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THE LEUVEN EXPERIENCE WITH A DICHOTOMY IN BRONCHIOLITIS OBLITERANS SYNDROME (BOS) AFTER LUNG TRANSPLANTATION REVEALED BY AZITHROMYCIN

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WINNING ABSTRACT: BOS is the most important cause of late mortality after LTx. Until 5 years ago, the prevalence was around 30% and 50%, 3 and 5 years after LTx. Introduction of azithromycin (AZI) improved the FEV_1 in 40% of BOS patients. AZI treatment may explain why in our center, the BOS prevalence at 3 years has decreased from 30% to 15% compared to the ISHLT registry. Opposed to the current belief about BOS, we hypothesize a dichotomy within BOS: Neutrophilic Reversible Allograft Dysfunction (NRAD) and fibropoliferative BOS (fBOS; table 1). This dichotomy is based on the discrepancy in AZI response and observations within our center consisting of clinical, biochemical and cellular (BAL) analysis.

NRAD makes a re-evaluation of the BOS definition (irreversible FEV_1 decline, neutrophilic inflammation, fibroproliferation) indispensable. As it is reversible, NRAD should be excluded from BOS and accepted as innate (non-specific) inflammation and as an important risk factor for the development of BOS. So after exclusion of other complications such as acute rejection, infection, gastro-oesophageal reflux and after a trial with AZI, BOS will remain what it is now (the fBOS). This implements a re-evaluation of the neutrophilic inflammation, as it is a prerequisite for AZI responsiveness. BOS can then histologically be characterized as pure inactive OB, which is hardly responsive to any treatment.

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SYNOPSIS OF MY JOB AND THE ROLE OF THE UNIT IN WHICH I WORK

I work in the Laboratory of Pneumology at the Katholieke Universiteit (Leuven, Belgium). Within the laboratory, research focuses on: chronic obstructive pulmonary disease (involvement of inflammatory and systemic mechanism); pulmonary hypertension (the role of the endothelium), interstitial lung disease (the involvement of innate inflammation); lung toxicology (mouse models of chemical-induced asthma, epidemiological research on cadmium and air pollution, and *in vitro* research on particulate matter and nanoparticles); and lung transplantation (inflammatory mechanisms in bronchiolitis obliterans syndrome (BOS)). In 2001, under the guidance of Professor Geert M. Verleden, we started experimental research on lung transplantation in our laboratory.

While writing this report, we are awaiting the 400th lung transplantation in our centre. During the last few years, ~40 lung transplantations have been performed each year, 2006 was the best-ever year, with 57 procedures carried out (fig. 1). As a result, our centre has extensive clinical experience (the programme started in 1991), both in the selection and follow-up of these patients, which is performed in the University Hospital Gasthuisberg (Leuven, Belgium).

Translational research is our philosophy in investigating BOS in our centre. The clinical programme is supervised by Professor G.M. Verleden and Professor L.J. Dupont. Under their guidance, and together with Dr Wim Wuyts and Dr Robin Vos, I manage the experimental section of the lung transplantation research, specifically the inflammatory mechanisms involved in acute and chronic rejection. It is with this philosophy of translational research that we were able to contribute to the understanding of BOS more specifically, by exploring the involvement of interleukin (IL)-17, IL-8 and neutrophils in combination with the possible modulatory effect of azithromycin.

SYNOPSIS OF MY RESEARCH AND HOW MY WINNING POSTER IS PART OF THIS

My PhD thesis is entitled ''Bronchiolitis Obliterans Syndrome after azithromycin and beyond: from clinical experience to basic science and back'' and was finalised in May 2007. An *in vitro* experiment with primary human airway smooth muscle

STATEMENT OF INTEREST: During his PhD, B. Vanaudenaerde received funding from the research foundation Vlaanderen (Belgium; grant no. G.0493.04).

