TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

REVIEW

Current views on chronic rejection after lung transplantation

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Keywords
azithromycin-responsive allograft dysfunction, bronchiolitis obliterans syndrome, chronic lung allograft dysfunction, chronic rejection, lung transplantation, restrictive allograft syndrome.

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Conflicts of interest
None of the authors have any conflict of interest regarding this study.

Received: 10 February 2015
Revision requested: 28 February 2015
Accepted: 7 April 2015
Published online: 23 Apr 2015

doi:10.1111/tri.12579

Summary

Chronic lung allograft dysfunction (CLAD) was recently introduced as an overarching term mainly to classify patients with chronic rejection after lung transplantation, although other conditions may also qualify for CLAD. Initially, only the development of a persistent and obstructive pulmonary function defect, clinically identified as bronchiolitis obliterans syndrome (BOS), was considered as chronic rejection, if no other cause could be identified. It became clear in recent years that some patients do not qualify for this definition, although they developed a chronic and persistent decrease in FEV_1, without another identifiable cause. As the pulmonary function decline in these patients was rather restrictive, this was called restrictive allograft syndrome (RAS). In the present review, we will further elaborate on these two CLAD phenotypes, with specific attention to the diagnostic criteria, the role of pathology and imaging, the risk factors, outcome, and the possible treatment options.

Introduction

Although lung transplantation has come of age, survival is still far behind other solid organ transplantations [1]. In fact, according to the recent registry report of the International Society for Heart and Lung Transplantation (ISHLT), 5-year survival is only 58% [2]. The major problem responsible for this rather low survival is the development of chronic rejection. This phenomenon, which develops in 50% of the patients at 5-year postprocedure, accounts for about 30% of the mortality between 3 and 5 years after the transplantation [2]. Originally, chronic rejection was defined as pathological obliterative bronchiolitis (OB) [3,4], for which the clinical correlate bronchiolitis obliterans syndrome (BOS) was proposed, characterized by an obstructive and persistent pulmonary function decline (>20% decline in forced expiratory volume in 1 second, FEV_1, compared to the best postoperative value) [5]. Over the last years, however, it became apparent that not every chronic decline in FEV_1 was obstructive nor irreversible [6], which led to new insights into chronic rejection after lung transplantation. These new insights form the basis of this review.

Chronic Lung Allograft Dysfunction (CLAD)

It is now acknowledged that there is a substantial percentage of patients with chronic FEV_1 decline after lung

transplantation for which the definition of BOS is not applicable. Indeed, these patients did not develop an obstructive pulmonary function pattern, but rather a restrictive decline in their pulmonary function. This led to a recently proposed new classification system [7] that defines subtypes of patients with chronic FEV_1 decline. Such phenotyping is interesting to investigate, as, for instance, the outcome after diagnosis may vary considerably (see further in this review). As a consequence, the term CLAD was introduced to describe any chronic decline in FEV_1, irrespective of its cause, for which further subtyping was proposed. Further phenotyping is then based upon subsequent investigation as outlined below [7]. As such, CLAD is defined as a persistent decrease in FEV_1 and/or forced vital capacity (FVC) of at least 20%, compared to the baseline values, considered as the mean of the two best postoperative measurements with at least 3 weeks in between [7]. Whether other forms of chronic decline in FEV_1 and/or FVC should also be considered as CLAD, although proposed in this study [7], received more critical comments from the lung transplant community. In fact, in the perspective paper where this classification was proposed [7], the authors considered CLAD as an overarching term, including all forms of chronic lung dysfunction post-transplant. This implies that the term CLAD could be used for every patient whose transplanted lung does not achieve or no longer maintains normal function for an arbitrarily defined period of time. In particular, the first part of this novel definition led to much speculation and questioning. Indeed, does a patient in whom the pulmonary function after double-lung transplantation only achieves a maximum of 60% of the predicted value suffer from CLAD? Such a patient series with an early obstructive pulmonary function after bilateral lung transplantation was recently described by Suhling *et al.* [8]. In that paper, the recipients were older at transplantation, had significantly decreased FEV_1, increased total lung capacity, and donor organs with lower pO_2 when ventilated with 100% oxygen before retrieval. If such a patient is indeed defined as suffering from CLAD, how then will we interpret a further pulmonary function decline from this (too low) baseline? The patient may then again suffer from (active) CLAD, whether obstructive, restrictive, or combined. Although it was suggested that CLAD may also be used in this specific situation, following the discussions that arose, it will most often be used to describe loss of function compared to the best post-transplant FEV_1 and/or FVC, hence in the context of chronic rejection.

