A NEW CLASSIFICATION SYSTEM FOR CHRONIC LUNG ALLOGRAFT DYSFUNCTION

Geert M. Verleden, Ganesh Raghu, Keith C. Meyer, Allan R. Glanville, and Paul Corris for the ISHLT pulmonary council

Key words: Lung Transplantation, classification system, CLAD, BOS, RAS

Running title: CLAD definition paper

Address for correspondence: Geert M. Verleden, MD, PhD
University Hospital Gasthuisberg
Lung Transplantation Unit
49, Herestraat
B-3000 Leuven, Belgium
Tel: + 32 16 34 6805
Fax: + 32 16 34 6803
Mail: geert.verleden@uzleuven.be
Summary
Although survival after lung transplantation has improved significantly over the last decade, chronic rejection is thought to be the major cause of late mortality. The physiological hallmark of chronic rejection has been a persistent fall in FEV1 associated with an obstructive ventilatory defect for which the term bronchiolitis obliterans syndrome (BOS) was defined to allow a uniformity of description and grading of severity throughout the world. Although BOS was generally thought to be irreversible, recent evidence suggests that some patients with BOS may respond to azithromycin with an improvement of their FEV1 with > 10%. In addition, a restrictive form of chronic rejection has recently been described, which does not fit the strict definition of BOS as an obstructive defect may not be apparent. Hence, the term chronic lung allograft dysfunction (CLAD) has been introduced to cover all forms of graft dysfunction but as yet has not been defined. In this manuscript we propose a definition of CLAD and describe a flow chart which may help to properly diagnose the different phenotypes of CLAD.
Introduction

Survival after lung transplantation remains significantly shorter than survival following transplantation of other solid organs. This disparity in survival has been attributed to the development of chronic rejection, which represents a major complication that limits the 5-year survival to approximately 55% (1). Initial investigations that examined lung tissue from recipients with persistent decline in allograft function after lung transplantation showed histopathologic changes of obliterative bronchiolitis (OB), which was perceived to be a consequence of chronic rejection that principally occurred via allo-immune mechanisms (2, 3). However, histopathological confirmation of OB is difficult to obtain via transbronchial lung biopsies due to limited sampling of lung tissue compared to surgical lung biopsy (4). Hence, an International Society for Heart and Lung Transplantation (ISHLT) committee introduced the term, bronchiolitis obliterans syndrome (BOS), which was originally meant to reflect chronic FEV1 decline due to the development of OB (5). The introduction of this clinical surrogate marker of chronic allograft dysfunction allowed the creation of a uniform system based upon FEV1 measurements that could be used worldwide to describe persistent decline in lung function due to progressive OB in the lung allograft. However, it has become clear that a number of allograft or extra-allograft abnormalities can also lead to persistent decline in FEV1.

As a consequence of new insights into the pathophysiology of BOS and the evolution of strategies to treat patients with BOS, an update of the initial statement on criteria for the diagnosis of BOS was published in 2002 (6), and a second revision is currently in preparation (7). During the process of preparing the latest revision, the ISHLT/ATS/ERS committee members acknowledged that there was a substantial cohort of patients with chronic FEV1 decline after lung transplantation for whom the previous definition of BOS was not the best descriptor. This statement will focus on the description of additional entities that can lead to chronic FEV1 decline after lung transplantation and introduce a new classification system that defines various terms that can be used to help the lung transplant community recognize distinct entities with important differences in their clinical manifestations and pathobiology. It is envisaged that a broadly accepted definition of chronic lung allograft dysfunction (CLAD) along with the recognition of different clinical entities that can lead to CLAD may facilitate investigations that seek to elucidate the pathophysiological mechanisms that lead to CLAD and thereby potentially suggest new strategies that may improve long-term survival after lung transplantation.
Lung allograft dysfunction

Lung allograft dysfunction may be an acute phenomenon (acute lung allograft dysfunction, ALAD), leading to an acute decline in FEV\(_1\) and/or FVC and may be due to various conditions that affect the graft. Such conditions include acute infection, pulmonary embolism, acute rejection, etc. These conditions are usually responsive to specific treatment, which may indeed restore the FEV\(_1\) and/or FVC to baseline values. If, however, the pulmonary function decline is not restored to > 90% of baseline and persists for 3 months, chronic lung allograft dysfunction (CLAD) is suspected.

