**Introduction**

Although survival has improved spectacularly following lung transplantation, it still limbs behind that after other solid organ transplantations (1). Although improvement in surgical techniques and clinical expertise led to an improved in initial survival, long-term overall outcome remains poor. Graft failure and chronic lung allograft dysfunction (CLAD) are the major culprits for this inferior long-term outcome (2). CLAD has been introduced recently as an overarching term encompassing all forms of chronic (>3 weeks) pulmonary function decline. Next to pulmonary function decline with a known cause (either graft-related, i.e., acute rejection, recurrence of native disease, infection, suture problems; or non-graft related, i.e., obesitas, pleural fluid, diaphragm dysfunction), there is also a large proportion of patients in whom no clear cause can be identified for the decline in pulmonary function which is therefore assumed to be due to chronic rejection. Within this review, we will describe historic and current evidence for CLAD classification and its clinical implications (diagnosis, pathology, radiology, risk factors and mechanisms) with a particular focus on treatment.

**History**

Historically, the term bronchiolitis obliterans syndrome (BOS) has been universally linked with chronic rejection post-transplant. BOS was defined as a persistent, obstructive decrease in forced expiratory volume in 1 second (FEV1) with at least 20% compared to the mean of the two best post-transplant values, in the absence of other
identifiable causes such as acute rejection, infection, suture problems… Further stratification was made according to the relative decrease in FEV₁ and consequently a grading system was introduced being BOS1 (FEV₁ 66–80% of best), BOS2 (FEV₁ 50–65% of best) and BOS3 (<50% of best). BOS was thought to be a functional reflection of obliterative bronchiolitis (OB). OB was considered to be the pathological hallmark of chronic rejection, but can also be found in other conditions such as pulmonary graft versus host disease after hematopoietic stem cell transplantation, auto-immune disorders especially rheumatoid arthritis, inhalation of toxins like sulfur mustard or as a post-infectious complication following childhood viral infection (3). OB is a pathological scarring or filling of the airway lumen with collagenous matrix leading to airflow limitation. The clinical definition of BOS based on serial pulmonary function measurements was deemed necessary given the poor sensitivity and specificity to diagnose OB on transbronchial biopsies (4). However, already in the initial pathological descriptions of explant lungs of patients suffering from chronic rejection some discrepancies were observed, such as the occurrence of a restrictive pulmonary function decline and significant pleural thickening (5,6). Nevertheless, in the following decades, the term BOS was universally utilized when referring to chronic rejection. The first elements to break this dogma came in 2003 when Gerhardt et al. found a proportion of patients with established BOS, who improved their pulmonary function upon azithromycin treatment (7). In some patients, this FEV₁ improvement was so pronounced that criteria for BOS were no longer fulfilled. This was confirmed by several other groups (8,9) and led to the first proposition of phenotypes of chronic rejection, leading to a novel phenotype called neutrophilic reversible allograft dysfunction or azithromycin responsive allograft dysfunction (10), which is nowadays considered as a reversible cause of CLAD and therefore is no longer thought to be a manifestation of chronic rejection (11). It was only in 2010 that a restrictive pulmonary function defect came apparent when Woodrow and colleagues defined a group of patients with so called ‘restrictive BOS’ (12). However, this description had no clinical implications (i.e., no survival difference) and therefore it was only in 2011 when Sato et al. identified a restrictive allograft syndrome (RAS) in patients with a decline in total lung capacity and infarct prognosis that general interest was aroused for what was thought to be a novel manifestation of chronic rejection (13). Typically, these patients presented with a restrictive pulmonary function, persistent CT infiltrates and most interestingly inferior survival compared to the obstructive (BOS) patients. Since literature on known causes of CLAD is rare, we will emphasize and contrast BOS to RAS and compare clinical characteristics, with special emphasis on treatment.

**BOS**

**Diagnosis, radiology and pathology**

BOS remains the most common phenotype of chronic rejection (65–75%). Typical characteristics include an obstructive pulmonary function defect and air trapping/mosaic attenuation on expiratory CT. Median survival after diagnosis is between 3–5 years. However, even within BOS there is significant heterogeneity: patients with an early (<2 years post-transplant) or a high grade onset (FEV₁ decline >35%) have inferior survival compared to patients with late and low grade onset (14). Analysis of explant specimens at redo transplantation has revealed OB in all BOS lungs (15), and the lesions seem to be segmental with 40–60% of the small airways appearing obstructed as of generation 6 on (16), which may explain the obstructive pulmonary function. OB is thought to be the end-result of persistent damage to the bronchial epithelium leading to an excessive inflammatory response, leading to local (myo-) fibroblast recruitment, fibrosis and ultimately complete obliteration of the airway lumen by fibrotic matrix.