TABLE 1 A hyphothesised dichotomy

	NRAD	fBOS
Airway inflammation	Neutrophils	No neutrophils
Clinical picture	Crackles, increased sputum production	No crackles, little sputum
Time after transplantation	Early	Late
Course	Slowly, several years	Fast, 6 months
Histology	Initially inflammation, finally fibrosis	Merely fibrotic
AZI therapy	Beneficial/reversible	Not beneficial/irreversible

NRAD: neutrophilic reversible allograft dysfunction; fBOS: fibropoliferative bronchiolitis obliterans syndrome; AZI: azithromycin.

cells to explore the potential role of IL-17 in the development of BOS was the starting point of my research [1]. This study led to a grant from the research foundation Vlaanderen (Belgium), making it possible to further explore the mechanism of BOS *in vitro* with the involvement of IL-8 chemokine and oxidative stress production through the mitogen-activated kinase intracellular pathway [2, 3]. Additionally, we installed a routine follow-up of our lung transplant patients with bronchoscopy and bronchoalveolar lavage (BAL). Analysis of the BAL fluid confirmed the involvement of IL-17 in patients who developed acute rejection or BOS [4, 5]. A study by GERHARDT *et al.* [6] has demonstrated, for the first time, a reversal of the disease by macrolides, especially azithromycin; we also focused our attention on the mechanisms of azithromycin. We demonstrated both *in vitro* and in patients with BOS that innate immunity with IL-8 and neutrophils are key elements to explaining the mechanism of action of azithromycin [7–10]. This led us to speculate about a dichotomy in the development of BOS based on the response to azithromycin therapy, as I presented at the 2007 European Respiratory Society Congress in my winning abstract.

HOW MY RESEARCH FITS INTO THE OVERALL RESEARCH OF MY WORKING GROUP/RESEARCH TEAM

As we demonstrated neutrophilic inflammation to be a key element in explaining the response to azithromycin, our group focused on several topics that are able to induce this neutrophilia. We confirmed that pseudomonal colonisation and bile acid reflux are involved [11–13]. A placebo-controlled, double-blind randomised clinical trial with azithromycin is currently being carried out in 75 patients, which aims to demonstrate a decrease in the prevalence of BOS at 2 and 3 yrs after transplantation.

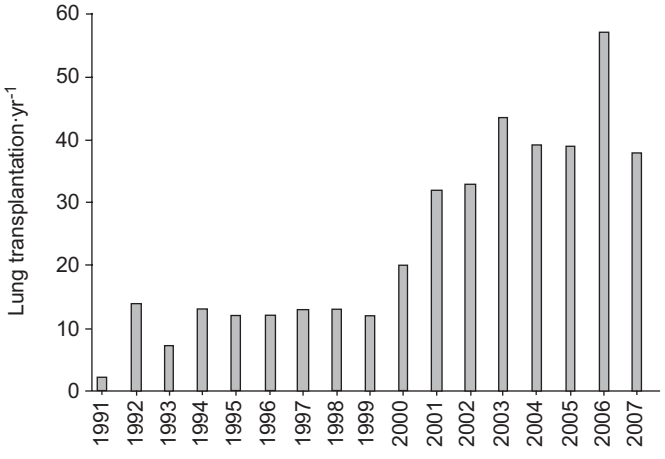

FIGURE 1. The number of lung and heart–lung transplantations performed at the University Hospital Gasthuisberg (Leuven, Belgium) from 1991–2007.

HOW MY RESEARCH WILL IMPACT ON CLINICAL OR RESEARCH PRACTICE

At the end of these experiments, we focused our attention on this dichotomy within bronchiolitis obliterans syndrome. It is surprising to see that the combination of clinical and experimental research has led to a better understanding of the pathophysiological mechanisms of bronchiolitis obliterans syndrome and also to the start of bronchiolitis obliterans syndrome phenotyping. Questions have also arisen concerning the definition of bronchiolitis obliterans syndrome, which probably needs further refinement. Thus, a working group, led by Professor G.M. Verleden, is being formed by the International Society for Heart and Lung Transplantation pulmonary council to revise bronchiolitis obliterans syndrome. This may ultimately lead to better management of the lung transplant patient.

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