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EDITORIAL

Azithromycin and bronchiolitis obliterans syndrome after lung transplantation: is prevention better than cure?

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Although lung transplantation is now accepted as an established therapy for selected patients with end-stage lung disease, long-term survival after lung transplantation remains limited by the development of bronchiolitis obliterans syndrome (BOS) in >50% of recipients [1]. BOS is the clinical manifestation of an inflammatory bronchiolitis associated with fibrotic remodelling of the small and medium-sized airways, and is characterised by progressive loss of allograft function with development of airflow obstruction [2, 3].

Until recently, the development of BOS was associated with an irreversible and relentless decline in lung function, which either eventually stabilised at a very low level or, in many patients, progressed to end-stage respiratory failure, accounting for the commonest cause of death after the first post-transplant year. BOS has historically been attributed to the effects of ongoing alloimmune injury as both the frequency and severity of acute rejection episodes have been associated with increased risk [4]. These observations lead to the paradigm that BOS is chronic rejection of the transplanted lung and, consequently, intensification of immunosuppression was used as an attempted therapy in many affected recipients. These approaches offered, at best, a slowing in the progression of the condition in some, but also contributed to infective complications that undoubtedly added to the overall mortality risk from BOS. Over the last decade, a number of clinical trials of more intensive immunosuppressive regimes from the time of transplant or after onset of BOS have failed to impact on the incidence of BOS or regaining of lost function [5, 6]. More recently, however, it has been appreciated that nonalloimmune insults to the lung allograft, such as the lung injury of primary graft dysfunction, viral and bacterial infections, and aspiration injury, also increase the risk of developing BOS [7]. This suggests that cross-talk between innate immune responses and alloimmunity may play a key role, and highlights the importance of inflammation in driving the process.

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Studies demonstrating a marked beneficial effect of the macrolide antibiotic, erythromycin, in improving lung function and survival in patients suffering from diffuse panbronchiolitis (DPB) appeared in the mid-to-late 1990s [8]. These reports were seized upon by the lung transplant community, as it was recognised that there are similarities between DPB and BOS in the nature of the airway inflammation present and the physiological defects that develop. This led to a number of small retrospective and prospective, open-label, non-placebo-controlled studies in lung transplant recipients with BOS using the newer 15-ringed macrolide, azithromycin, which reported a significant response in 30–80% of those treated in improving lung function by a mean of 4–18% across the study groups [9–12]. It became clear that the improvement in forced expiratory volume in 1 s (FEV_1) in the responders was highly clinically, as well as statistically, significant, ranging from 15% to >30%. These observations potentially represented a major therapeutic advance as the first intervention that had ever been shown to reverse the loss of lung function in patients with BOS. In a subsequent small prospective study designed to examine potential mechanisms of macrolide action in BOS, it was shown that responders had a higher bronchoalveolar lavage (BAL) neutrophil count before treatment was started and, at the end of 3 months of treatment, their airway neutrophilia was dramatically reduced, and their BAL concentration of the neutrophil chemokine interleukin (IL)-8 was lower [13]. However, although the role of azithromycin as a potential therapy in BOS looked very promising after these reports, the lack of a randomised, placebo-controlled trial that also addressed safety concerns left doubt as to whether azithromycin should become a new standard intervention in BOS.

The international lung transplant community has, therefore, been calling for adequately powered randomised clinical trials of macrolides in BOS for some time [14, 15]. All too often in these scenarios, the chance to provide high-quality evidence of the effectiveness and safety of an intervention can be lost as it creeps into standard clinical practice based on the results of small retrospective studies.

In this issue of the *European Respiratory Journal (ERJ)*, Vos *et al.* [16] from the Lung Transplant Programme in Leuven, Belgium, present the results of the world's first randomised, double-blind, placebo-controlled study investigating the role of azithromycin given as prophylaxis to lung transplant recipients to prevent the development of BOS. Although this

study primarily addresses a different question, as to whether azithromycin offers effective protection against the development of BOS as opposed to its effectiveness as a treatment for BOS, it marks a major step forward for the international lung transplant community.

This single-centre study was powered to show a difference in both BOS-free and overall survival in the first two post-transplant years between recipients receiving low-dose azithromycin (n=40) or placebo (n=43) continuously from the time of post-transplant hospital discharge. The dose of azithromycin, 250 mg on Mondays, Wednesdays and Fridays, was the same as used in the previous retrospective and prospective nonrandomised studies. Additionally, any patient in the trial who developed BOS was switched from trial medication to open-label azithromycin and followed for the remainder of the 2 yrs of the study.

The trial showed that, over the 2-yr follow-up period, those who received azithromycin had a significantly lower incidence of BOS: 12.5% compared to 44.2% in those who received placebo. The primary outcome measure of BOS-free survival was significantly better in patients on azithromycin, with a hazard ratio of 0.27 (95% CI 0.092–0.816). However, in contrast, there was no significant difference in overall survival between the two treatment arms. The study was well performed and the possible confounding factors that might have impacted on the incidence of BOS were corrected for or examined carefully to ensure that there were no systematic differences in the groups. The incidence of acute rejection episodes, lymphocytic bronchiolitis, pneumonitis, bacterial colonisation and gastro-oesophageal reflux as secondary outcome measures were also comparable between the study groups. Furthermore, the patients receiving azithromycin had significantly better lung function, as measured by FEV_1, at the end of the study and a significantly lower degree of airway neutrophilia over the duration of the study. The study treatment was generally well tolerated, although the gastrointestinal side-effects of nausea and diarrhoea were nonstatistically higher in the treatment group, affecting 7.5% compared to 2.5% in the placebo group. This is most likely due to azithromycin's action on gut motility.