It should be noted that for every decline in FEV\(_1\) and/or FVC, the authors suggest to use the postbronchodilator lung function (after 200-400 microgram of albuterol), as comparator to the best postoperative values.

Chronic lung allograft dysfunction (CLAD)

CLAD is a term that was first introduced in the lung transplant literature in 2010 (8) although no precise definition currently exists. This document will propose such a definition in the attempt to draw together some disparate notions regarding this condition and standardize its use in describing loss of lung allograft function. We recommend that CLAD should not be used as a synonym for BOS. The authors strongly support the continued use of the term BOS where appropriate and according to the ISHLT definition (6). CLAD is an overarching term that, in common usage, embraces all forms of chronic lung dysfunction post-transplant and, therefore, includes all cases of BOS. It is recognized that individual patients may have more than one reason for declining graft function. For example, OB can manifest as BOS with associated chronic graft infection, and appropriate treatment and suppression of infection may lead to improved allograft function. It is important to fit the usage of the CLAD acronym to the common English meaning of the words, hence an alternative definition can be “a transplanted lung that does not achieve or maintain normal function for an arbitrarily defined period of time”. Hence a lung that does not achieve normal function (e.g. due to severe primary graft dysfunction) could be described as having CLAD, even if the FEV\(_1\) is slowly improving but remains with a function that is significantly impaired when measured against predicted normal physiological indices. Therefore, the term, CLAD, could be used in this situation, although it would most often be used to describe loss of function from the best post-transplant FEV\(_1\) achieved once the function of the implanted allograft has stabilized. The use
of the term CLAD, per se does not (and cannot) make any assumptions regarding the potential reversibility or irreversibility of the underlying causes of allograft dysfunction. Nor is the term CLAD so specific as to justify its use as a diagnosis. CLAD is simply a descriptor of sustained lack of normal function of the transplanted lung or, more commonly, a persistent decline in comparison to the best post-operative FEV₁. Every effort should be made to identify the specific cause of persistent decreased function in the hope that appropriate and successful therapeutic interventions can be undertaken to restore and optimize graft function. Various conditions that may cause CLAD are described below, and a diagnostic algorithm is given in Figure 1.

Chronic implies an arbitrary duration of time, and from combined experience, we consider three months to be a sufficiently prolonged period to label allograft dysfunction as “chronic”. Persistence of causes of graft dysfunction (figure 1, specific causes) for longer than three months that may be potentially reversible, still fits this definition. Although a number of broad phenotypes have been described that fit the term CLAD, we recommend that the term should not be used as an all embracing term for graft dysfunction etiologies. Rather, its detection should stimulate a rigorous evaluation to determine the reason(s) why a dysfunctional lung allograft does not work or no longer works properly. It is emphasized that CLAD may not be a permanent situation, as it may be reversible upon specific treatment. It should be recognized that despite use of the term, “chronic,” we do not advocate waiting three months prior to investigating the cause of declining function or persistent graft dysfunction and initiating potential therapies to improve/restore lung function.

In contrast to the BOS classification, which is solely based on FEV₁ decline, we suggest that when CLAD has been identified, further investigation, including full pulmonary function testing (measurement of total lung capacity and residual volume in addition to spirometry), bronchoalveolar lavage (BAL) with total and differential cell count, and high-resolution chest tomography (HRCT) of the thorax with inspiratory and expiratory imaging may provide additional information that facilitates the identification of specific CLAD phenotypes (Fig. 1). We also suggest that a decline of 10% in FEV₁ and/or FVC from stable baseline function (suspected CLAD) should trigger an investigation to identify a cause or causes of functional decline such as acute cellular, antibody-mediated rejection, lymphocytic bronchiolitis, and azithromycin- responsive allograft dysfunction (ARAD).
**Bronchiolitis obliterans syndrome (BOS)**