**Risk factors and mechanisms of BOS**

Many risk factors for BOS have been identified such as acute rejection [specifically acute rejections associated with pulmonary function decline (17)], lymphocytic bronchiolitis, infection and colonization with micro-organisms (i.e., *Pseudomonas aeruginosa* and *Aspergillus fumigatus*), donor and recipient genetics, primary graft dysfunction, particulate matter and presence of HLA antibodies, or antibodies to self-antigens (18). Especially regarding the latter, progress has been made in recent years. De novo development of donor specific antibodies occurs frequently (35–60%) and is independently associated with CLAD (19,20). Similarly, antibodies to self-antigens (like K-α1 tubulin and collagen V) have been demonstrated to increase the risk for subsequent BOS development (21).

Since the mechanisms of BOS remain mostly elusive, novel evidence is accumulating with the use of the mice
orthotopic lung transplant model. Depending on the type of mismatch, immunosuppression and the duration of follow-up, lesions compatible with OB can be found in transplanted mice lung. Given the advantage of genetics knockouts in mice and possibility of invasive sampling, this model is an excellent set-up to study underlying mechanisms. For example, it was shown that progressive loss of self-tolerance through epitope spreading promotes airway fibrosis (22).

In another experiment, it has been shown that the murine lung allograft fibrosis originates mostly from the donor (23). However, these results cannot be directly extrapolated from mice to men as in humans, 32% of OB lesions are occupied by recipient and not donor fibroblasts (24). Moreover, human OB mostly develops in small airways, yet mice lack small airways, which is an additional problem to overcome. Therefore, experimental research has also focused on in vitro culture of bronchial epithelial cells, which showed that transition of epithelial cells to a mesenchymal phenotype can contribute to the fibroblast accumulation in OB lesions (25).

Interestingly, Pseudomonas can significantly aggravate this so called epithelial-mesenchymal transition (EMT), which is important given that colonization with pseudomonas occurs frequently post-transplant and is independently associated with a higher prevalence of BOS (26).

For decades, the search has been ongoing to identify an appropriate marker for BOS. Given the heterogeneous nature of CLAD, it comes to no surprise that at this moment, there are no universally applied biomarkers for BOS diagnosis. Broncho-alveolar lavage (BAL) analysis may provide insights in the lung micro-environment (27). Using BAL, the first important markers for BOS came to light, which included neutrophils and markers of neutrophil activation (CXL-8, MMP-9) (28). Later, however, it became clear that patients with elevated BAL neutrophilia and IL-8 are those who display the best response to treatment with neomacrolides (most commonly azithromycin which was denominated azithromycin responsive allograft dysfunction or neutrophilic responsive allograft syndrome, see above). The same was later seen with BAL IL-17, which has been implicated in BOS. IL-17 is a major pro-inflammatory molecule inducing the release of IL-8, but is also implicated in the response to self-antigens. However, IL-17 staining in the lamina propria later revealed no difference in BOS compared to stable patients (29), while orthotopic lung transplantation in major mismatch mouse strains did not reveal a difference between wildtype and IL-17 knockout mice (30). In patients with lymphocytic bronchiolitis, who were treated with azithromycin, IL-17 positive cells disappear from the lamina propria and FEV1 increases (31).

Nevertheless, these patients may later still develop BOS, without IL-17 involvement. Evidence from other groups nevertheless suggest an important role for IL-17 in CLAD as treatment with an anti-IL-17 antibody or with halofuginone (which reduces IL-17), may attenuate features of chronic rejection in a murine transplant model (32,33).

Overall, none of the historically identified proteins seem to be a good biomarker for BOS development. In fact, a recent BAL cytokine and chemokine analysis, could not detect any molecule that was differentially regulated between stable (non-rejecting) patients and patients with BOS (34). Consequently, some groups have tried to identify blood markers blood for BOS development, but so far none have proven to be very sensitive and specific.

