who have higher mortality risk.[92,93] It is these patients who are most likely to benefit from interventions such as early commencement of TB treatment. By virtue of its low cost (approximately US$3.50 per test), technically simple operation, and rapid results, TB LAM appears to be well suited for use in low-resource settings with high HIV-TB burden.

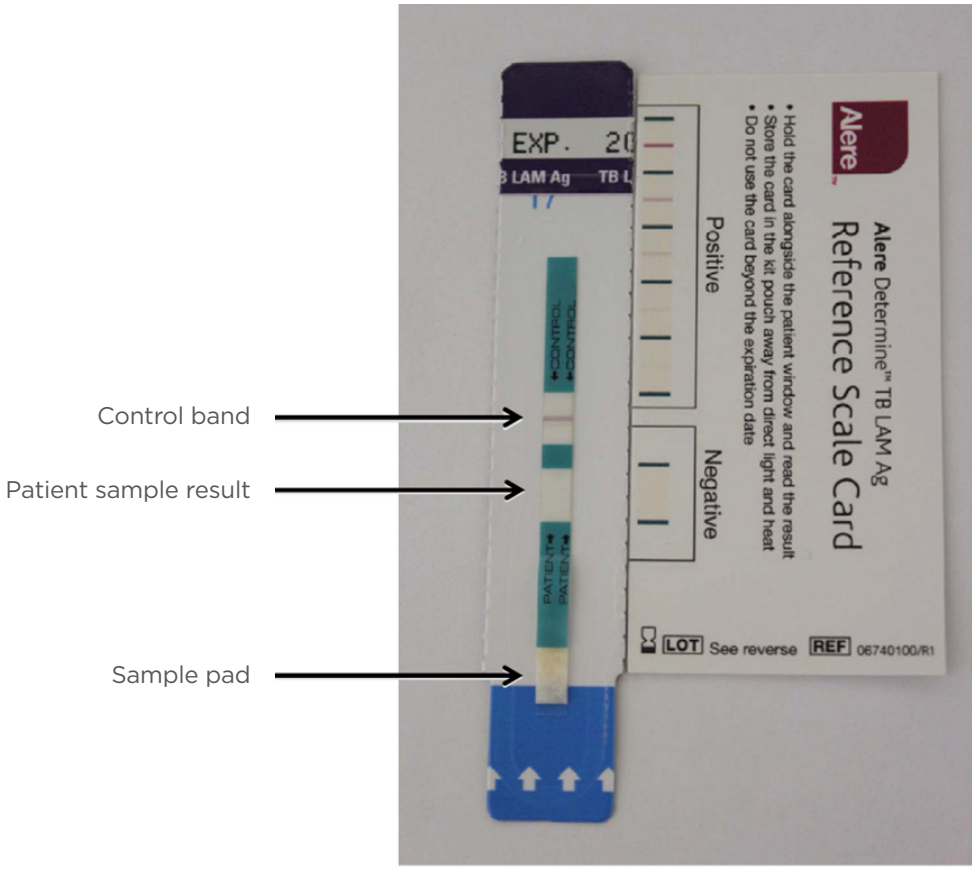

Figure 2: Photograph of a Determine TB LAM test strip showing the sample pad to which 60 µl of the test urine is applied, patient sample result, control band, and manufacturer's reference card.
TB: tuberculosis; LAM: lipoarabinomannan.
Reproduced from Lawn[75] with authors' permission.

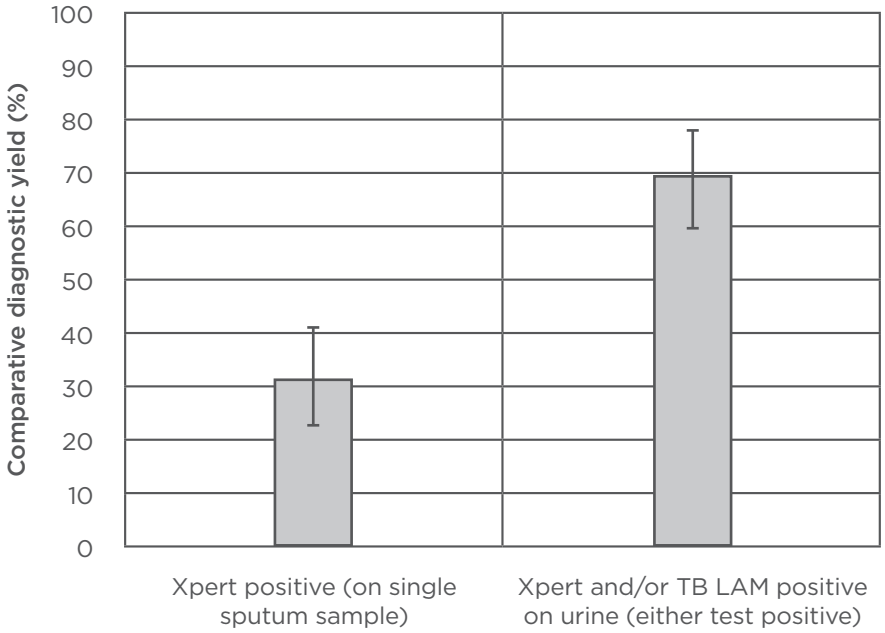

Figure 3: Graph showing relative diagnostic yields (and 95% confidence intervals) of a sputum-based diagnostic (Xpert MTB/RIF assay) and urine-based diagnostic (Xpert MTB/RIF or Determine TB LAM assays), both compared with HIV-associated tuberculosis diagnosed by any microbiological test.
MTB/RIF: mycobacterium tuberculosis/rifampicin; TB: tuberculosis; LAM: lipoarabinomannan.
Data from Lawn et al.[76]