When the FEV₁ decline (≥20%) is not only **persistent**, this means in two measurements at least 3 weeks apart, but also **obstructive**, we recommend that BOS should continue to be used as the preferred term to describe this phenotype of CLAD. It is clear that not every patient should/will go through a stage of suspected CLAD, as some patients may already have lost > 20% of their FEV₁ when they are picked up. Our revised definition of BOS, as given in the soon-to-be-published 2013 revision document (7), emphasizes that a decline in lung function that meets BOS criteria may be partially or even completely reversible (in contrast to previous definitions where this possibility was considered but not formally addressed) if it responds to specific treatment options (e.g. azithromycin). Indeed, in the 2001 revision of the BOS statement, although it was acknowledged that BOS may be reversible, reversibility was considered an exception (6), which is indeed logical if one assumes that BOS reflects the development of OB with irreversible small airway obliteration. It has become clear that small airway disease is not necessarily fibrotic and irreversible, especially in early stages. Additionally, if evidence of infection is detected, aggressive measures to treat infection may improve lung function, and new evidence suggests that lung function decline consistent with the diagnosis of BOS can stabilize or improve in some recipients (9, 10).

Several groups have now shown that about 40% of patients with BOS respond to azithromycin with an increase in their FEV₁ of at least 10%, and that some patients may experience complete reversal of their decline in FEV₁ and return to BOS stage 0 (11, 12, 13). A persistent increase in BAL neutrophil percentage has been recognized to predict an increased risk for the development of BOS (14, 15, 16), and when a BAL neutrophil percentage > 15% is detected, administration of azithromycin has been associated with a significant improvement in FEV₁ in a substantial number of patients (17, 18). Other reports, however, have shown that BAL neutrophilia in the setting of BOS is mostly due to coexistent infections, which therefore need to be carefully excluded (19). Additionally, the role of BAL neutrophilia in predicting the response to azithromycin is not corroborated in other studies for instance in the Meloni study, a significant FEV₁ response to azithromycin could also be demonstrated when BAL neutrophilia was not detected and vice versa (20).

Responders to azithromycin (defined as a FEV₁ increase of ≥ 10% after a 2-3 month treatment) were initially classified as having neutrophilic reversible allograft dysfunction (NRAD) (21), but should be renamed as azithromycin reversible allograft dysfunction
(ARAD) or azithromycin-responsive BOS, keeping in mind that this phenotype can only be determined retrospectively after the decline in FEV$_1$ has been diagnosed and subsequently responded to azithromycin. These findings also suggest that all patients who experience lung function decline consistent with CLAD, with or without BAL neutrophilia, should receive a trial with azithromycin for at least 2-3 months. Long-term follow-up of azithromycin responders will be required to inform us as to whether azithromycin therapy simply delays the inevitable development of BOS, which was indeed recently suggested in a placebo-controlled trial with azithromycin during the first 2 years after lung transplantation (22) and whether the natural history is identical to that of BOS when BAL neutrophilia is not detected.

We suggest that patients who do not respond to azithromycin represent a phenotype of BOS that is characterized by fibrotic OB (20).

Imaging with HRCT may also be useful to assist in the identification of BOS phenotypes. One phenotype may show a combination of air trapping, tree-in-bud opacities, and peribronchiolar infiltrates that are compatible with the presence of bronchiolitis, and treatment with azithromycin may improve and even clear the peribronchiolar infiltrates and tree-in-bud opacities (23). Conversely, as previously reported, BOS 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 (23, 24).

It must also be recognized that both of these conditions may co-exist, so that such recipients may improve their FEV$_1$ > 10% upon azithromycin treatment, but complete reversal is unlikely to occur. These patients are more likely to have persistent BOS due to the presence of a significant degree of irreversible OB lesions (21).