### Treatment

The widespread use of the neomacrolides has significantly impacted CLAD incidence and long-term survival. In fact, a randomized placebo controlled prevention trial with azithromycin initiated at hospital discharge following transplantation has shown that patients taking azithromycin demonstrate better pulmonary function, as well as decreased BAL neutrophilia and lower CLAD prevalence (35). A recent post-hoc analysis of this trial revealed that these long-term beneficial effects persisted and that azithromycin was able to significantly postpone the development of CLAD (36).

Treatment with macrolides in established CLAD also seems to be an adequate treatment option (37) but given the rarity of randomized controlled trials in this field, we do not know if either prophylactic or targeted treatment is superior. An expert task force concluded that currently available therapies have not shown a significant benefit in preventing or treating BOS, although investigation of possible underlying gastro-oesophageal reflux and a trial with macrolides in BOS is recommended (18). Some other therapies have shown promise in smaller, mostly single-center studies which are briefly discussed below.

Despite attenuation of neutrophils by azithromycin, in a subset of patients elevated airway neutrophilia later can redevelop. These patients usually present with a colonized graft (mostly pseudomonas) and demonstrate inferior survival compared to patients without neutrophilia (38). Interestingly, IL-1α is increased in BAL of those patients indicating that these alarmins might play an important role in the pathophysiology of BOS (39). Macrolide treatment does not seem to affect these patients (40). However
extracorporeal photopheresis (ECP), a leukapheresis-based procedure, was beneficial (41), and seems to be mainly an adequate treatment for patient with macrolide resistant airway neutrophilia (42). Part of this beneficial effect can be explained by effects of ECP on reducing inflammatory cytokines, chemokines and donor specific antibodies (43).

Montelukast, a cysteinyl leukotriene inhibitor is another possible treatment for BOS. A case series demonstrated a less pronounced decrease in pulmonary function in patients treated with montelukast compared to never treated patients (44). In a randomized placebo-controlled trial, montelukast was shown to be beneficial especially BOS stage 1 compared to placebo, but in later BOS stages no beneficial effects were seen (Ruttens et al. submitted).

As a last option for BOS, redo transplantation can be considered, amounting to about 5% of the total number of transplantations being performed annually. Although survival is not as good compared to a primary transplantation, for a well-selected group of patients redo transplantation may be the only option to improve outcome and quality of life (45). Given the scarcity of donor organs, this is not an option offered at every transplant center.

Restrictive CLAD (rCLAD)

Diagnosis, radiology and pathology

Besides the most commonly known BOS phenotype, the rCLAD seems to be gaining a lot of interest lately. Diagnostics remains troublesome at the moment. The initial report by Sato et al. used a decline in TLC of at least 10% to diagnose patients suffering from a restrictive pulmonary function defect (13), while Todd et al. used a FVC decrease >20% (46) and Verleden et al. used a combination of TLC and FEV/FVC (47). The common denominator in all these patients is the presence of persistent pleuroparenchymal infiltrates on CT imaging. Therefore Suhling et al. proposed to use a combination of pulmonary infiltrates on CT and pulmonary function measurements, specifically a TLC decrease >20% (48). In single-lung transplanted recipients, accurate rCLAD diagnosis is more complicated, given the confounding effect of the native lung, but a FVC decrease >20% was also associated with a poor outcome in a multi-center cohort study (49). This poor outcome is also a common denominator in all aforementioned studies: independent of the criteria used to diagnose restriction, outcome was worse in patients with a restrictive (rCLAD) vs. an obstructive (BOS) pulmonary function defect, with a median post-diagnosis survival of 6–18 months in rCLAD compared to 3–5 years in BOS (50). Prevalence of rCLAD is quite similar across different centers with 25–35% of CLAD patients affected (50). It is important to note that this classification is not absolute and that patients can evolve at any time during their post-transplant course from BOS to RAS or vice versa. Most often, patients evolve from an obstructive to a restrictive form of CLAD, however the opposite has also been described (13). Evolution from BOS to rCLAD is very difficult to diagnose, given the underlying severe obstruction, but it does not seem to imply a worse prognosis (51). A representative case with an initial BOS diagnosis is shown in Figure 1, as well as his evolution towards later rCLAD.