Given its modest sensitivity and potential overlap with NTM, its role in resource-rich settings is less clear, although it could be utilised as a 'rule-in' test to expedite TB treatment when NAATs have failed and cultures and speciation are pending.[94]

OTHER FUTURE DIAGNOSTIC TECHNOLOGIES

Several evolving technologies offer potential benefits for HIV-TB. Serological assays have a long history and have transformed the diagnosis of HIV and other infectious diseases. Current TB serological assays have limited accuracy and WHO has issued strong guidance against their use.[95] However, this remains an important area for further research and development since improved serodiagnostics offer huge potential to be developed as PoC tests, which could diagnose EPTB (useful for HIV-TB).[95,96] HIV-TB presents particular challenges for serological assays, for example the heterogeneity of immunological responses to MTB.[97,98]

A recent case–control study examined antibody responses to several MTB proteins in HIV-TB and demonstrated moderate sensitivity.[99] Serological positivity was also common in HIV-infected controls, but the cross-sectional design was unable to differentiate poor specificity from early, subclinical disease that may have progressed to active TB.[99] It appears that a pooled group of antigens gives a superior performance, and multiplex PoC diagnostics are being developed which can assess up to 57 MTB antigens with 150 µl of whole blood added directly to a cartridge.[27] MTB's production of specific volatile organic compounds has the potential to be used in PoC tests to discriminate patients with active TB infection.[27,100] Other 'breath tests' have already shown that they can discriminate isoniazid-susceptible MTB, isoniazid-resistant MTB, and other common lung pathogens.[101]

DOES EMPIRICAL TUBERCULOSIS TREATMENT NEGATE THE NEED FOR NEW TUBERCULOSIS DIAGNOSTICS?

There was much enthusiasm following the promising results of early assessments of the Xpert MTB/RIF assay and WHO endorsement. Subsequent substantial investment by donors and governments has led to rapid global implementation.[102] Several studies have modelled a positive impact of scaling-up of Xpert on economic, individual, and population-level outcomes.[103–107] However, whilst data from high HIV-

burden settings have demonstrated that Xpert has superior diagnostic accuracy to sputum-smear microscopy and can improve time to diagnosis, any impact on morbidity or mortality is yet to be demonstrated.[108-112]

One major hypothesis for this failure to observe a beneficial impact is the use of 'empirical' treatment for TB clinically diagnosed by clinicians in the absence of positive TB diagnostics, especially in the context of HIV coinfection.[113] Empirical TB treatment (defined as the decision to commence TB treatment in the absence of bacteriologically confirmed TB using smear microscopy, culture, or WHO-endorsed rapid diagnostics[114]) remains poorly studied but is likely to be done in a non-standardised fashion and be influenced by several factors, including poor sensitivity of traditional TB diagnostics, high TB prevalence (a high pre-test probability), high mortality in HIV-TB (therefore little to lose by treating TB), cadre of healthcare worker, and availability of adjunctive therapies. A meta-analysis of the WHO algorithm[13] for smear-negative TB in high HIV-prevalent settings estimated pooled sensitivity of 61% (95% CI: 55-67%) and specificity was 69% (95% CI: 66-72%).[115] It is difficult to standardise empirical treatment, and therefore difficult to estimate accuracy and account for it in models.

The TB-NEAT study[110] noted that of the smear-negative patients detected by Xpert, 93% were treated empirically by clinicians. A legitimate concern is that Xpert may simply be confirming a TB diagnosis in those who would receive TB treatment anyway, negating the potential benefit of Xpert (or other new diagnostics) on individual and population-level outcomes.[113] Models attempting to adjust for empirical therapy have also reflected this.[116] However, empirical TB treatment also has potential harms through overtreatment, including cost to patients and healthcare systems, unnecessary drug toxicities which are more common in the context of concomitant antiretroviral therapy, and inappropriate treatment of unsuspected MDR-TB. Post-mortem studies also demonstrate that empirical treatment misses large numbers of HIV-TB cases.[14-16] Further studies from a wide variety of settings are required to understand empirical TB treatment, in particular the impact of new diagnostics such as Xpert. However, there is a real danger that certain features of the design and conduct of TB diagnostics intervention trials may actually increase the likelihood of empirical TB treatment being prescribed.[117] Thus, future trial designs for new diagnostics must account for empirical treatment, as should studies modelling implementation and scale-up.

CONCLUSION

In conclusion, HIV-TB remains a major public health burden. Improved diagnostics are considered crucial to addressing this public health challenge, especially in resource-limited settings. At present the most progressive technologies are the Xpert MTB/RIF assay and urine TB LAM assays, although clinical impact at an individual or population level has yet to be demonstrated. After decades of neglect the TB diagnostics pipeline looks promising, with several new diagnostic technologies that could be applicable to the challenges of HIV-TB. However, future diagnostics need evidence of impact beyond simple diagnostic accuracy before scale-up and widespread implementation. This includes clinical trials assessing clinical outcomes in a variety of settings and must take into account the use of empirical treatment.

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