Other conditions have been described that may evolve and cause a decline in allograft function that fits criteria for CLAD and BOS (persistent and progressive obstructive FEV$_1$ decline). These include exudative (25) or follicular (26) bronchiolitis, in which the small airways are compressed by lymphoid follicles in the airway wall, whereas the airway mucosa itself remains normal. Thoracic HRCT may help identify these specific CLAD phenotypes that can be associated with intense airway inflammation and BAL neutrophilia (25, 26). The exact pathogenesis of these conditions when they occur following lung transplantation is
unknown. Whether these represent chronic rejection processes versus local immunological (and possibly autoimmune) phenomena remains to be elucidated.

**Restrictive allograft dysfunction**

Recent publications have reported that some patients may experience a *persistent* decline in vital capacity (VC) and total lung capacity (TLC) that is accompanied by a decline in FEV$_1$ of $>$ 20%, which has been termed restrictive allograft syndrome (RAS) (27). In comparison to BOS, we also recommend that there should be two pulmonary function measurements at least 3 weeks apart showing this decline. Depending on the relative change between the FEV$_1$, the FEV$_1$/FVC ratio and the TLC, these findings may lie outside the usual definition of BOS, which specifies a decline in FEV$_1$ with evidence of an *obstructive* ventilatory defect. When lung volumes are not specifically measured, a restrictive ventilatory defect is more firmly suggested if the FEV$_1$ and FVC simultaneously decrease while the FEV1/FVC ratio remains normal or increases above the normal range (28).

The majority of the recipients with RAS reported by Sato et al. had persistent (over a 3 to 6 month period) infiltrates on thoracic HRCT (ground glass opacities, interstitial infiltrates, possible honeycombing) with upper lung zone predominant fibrotic changes as described previously (29). The presence of HRCT findings of parenchymal fibrosis is consistent with histopathological findings from two autopsy case series that examined post mortem lungs from lung transplant recipients (30, 31). Additionally, it was recently demonstrated that a temporal sequence of diffuse alveolar damage followed by the development of pleuroparenchymal fibroelastosis may occur in the histopathologic evolution of RAS (32).

Whether these infiltrates and the accompanying restrictive pulmonary function defect represent chronic rejection remains to be determined. Nonetheless, the Toronto group has clearly demonstrated the presence of diffuse alveolar damage and extensive fibrosis (in the alveolar interstitium, visceral pleura and interlobular septae) with or without scattered OB lesions in their recipient cohort with RAS, which suggests that chronic rejection may indeed be involved in the pathogenesis of this CLAD phenotype (27).

The importance of recognizing this specific type of CLAD is suggested by the significantly worse survival of patients with RAS compared to recipients with obstructive BOS (27, 28).
However, this finding has yet to be substantiated by reports published by other transplant centers.

Therefore, using comprehensive pulmonary function testing plus BAL and HRCT to identify and differentiate graft dysfunction phenotypes such as those associated with a significantly increased BAL neutrophil count and features of obstructive BOS from those associated with a predominant restrictive physiology due to parenchymal fibrosis, can facilitate the detection of CLAD phenotypes for which prognosis and treatment may vary.

It should be noted that some patients who develop the RAS phenotype initially display a typical FEV\textsubscript{1} decline that is compatible with BOS criteria but subsequently develop persistent parenchymal infiltrates later in their course that may precede the evolution of a restrictive physiology. These changes may occur over a somewhat prolonged period of time, but the development of infiltrates on HRCT is fairly predictive of the conversion from obstructive BOS to the RAS phenotype, even though the pulmonary function decline may not be consistent with a restrictive pattern when the HRCT changes are first detected (27, 28).