Nowadays diagnostic guidelines for rCLAD are lacking, CT is not implemented as a diagnostic criterium for rCLAD. However, rCLAD typically shows significantly different radiology compared to BOS, as there are signs of (sub)pleural thickening and pleuroparenchymal infiltrates. The land-mark study of Sato et al. showed an apical predominance in a significant subset of patients (13), which was in line with the earlier observation of upper-lobe dominant fibrosis post-lung transplantation (52). However, we recently demonstrated that there are also patients with diffuse or basal-dominated infiltrates on CT and interestingly, these patients had a worse outcome compared to patients with apical dominated fibrosis (51), while the degree of consolidation, ground glass or reticulation did not correlate with survival post diagnosis (53). CT could also be used as alternative tool to diagnose rCLAD, as lungs have significantly lower lung volume compared to baseline, while the volume of lungs in BOS remains stable or even increases (54). This could provide an easy to interpret, add-on tool to diagnose rCLAD when pulmonary function tests are inconclusive. Thorough investigation of rCLAD explant lungs using CT and microCT demonstrated disappearing airways on CT, with OB in 30–40% of the remaining airways. Further, microCT showed a decrease in the number of terminal bronchioles (the last conducting airway before the alveoli). Therefore, this indicates that the airways are also involved in rCLAD, although the proportion of OB lesions was not that high as in pure BOS (55). Next to this airway involvement, the alveoli looked completely different reflecting interstitial and/or alveolar fibrosis.

On pathological examination pleuroparenchymal fibro-elastosis is the most common histological pattern of rCLAD (56). Molecular analysis of this alveolar fibro-elastosis pattern revealed that the initial changes are a non-
A patient underwent heart lung transplantation for Eisenmenger’s syndrome with an initial uneventful follow-up post discharge who developed BOS. 5 years post-transplantation (pulmonary function evolution in A), but without decrease in TLC (B). However 10 years post-transplantation there was a sudden TLC drop (red line indicates 10% decrease) and therefore diagnosis was changed to rCLAD/RAS. CT evolution is shown in panel 1C-D-E-F. Initially, the patient had a normal CT (C), which remained unchanged after BOS diagnosis (D). However, when the decrease in TLC was found, persistent apical infiltrates were seen on CT (E), which deteriorated at the last CT before successful redo transplantation (F). The histological analysis of this explant lung confirmed rCLAD diagnosis as a pattern of pleuroparenchymal fibro-elastosis and OB was observed.

Specific fibrin reaction to a yet unknown injury, which progresses to a failed attempt to resolve this, resulting in manifest fibro-elastosis (57). The Melbourne group first described such a pattern consistent with acute fibrinous and organising pneumonia (AFOP) on transbronchial biopsy, which is also associated with a non-obstructive pulmonary function decline, persistent infiltrates and poor outcome in surviving patients (58). Thus, AFOP and rCLAD are likely to represent two entities (acute-chronic) of the same fibrotic spectrum.

**Risk factors and mechanisms**

The body of evidence for risk factors specific for rCLAD is not that robust as for BOS, although it seems that many risk factors are similar between both phenotypes. Indeed, acute cellular rejection, lymphocytic bronchiolitis, colonization with Pseudomonas, infection, and BAL neutrophilia were equally important for later BOS and rCLAD (59). Of interest BAL and blood eosinophilia, a cell that is mostly discarded in lung transplantation because of its low relative abundance, shows a strong association with subsequent development of rCLAD (60). Moreover, in patients diagnosed with rCLAD, BAL and blood eosinophilia are also able to dissect those rCLAD patients with the worst prognosis, indicating that eosinophilia could serve as an easy marker for rCLAD development and prognosis following diagnosis (51). Other studies focused on particular (inflammatory) cytokines and chemokines. For example, specific increase in pro-inflammatory alveolar alarmins (61), IL-6 and IP-10 (34) could be important in the pathophysiology of rCLAD. An immunohistochemistry study of rCLAD explant lungs revealed pronounced inflammation, with a significant increase in macrophages, neutrophils, mast-cells, eosinophils, CD8 T-cells and interestingly B-cells. These B-cells were organized in
lymphoid follicles, which is a common finding in other chronic respiratory diseases (62). Given this presence of lymphoid follicles, it comes as no surprise that immunoglobulin levels were also increased in rCLAD (63). Therefore, this raises the question to which extent rCLAD overlaps with chronic antibody-mediated rejection (AMR). AMR is an acute or subacute form of graft injury wherein antibodies against donor human leukocyte antigens cause characteristic lung histology (for instance neutrophilic capillaritis) with or without evidence of endothelial C4d staining (64). The presence of HLA antibodies seems to be more associated with rCLAD compared to BOS which is in line with the hypothesis of (at least part) overlap (65).