The impact of azithromycin in protecting the lung transplant recipient from developing BOS is profound at 2 yrs. However, it will be essential to follow this cohort further to determine for how long this protective effect is maintained. If this effect is maintained to 5 yrs, then it will, without doubt, offer the potential to revolutionise the post-transplant outcomes for >1,000 new lung transplants performed worldwide each year. The fact that the benefits of azithromycin therapy did not translate into differences in overall survival is not surprising and might be explained in two ways. First, the 2-yr duration of the study was too early to observe an effect on mortality as an end-point in those who developed BOS and, secondly, the study protocol dictated that any patient developing BOS had study medication stopped and were started on open-label azithromycin. Of the patients who developed BOS during the 2 yrs of the study, >50% of them improved their FEV_1 once open-label azithromycin was commenced. A recent publication has shown, in a retrospective, nonrandomised observational study, that azithromycin was associated with a reduced risk of mortality in patients with BOS [17].

Although this trial by VOS *et al.* [16] was not designed to address the mechanistic understanding of macrolide action in the transplanted lung, the results mark a significant advance in our appreciation of how macrolides such as azithromycin can work clinically. Debate continues as to the key action of macrolides in mediating beneficial effects in inflammatory lung disease. The dose used in this clinical trial and other studies was well below the mean inhibitory concentration for common respiratory pathogens and, in addition, azithromycin has no direct antibiotic action against *Pseudomonas aeurginosa*, which is commonly found in lung transplant recipients [8, 18].

Observations from studies in a number of chronic inflammatory lung diseases support the assumption that it is the recognised anti-inflammatory or immunomodulatory actions of macrolides that are the key [19]. This includes the fact that it can take several months for macrolides to have their effect and that beneficial effects can be seen in both colonised and noncolonised patients. Clinical improvement can still be seen even when respiratory pathogens persist, especially with organisms, such as *Pseudomonas*, that are resistant to macrolides [20]. However, the concentration of azithromycin in macrophages is significantly higher than in serum, and the local antibiotic effect in the airway may, therefore, be substantial, even at low dose [21]. In addition, it must also be remembered that some actions of macrolides are not antibiotic but still antimicrobial. These include the ability to reduce bacterial adherence to airway epithelium, inhibition of bacterial biofilm formation and reduced transcription of quorum signal molecules, which are critical for determining bacterial communication and, hence, behaviour [20]. It is, therefore, still possible that the beneficial effects of macrolides in the airway are due to their actions on pathogens.

The immunomodulatory actions of macrolides are broad-ranging. They are able to inhibit intracellular signalling in a least two important inflammatory pathways; blocking extracellular signal-regulated kinase (ERK)1/2 phosphorylation in the mitogen-activated protein kinase pathway and inhibiting inhibitor of κB (I-κB) phosphorylation, a key step in nuclear factor-κB signalling. The consequence is a reduced secretion of cytokines and chemokines, such as IL-1β, IL-8, tumour necrosis factor-α and granulocyte–macrophage colony-stimulating factor, from epithelium and inflammatory cells in response to stimuli [22]. The immunomodulatory action of macrolides on neutrophils is particularly relevant in inflammatory lung disease. Macrolides have been shown to reduce neutrophil adhesion, increase neutrophil apoptosis and increase the phagocytosis of apoptotic neutrophils by macrophages [23]. Finally, it is worth noting that macrolides can reduce the clearance of corticosteroids, potentially enhancing their anti-inflammatory effect. An excellent review by KANOH and RUBIN [20] provides much more detail on all the immunomodulatory actions of macrolides.

The trial presented in this issue of the *ERJ* [16] shows that prophylactic use of azithromycin is effective at lowering the incidence of BOS over the first two post-transplant years. This suggests that very long-term therapy with macrolides may, therefore, be required in clinical practice. Concerns about side-effects, such as cardiac arrhythmias, severe gastrointestinal upset or emergence of resistant organisms, did not materialise

within the study timeframe. This may be due to the use of azithromycin as the macrolide of choice, as it has been associated with reduced risk of cardiac arrhythmias and improved gastrointestinal tolerability compared to older agents, such as erythromycin [24]. However, concerns remain about the potential for emergence of bacterial resistance with very long-term use. This could be the case if the prophylactic approach was applied to much more common inflammatory lung diseases, such as chronic obstructive pulmonary disease. There is evidence that marked resistance of *Staphlycoccus aureus* and *Haemophilus influenzae* to macrolides develops in cystic fibrosis patients receiving long-term macrolides, with resistance to *S aureus* reaching 100% within 3 yrs [25]. In addition, concerns have been raised as to whether long-term macrolide use might induce resistance in nontuberculous mycobacteria, which are emerging as an increasingly important class of lung pathogens in the cystic fibrosis population [26].

These concerns will only be addressed by very long-term studies that are specifically focused on measuring changes in resistance patterns, and these will be expensive and difficult to perform. This raises the possibility that development of nonantibiotic macrolide compounds that still possess all or most of the anti-inflammatory and immunomodulatory actions would have significant advantages. Use of such novel macrolides would also help address important questions about how macrolide therapy is working in chronic inflammatory lung disease. In the meantime, VOS *et al.* [16] from Leuven are to be congratulated on delivering an extremely well performed and clearly reported study, which the international lung transplant community has been long awaiting.

STATEMENT OF INTEREST

None declared.

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