It should be clear that the term CLAD is not to be used as a diagnosis, but rather presents most commonly a persistent decline in comparison with the best postoperative pulmonary function values. As a consequence, every possible effort should be undertaken to identify the specific cause of persistent (accepted to be at least going on for 3 weeks) decreased function. Only when no specific cause is found (see Table 1), the patient's decline in pulmonary function may be attributed to CLAD.

Even when the FEV_1 and/or FVC decline takes longer than 3 weeks, specific treatment may still lead to reversibility [9]. This may point to other etiologies of pulmonary function decline, which are discussed further under gastroesophageal reflux and azithromycin-responsive allograft dysfunction (ARAD). These conditions need to be ruled out, and in most patients, this may postpone the identification of CLAD as it may take several weeks before the pulmonary function begins to improve. So in retrospect, after adequate treatment and subsequent improvement, the patient may not have had CLAD, as this is essentially considered to be nonreversible.

The detection of CLAD implicates the start of an evaluation to determine the reason(s) why the lung function decreased. Although the term "chronic" reflects a process that takes at least 3 weeks to develop, it is recommended to investigate the cause of the declining pulmonary function as soon as it was detected in order not to withheld potential therapies that might improve/restore lung function. As such, a decline of 10% in FEV_1 and/or FVC from stable baseline function (suspected CLAD) should already trigger further investigation (see below) to identify a possible cause [7].

When CLAD is the likely cause of FEV_1 decline (>20% from baseline) by exclusion of other conditions (Table 1), further investigation is definitely needed to gather additional information that facilitates the identification of specific CLAD phenotypes. Such investigations must include full pulmonary function testing, bronchoscopy with transbronchial and endobronchial biopsies, bronchoalveolar lavage (BAL) with viral/bacterial/fungal cultures and total and differential cell count, and CT of the thorax with inspiratory and expiratory imaging [7]. Only by integrating

Table 1. Confounding factors leading to nonchronic rejection FEV_1 decline [adapted from ref 7].

Allograft-Related
Persistent acute rejection
ARAD*
Infection/colonization
Anastomotic stricture
Disease recurrence
follicular bronchiolitis
Extra-allograft
Pleural disease
Diaphragm dysfunction
Native lung hyperinflation
Other causes

*ARAD, azithromycin-responsive allograft dysfunction.

these findings in the CLAD definition, it will be possible to distinguish BOS from other phenotypes of chronic rejection. Figure 1 gives a schematic overview of CLAD and its different subtypes.

Bronchiolitis obliterans syndrome (BOS)

Obliterative bronchiolitis, considered as the pathological correlate of chronic rejection [3,4], is difficult to prove on transbronchial biopsies, because of its low sensitivity [10]. As a consequence, an expert group of the ISHLT described bronchiolitis obliterans syndrome (BOS) as the clinical correlate of OB or chronic rejection [5]. This terminology was introduced to uniform the diagnosis all over the world and to permit research to identify risk factors to prevent, and underlying mechanisms to understand and ultimately treat chronic rejection using the same definition. BOS was defined as a persistent, mostly irreversible, progressive and obstructive decline in FEV_1 after lung transplantation and has for a long time been considered as the hallmark of chronic rejection. The diagnosis and severity of BOS was initially divided into 4 grades, only based on FEV_1 decline, compared to the baseline post-transplant FEV_1 (mean of the two best FEV_1 measurements with at least 3 weeks in between) [5]. In a first revision of the BOS definition, a fifth category was added, namely BOS O-p or potential BOS, which also included measurements of forced expiratory flow at 25 and 75% of vital capacity ($FEF_{25–75}$) [11]. BOS O-p, which was introduced as a trigger for further etiologic investigations and specific treatments, may now be replaced by suspected CLAD, defined by a persistent 10% drop in FEV_1 and/or FVC [7]. Of course, other explanations for a chronic decline in FEV_1, such as chronic infection or hyperinflation of the native lung, anastomotic strictures, acute rejection, and infection, should be excluded before BOS can be diagnosed. These conditions are in fact already excluded by identifying a patient as having CLAD (Table 1).