Of interest is also that in BAL, VEGF levels are decreased in rCLAD patients (34), which is in line with the hypothesis that the capillary network is of importance, which was also demonstrated in a descriptive pathological study (66). In contrast, the lymphatics do not seem to be altered in rCLAD, which is surprising given the predominant distribution (pleural and septal) of fibrosis in rCLAD (67). Despite these interesting observations, more research is needed to elucidate the pathophysiological mechanisms in rCLAD.

Treatment

Similar as in BOS, treatment of rCLAD remains troublesome. The disease course is very unpredictable, given that the disease evolution follows a stepwise pattern of decline: an acute phase characterised by acute lung injury (diffuse alveolar damage, DAD), followed by a resolution stage, during which fibrosis further develops (68). Therefore, patients who at first seem stable can evolve rapidly to a more severe (sometimes even life-threatening) disease stage requiring urgent redo transplantation or death. In that respect, it is important to realize that survival after redo transplantation for rCLAD is inferior compared to BOS, which by itself is already worse compared to survival after primary transplant [3-year survival of 67% in BOS and 33% in rCLAD (69)]. Also, CLAD more frequently redevelops following redo transplantation for rCLAD, again limiting long-term survival. Given these disappointing results, anti-fibrotic treatment may be a good option, based on the positive experience in IPF patients, where it has been shown to slow down the FVC decline (70). Although the experience in treating rCLAD patients is limited at this moment, case reports of successful treatment with pirfenidone (71) and nintedanib (72) described stabilization of the disease, which may be considered a success given the bad prognosis after diagnosis. Nevertheless, no large cohorts have been described so far and therefore more evidence is needed before antifibrotics can be introduced in general clinical practice. ECP therapy does not seem to be able to slow down rCLAD progression and therefore does not seem a viable option (42). Another drug with potential to slow down disease progression is alemtuzumab (Campath-1H), an antagonist of CD52 which is expressed on B-cells, lymphocytes, dendritic cells and monocytes. This drug was found to improve interstitial changes and lung function in four patients who likely had rCLAD (73), while it was also described in successful treatment of persistent acute rejection (74). Another approach of treating rCLAD might be trying to decrease or erase HLA antibodies by using plasmapheresis, intravenous immunoglobulins and rituximab, which has shown to be partly successful in at least reducing the antibody titre (75). However, true efficacy in treating or stabilizing rCLAD remains unknown. Therefore, at present, there are little effective therapeutic options for rCLAD. Hopefully, a better understanding of the pathophysiological mechanisms will lead to a rapid and efficient therapeutic strategy which is desperately needed given the poor outcome of these patients.

Conclusions

BOS and rCLAD are separate entities within CLAD, with their own clinical, radiological and pathological characteristics (see Figure 2 for illustration). To what degree these syndromes differ is at this moment unknown. Given the overlap in risk factors and the fact that OB lesions are detected in both syndromes, and the possible evolution of one syndrome to another, there is likely at least some degree of overlap between BOS and rCLAD. More importantly, rigorous identification of the different phenotypes is clearly needed for both clinical and scientific purposes. Further advance in this field is limited by the absence of uniform diagnostic criteria for rCLAD, which makes the design of multicentre studies nearly impossible. Yet, given the rather low incidence of rCLAD in individual centers, monocentric studies are currently hampered by the number of patients that can be included. Only by doing so, we can adequately power and design clinical trials which are desperately needed given the disappointing outcome after lung transplantation compared to other solid organ transplantations. These different phenotypes are nonetheless an indication that the future will probably lie in individualized therapy, needed to further improve survival.
Acknowledgements

Funding: SE Verleden is sponsored by grants from the FWO (FWO12G8715N and 1515816N). R Vos is supported by UZ Leuven (STG15/023). BM Vanaudenaerde is sponsored by the KU Leuven (C24/15/030).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References


Figure 2 Comparison between BOS and rCLAD. Gross image of a BOS lung (A), with the CT showing typical hyperinflation (B), while the explant lung specimen shows obliterative bronchiolitis (arrow) (C). Gross lung image of RAS/rCLAD lung (D), with the CT showing ground glass and reticulation (E) and the pathology showing newly formed fibrosis (arrows). H&E stainings are used in C and F.


30. Yamada Y, Vandermeulen E, Heigl T, et al. The role of