**Future needs**

Using the term CLAD to stimulate a search for a specific phenotype based upon a combination of the pattern of pulmonary function decline, BAL cellular analysis, and HRCT acquisition may allow identification of phenotypes for which prognosis and treatment interventions vary significantly. We suggest that the term CLAD should be used as a suitable alternative to BOS Stage 0p and that the detection of FEV\textsubscript{1} decline of more than 10% from a stable, best FEV\textsubscript{1} value should trigger an evaluation that seeks to determine the cause(s) that explain the decline in lung function. However, we also recognize that not every patient can necessarily fit cleanly into a specific phenotype (e.g. BOS, RAS, ARAD), and phenotypes may overlap significantly. Moreover, some situations are likely to still be very difficult to decipher and classify. One example would be a patient who develops multiple sequential acute rejection episodes and eventually develops a persistent decline in FEV\textsubscript{1} in the setting of ongoing acute rejection. Such a situation has been very well illustrated by Martinu et al. with their examination of pathologic correlates of BOS in explants of patients who have undergone retransplantation (31). Their findings seem most consistent with CLAD related to underlying persistent acute rejection. Similarly, patients who develop recurrent infectious episodes or persistent infection may have CLAD. Some infections may be occult (eg chlamydia)( 33)
versus infections with more prominent clinical manifestations (e.g. Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus fumigatus) even in the setting of development of bronchiectasis (34, 35, 36).

It may also prove difficult to classify patients with significant variability in pulmonary function over time as meeting criteria for CLAD. By definition, CLAD is not present unless dysfunction is sustained, and alternative etiologies for impaired allograft function must be ruled out. The combination of BAL data and HRCT may increase the clinician’s ability to make an accurate and confident diagnosis in specific situations. Additionally, the presence of significant anastomotic dysfunction due to stricture or bronchomalacia can lead to reduced FEV$_1$ and is perfectly in keeping with the definition of CLAD if persistent.

Many issues have yet to be resolved. These include the classification of RAS into different stages or levels of severity, as has been done for both obstructive BOS staging (5, 6) and for primary graft dysfunction (37). Another issue is the identification of HRCT imaging patterns that may be highly specific for individual CLAD phenotypes. Table 1 summarizes the key features of the CLAD phenotypes.

There are undoubtedly more non-classifiable conditions that fit under the broad umbrella of CLAD. We hope future investigations will refine this proposed model for phenotyping CLAD and identify the different pathophysiological mechanisms underlying these phenotypes. Well-powered, collaborative multicenter studies are best suited for investigations that will improve our understanding of CLAD and will likely foster better treatment options and improved long-term survival in our lung transplant recipients.