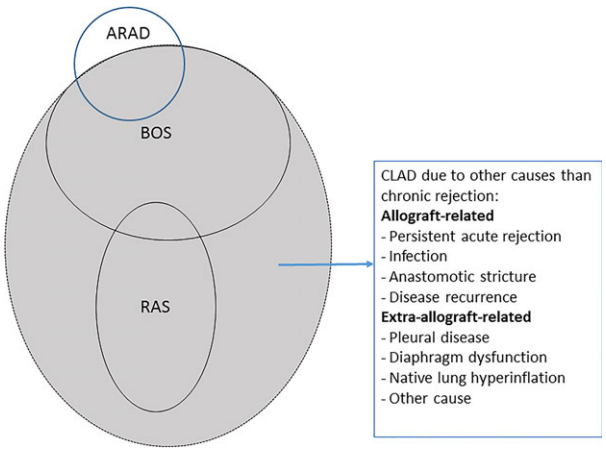

Figure 1 This Venn diagram describes CLAD and illustrates the non-chronic rejection causes of CLAD and the interrelation between RAS and BOS as subphenotypes of chronic rejection causes of CLAD. ARAD should be retrospectively excluded by a 3-month trial with azithromycin, before CLAD can be diagnosed. Also specific causes of CLAD should be excluded before to accept CLAD as a manifestation of chronic rejection and before subphenotyping into BOS and RAS can be performed. RAS, restrictive allograft syndrome; BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; ARAD, azithromycin-responsive allograft dysfunction.

As a consequence of new insights into the pathophysiology of BOS and the evolution of strategies to treat patients with BOS, a second revision, approved by the International Society for Heart and Lung Transplantation (ISHLT), the American Thoracic Society (ATS), and the European Respiratory Society (ERS), was recently published [9]. Although BOS seems to stand for an easy to interpret definition, it is clear that this definition remains difficult to apply in some patients, as recently evidenced by Kapila *et al.* clearly demonstrating that there are potential limitations with the current criteria for diagnosing BOS and that further refinements of these diagnostic criteria will be necessary to allow an improved ability to identify and characterize patients who develop or are at risk for BOS [12].

Bronchiolitis obliterans syndrome due to OB is mostly characterized by air trapping on expiratory HRCT that is best demonstrated by mosaic attenuation when imaging is combined with a breath-hold at end-expiration [13]. During further evolution of BOS, bronchiectasis may also develop. Recently, a micro-CT study of BOS explant lungs revealed that >60% of the airways from the 6th generation onwards showed obstruction, with characteristic OB lesions (constrictive bronchiolitis), but also airway collapse lesions (without increased collagen deposition), the exact significance or pathophysiology being unknown up to now [14].

More risk factors have been identified, and over the years, new risk factors were added to this list. In the most recently updated BOS document, a review of all acknowledged, possible and probable risk factors was published [9]. This list is certainly not exhaustive, and other risk factors may still emerge. One of the recently added risk factors is air pollution, which may account for a 25% excess of BOS and mortality after lung transplantation [15,16]. Also various genetic risks have been identified which may promote the development of BOS in an appropriate clinical setting, such as a SNP (single nucleotide polymorphism) of TGF (transforming growth factor)-β1, INF(interferon)-γ, TLR (Toll-like receptor) TLR-2, TLR-4 and TLR-9 and IL (interleukin)-17R [17–20] (Table 2).

With respect to these risk factors, there is increasing knowledge on the role of antibody-mediated rejection, with the development of donor-specific antibodies (DSA),

Table 2. Risk factors for the development of BOS [adapted from ref 9].

Primary graft dysfunction
Acute cellular rejection
Lymphocytic bronchiolitis
Antibody-mediated rejection (e.g., *de novo* donor-specific anti-human leukocyte antigen antibodies)
Gastroesophageal reflux and microaspiration
Infections/colonization
Persistent neutrophil influx and sequestration
Autoimmunity (e.g., collagen V sensitisation)
Air pollution
Genetic factors

identified as an independent risk factor for the development of BOS and also death. Patients with early but also late development of DSA and especially those with persistent DSA are prone to develop BOS [21–23]. Also the role of non-HLA antibodies to self-antigens such as K-α1 tubulin and Collagen V has been identified in lung transplant patients diagnosed with BOS suggesting a pathogenic role for these antibodies [24].

Once BOS is diagnosed, the median survival is restricted to approximately 2.5 years [25], but it is known that early-onset BOS (within 2 years after transplantation) or BOS onset grade 2 or 3 (high-grade onset) is predictive of significantly worse survival [25,26]. The treatment remains difficult, as the exact pathophysiology is obscure; however, several modalities such as switching immunosuppressive treatments, addition of methotrexate, cyclophosphamide, montelukast, total lymphoid irradiation, and extracorporeal photopheresis have been tried, most of which with minimal success rate, leading at best to a (temporary) arrest of the declining FEV_1 [27]. Retransplantation in well-selected patients with BOS seems the only possibility to achieve long-term survival [9,28].

In the recently published consensus paper on BOS, it is emphasized that a decline in lung function that meets BOS criteria may be partially or even completely reversible [9] (in contrast to previous definitions where this possibility was only considered) [5,11]. This also contrasts with the perspective paper on the new classification of CLAD, as conditions that may lead to a reversibility of FEV_1 upon treatment should in fact be excluded before CLAD can be identified, although this may be in retrospect as discussed above [7]. Two such specific treatment options (fundoplication and azithromycin) were in fact the first challenges for the definition of BOS and will be reviewed in more detail.

Gastroesophageal reflux (GER) and BOS

Palmer *et al.* published for the first time the possible role of fundoplication as treatment for BOS in a lung transplant patient [29]. They described a patient who needed retransplantation because of a progressively declining FEV_1, suggestive for BOS, which was also confirmed on pathology of the explanted lung that showed OB lesions. Three months after the redo transplant, the FEV_1 again deteriorated and transbronchial biopsies showed peribronchial mixed inflammation (no rejection nor infection), while a chest CT scan revealed evidence of mild bronchiectasis in the posterior segments of the lower lobes. One month later, GER disease was diagnosed and the patient underwent fundoplication, after which the FEV_1 returned to normal values. A repeat transbronchial biopsy showed absence of inflammation. Unfortunately, results of cell differentiation in the BAL fluid of this patient before and after the fundoplication procedure are not available.

Since the publication of that particular case, various case series on the possible role of fundoplication to prevent or to treat BOS have been published [30–34], mostly retrospective studies without a control arm, which sometimes show amelioration of FEV_1 after fundoplication, but otherwise have no effect at all on a declining FEV_1 nor on survival. In the 2014 BOS revision document, it is advised to investigate the presence of GER once suspected CLAD is present and to check for GER once BOS has been diagnosed and if present to eventually treat by fundoplication [9].

Neutrophilic reversible allograft dysfunction (NRAD)

The potential beneficial effect of azithromycin, on the other hand, is less doubtful. Several groups have indeed demonstrated that some 40% of patients with BOS may respond to azithromycin with an increase in their FEV_1, with some patients even experiencing a complete reversal of their FEV_1 [reviewed in 35]. Initially, it was thought that only patients with excess BAL neutrophilia (>15%) might benefit from azithromycin treatment [36]. This was a consequence of the fact that a persistent increase in BAL neutrophil percentage is accepted as an increased risk for the development of BOS [37]. Other reports, however, have suggested that BAL neutrophilia in the setting of BOS may rather be due to coexistent infection, which therefore needs to be carefully excluded [38]. Additionally, the role of BAL neutrophilia in predicting the response to azithromycin was not that clear in other studies; Meloni *et al.* demonstrated a significant FEV_1 response to azithromycin in patients without BAL neutrophilia and vice versa [39]. Also in the double-blind placebo controlled azithromycin trial of the Newcastle group, BAL neutrophilia was not predictive for an effect on FEV_1 in patients with BOS [40].

Patients who respond to azithromycin with a FEV_1 increase of $\geq$ 10% after a 2–3 months treatment were initially classified as having a specific phenotype of BOS, called neutrophilic reversible allograft dysfunction (NRAD) [41].

Based on the fact that BAL neutrophilia may not be that predictive for the response to azithromycin, it was recently suggested to rename this condition as ARAD (azithromycin-responsive allograft dysfunction) [7]. As a consequence, NRAD or ARAD may now be looked at as a potential confounder of BOS (and hence, CLAD) that should be actively excluded before CLAD/BOS can be diagnosed. It is therefore advised to undertake a 3-month trial of azithromycin (250–500 mg three times per week) in all patients who experience lung function decline consistent with CLAD/BOS, irrespective of the BAL neutrophil percentage [7,9]. Azithromycin should always be used carefully, as it may significantly increase the chance of sudden cardiac death [42], although the paper of Svanström could not corroborate this finding and rather attributed the increased episodes of sudden cardiac death to the infection for which azithromycin was initiated [43]. So far, the risk in lung transplant patients who receive a lower dose (e.g., 3 times a week instead of daily) remains small and should therefore be balanced against the potential benefit of azithromycin.