**Conclusion**

In the current manuscript, we have offered a tool for better phenotyping pulmonary function decline in lung transplant patients. We identified “Acute Lung Allograft Dysfunction” (ALAD) which may be due to acute rejection, infection, pulmonary embolism, etc and may be reversible upon specific treatment. If reversibility is $<$90% of postoperative best FEV$_1$ and or FVC, ALAD may become “chronic lung allograft dysfunction” (CLAD), which is an umbrella term to define a persistent (at least 3 months), often unexplained decline in pulmonary function (FEV$_1$ and/or FVC) $\geq$ 10% from baseline (baseline defined as the average of the two best post-transplant values for FEV$_1$ and FVC obtained at least 3 weeks apart).
Diagnosis of CLAD should be the trigger to initiate further investigations into the cause and may therefore replace BOS 0p in some circumstances. CLAD may still be reversible upon specific treatment (reversibility defined as an FEV$_1$ and/or FVC increase to >90% of the best postoperative values) and such causes may include infection, pulmonary embolism, gastro-esophageal reflux, extra-allograft disorders (pleural disorders, obesity, diaphragmatic dysfunction, native lung disorders, ascites,...). Azithromycin-responsive allograft dysfunction (ARAD) is also part of the treatable/reversible causes and a BAL neutrophilia of > 15% may predict a response to azithromycin, although reversibility may also occur when BAL neutrophilia is absent. Therefore, the authors support an azithromycin trial for 2-3 months (which may improve and stabilize allograft function) before a diagnosis of CLAD due to BOS or RAS is made. If, despite treatment, the FEV$_1$ keeps declining with ≥ 20% from baseline, every effort should be undertaken in all cases to further phenotype CLAD so that in the future specific therapeutic options may be developed and employed.
<table>
<thead>
<tr>
<th>Entity</th>
<th>Classic BOS</th>
<th>ARAD</th>
<th>RAS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulmonary function</strong></td>
<td>- Obstructive (FEV1 ≤80% of stable baseline value)</td>
<td>-Obstructive (FEV1 ≤80% of stable baseline value)</td>
<td>-Restrictive (TLC ≤90% of stable baseline value)</td>
</tr>
<tr>
<td>HRCT Imaging</td>
<td>- Air trapping usually present</td>
<td>- Changes of constrictive bronchiolitis (“tree-in-bud”, peri-bronchiolar infiltrates often present)</td>
<td>- Infiltrates usually present</td>
</tr>
<tr>
<td></td>
<td>- No/minimal infiltrates</td>
<td>- ± air trapping</td>
<td>- ± bronchiectasis</td>
</tr>
<tr>
<td>HRCT Imaging</td>
<td>- ± bronchiectasis</td>
<td></td>
<td>- ± air trapping</td>
</tr>
<tr>
<td>Histopathology</td>
<td>- OB (difficult to diagnose via transbronchial biopsy)</td>
<td>- Cellular bronchiolitis</td>
<td>- Parenchymal/pleural Fibrosis ± OB</td>
</tr>
<tr>
<td>Clinical course</td>
<td>- Typically progressive but may stabilize</td>
<td>- High likelihood of significant response to azithromycin (may no longer meet criteria for persistent BOS if recipient is an azithromycin responder)</td>
<td>- Tends to be relentlessly progressive</td>
</tr>
<tr>
<td></td>
<td>- Recipients may have coexistent chronic bacterial infection</td>
<td></td>
<td>- May start as/coincide with BOS</td>
</tr>
<tr>
<td></td>
<td>- May evolve to RAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>- Usually responds poorly to pharmacologic therapies</td>
<td>- BAL neutrophilia (e.g. ≥15% on differential cell count) may correlate with response to azithromycin therapy but is not necessary for a response</td>
<td>- Correlates with the presence of early DAD post-transplant</td>
</tr>
</tbody>
</table>

*Infection, other pathologies (e.g. acute cellular rejection, lymphocytic bronchiolitis, antibody-mediated rejection), and/or other causes of allograft dysfunction (e.g. significant gastroesophageal reflux, pleural disorders, anastomotic dysfunction, obesity, thromboembolic disease, recurrent primary lung disease, etc.), must be ruled out

Abbreviations: BAL = bronchoalveolar lavage; BOS = bronchiolitis obliterans syndrome; CLAD = chronic lung allograft dysfunction; DAD = diffuse alveolar damage; ARAD = azithromycin-responsive allograft dysfunction; OB = obliterative bronchiolitis; RAS = restrictive allograft syndrome
References


36. Miller WT Jr, Kotloff RM, Blumenthal NP, Aronchick JM, Gefter WB, Miller WT. Utility of high resolution computed tomography in predicting bronchiolitis obliterans

Figure 1

This flow chart represents an approach that can be used to evaluate a lung transplant recipient’s decline in postbronchodilator FEV$_1$ and/or FVC of $\geq 10\%$. This may be acute (ALAD) and may normalize with treatment. However, if the lung function decline persists for 3 months without the FEV$_1$ or FVC returning to $> 90\%$ of the postoperative best values, CLAD is suspected. Extended PFT (spirometry and lung volumes), HRCT, and bronchoscopy with BAL and transbronchial biopsies may identify a cause or causes of suspected CLAD that may still be reversible upon specific treatment. If despite treatment, or without identifying a clear cause, the FEV$_1$ and/or FVC declines further to $\leq 80\%$ of the postoperative best values, a specific CLAD phenotype should be identified.

Abbreviations: ALAD = acute lung allograft dysfunction; BOS = bronchiolitis obliterans syndrome; CLAD = chronic lung allograft dysfunction; CXR = routine chest X-ray; FEV$_1$ = forced expiratory volume in 1 second; HRCT = high-resolution computed tomography of the thorax; PFT = pulmonary function testing; SLT = single lung transplant; ARAD = azithromycin-responsive allograft dysfunction; RAS = restrictive allograft syndrome.