HRCT may also help to identify ARAD as it may show a combination of air trapping, tree-in-bud opacities, and peribronchiolar infiltrates that are compatible with the presence of inflammatory bronchiolitis, which may all improve upon azithromycin treatment [44].

Bronchiolitis obliterans syndrome and ARAD may coexist, meaning that patients treated with azithromycin may improve their FEV_1 >10% (ARAD part), although complete reversal of FEV_1 is unlikely and they remain with an obstructive pulmonary function (BOS part) [6,7,9,41].

Restrictive allograft syndrome

Although numerous previous reports, describing lung transplant patients with a declining pulmonary function, demonstrated signs of interstitial fibrosis on biopsies, persistent pulmonary infiltrates on CT scan, and a restrictive pulmonary function defect, these findings were mostly regarded as atypical and no attempts were made to further characterize these patients [4,45–47]. More recently a restrictive pulmonary defect with persistent infiltrates on CT scan was published as a small case series under the acronym upper lobe fibrosis [48]. It was Sato *et al.*, however, who described for the first time the fate of patents with this phenotype of CLAD, which they called restrictive allograft syndrome (RAS) [49], although it might be better to rename it as restrictive CLAD (rCLAD) [7,9,50]. These patients experienced a chronic decline in FEV_1 of at least 20%, which, however, was not obstructive, but rather restrictive, which they characterized by a decline in total lung capacity (TLC) of at least 10%, compared to the postoperative baseline TLC. Most of these patients also presented with persistent infiltrates on CT scan. This cohort of patients constituted some 30% of all patients with CLAD, and they had a mean survival postdiagnosis of only 1.5 years, compared to 4 years with BOS [49].

The problem with this definition of restriction was the fact that in most centers, TLC is not routinely measured and also not available as comparator before the FEV_1 starts to decline. Moreover, this definition is hard to use in patients that underwent single-lung transplantation [7,50]. As a consequence, the Leuven group introduced the FEV_1/FVC (Tiffeneau) index as an indicator of restriction, meaning that patients who experience a decline in FEV_1 with an increasing index, could be defined as having at least a restrictive component in their spirometry. This could also be used after single-lung transplantation, taking into consideration that the index will mostly not normalize, but simply increase when the FEV_1 deteriorates. Also in the Leuven experience, 28% of CLAD patients were diagnosed with RAS, most patients had persistent infiltrates on CT scan and experienced a worse survival compared to BOS (0.7 years compared with 3 years for BOS) [51].

The group from Duke defined patients with RAS as those who had a decline in FVC >20%, compared to baseline at diagnosis of CLAD. This definition seems indeed easy to use, as these parameters are always available during routine follow-up. Again, in this cohort of patients, rCLAD occurred in 30% of the investigated CLAD patients, resulting in a survival of 0.8 year compared with 3 years for BOS [52]. A summary of these possible diagnostic investigations, together with their advantages and disadvantages, was recently published [50].

CT scan in patients with RAS/rCLAD mostly demonstrates persistent infiltrates, varying from appearance of central and peripheral ground glass opacities, and nonseptal lines at diagnosis to (traction) bronchiectasis, central and peripheral consolidation, architectural deformation, volume loss and hilus retraction to pleuroparenchymal fibro-elastosis during further evolution. None of these findings seemed to predict survival after diagnosis [53].

Biopsy findings might also help to diagnose RAS/rCLAD, although these findings again may be nonspecific [54,55]. Recently, acute fibrinoid-organizing pneumonia (AFOP) was diagnosed on transbronchial biopsies in patients with FEV_1 decrease ≥20% and FEV_1/FVC >0.70 (nonobstructive) in combination with bilateral infiltrates on CT, not compatible with BOS. Biopsies were characterized by patent bronchioles with peribronchial and alveolar fibrin deposition with little or no concomitant inflammation. Whether AFOP is pathologically linked with RAS/rCLAD will need further investigation, but also these patients had a worse prognosis after diagnosis with a median survival of only 0.3 years [56].

It does not need to be said that in all these patient cohorts, other causes of restriction such as diaphragm paralysis, resection, myopathy need to be excluded, which is the case if, per definition, we first identify CLAD as defined above, and only then try to subphenotype into BOS (or obstructive CLAD and RAS (or restrictive CLAD) [7]. Figure 1 schematically represents CLAD and its various causes, including chronic rejection (BOS and RAS/rCLAD) and nonchronic rejection causes.

Several risk factors for the development of RAS/rCLAD have so far been identified, such as severe lymphocytic bronchiolitis, late-onset DAD, BAL eosinophilia, increased BAL protein levels of alarmins, such as S100A8, S100A9, S100A8/A9, S100A12, S100P and high-mobility group box 1 (HMGB1), sarcoidosis or interstitial lung disease as indication for transplantation, CMV donor/receptor mismatch, younger age, female gender, whereas other risk factors are in common with the risk for BOS development, such as increased BAL neutrophilia, acute rejection, pseudomonal colonization, and pulmonary infections [57–60]. All of these so-called specific risk factors were derived from rather small monocentric studies and were not corroborated in other cohorts of rCLAD/RAS patients. As a consequence, their significance as to the specific development of rCLAD/RAS remains speculative.

Multicentric, prospective studies will be needed to confirm the worse outcome of rCLAD compared with patients with BOS. One of the reasons might be that these patients may have several acute exacerbations, with a stepwise decrease in pulmonary function, rapidly leading to death or need for urgent retransplantation [61].

The treatment of this CLAD phenotype remains somewhat enigmatic, as no specific options have been identified that may halt the progression of the disease. The same therapeutic interventions as for BOS are usually been applied, however, with varying and mostly no success. Some anecdotal reports point to possible improvement with pirfenidone [62], an antifibrotic agent, recently approved for the treatment of IPF or alemtuzumab (Campath-1H), an antagonist of CD52, which was found to improve interstitial changes and lung function in 4 patients who likely had rCLAD [63]. The possibility of antibody-mediated rejection as a potential confounder or even risk factor for RAS/rCLAD remains challenging. Indeed, several centers mentioned that patients with RAS/rCLAD were more likely to have donor specific antibodies [48], which may open new treatment options. Further multicentric and concerted actions will be required to find a possible solution for this devastating phenotype of CLAD.

Importantly, compared to BOS, the results of retransplantation for RAS/rCLAD are much worse [64]. As a consequence, strict criteria should be applied when to consider a patient with RAS/rCLAD for retransplantation.

Conclusion

Chronic lung allograft dysfunction as a denominator for chronic rejection is now well accepted in the lung transplantation field, and there is convincing evidence that phenotyping into BOS and RAS is very meaningful, as it may impact on survival. Furthermore, careful exclusion of other causes of persistent pulmonary function decline, including ARAD, is necessary before one can diagnose chronic rejection as the cause of CLAD.

Several issues remain, however, unanswered. Although BOS is very well described as a progressive and obstructive pulmonary function defect, for which no other cause than chronic rejection can be identified, its exact pathophysiology and treatment options are still debatable.

The diagnostic criteria for RAS/rCLAD as a restrictive pulmonary function defect need to be refined so that they can easily be used in all centers worldwide. Indeed, the use of TLC decline as a criterion to identify RAS is difficult as this measurement is not always available in retrospect. An FVC decline of >20 at diagnosis of CLAD may be more realistic, although prospective studies also need to show its specificity. Although the prevalence of RAS/rCLAD is some 25–30% among all patients with CLAD, reports are emerging to show much lower percentages, which can so far not be explained. Although the prognosis of patients with RAS/rCLAD is usually worse compared to BOS, this seems not the case for every patient with RAS/rCLAD. Some patients indeed develop CT scan changes consistent with the development of RAS such as pleuroparenchymal fibro-elastosis, without having significant changes in their pulmonary function, hence not qualifying for RAS/rCLAD. Specific risk factors for RAS/rCLAD are emerging; however, no real treatment options do exist, although it became apparent that retransplantation carries a worse prognosis compared to retransplantation for BOS. Subtyping of CLAD is also time specific and can change along the course of the disease, as some patients may evolve from one phenotype to the other, especially from BOS to RAS/rCLAD [7,65].

We definitely need prospective multicenter studies tackling all these questions [66,67]. Only then, we may further improve the outcome of CLAD and more specifically the outcome of patients with RAS/rCLAD.

Funding

SEV is a post-doctoral fellow of the FWO (12G8715N). RV is supported by the Research Foundation Flanders (FWO) (KAN2014 1.5.139.14) and Klinisch Onderzoeksfonds (KOF) Leuven. GMV is supported by the FWO (G.0723.10, G.0679.12 and G.0679.12).

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