Lung allograft loss: naming helps seeing... and vice versa!

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Peter Dorfmueller¹ and Tom Kotsimbos²

Affiliations: ¹Dept of Pathology and INSERM UMR_S 999, LabEx LERMIT, Marie Lannelongue Hospital, Le Plessis-Robinson, France. ²Dept of Medicine, Central Clinical School, Monash University, Dept of Allergy, Immunology and Respiratory Medicine, Alfred Hospital, Melbourne, Australia.

Correspondence: Dept of Medicine, Central Clinical School, Monash University, Dept Allergy, Immunology and Respiratory Medicine, Alfred Hospital, Melbourne, Victoria, 3004, Australia. E-mail: tom.kotsimbos@monash.edu

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The many forest fires of chronic lung allograft dysfunction: where are they, when were they lit and how do they burn? <http://ow.ly/Sob6f>

The limits of my language is the limit of my imagination — Ludwig Wittgenstein

There is a crack in everything – that is how the light gets in — Leonard Cohen

The loss of allograft function in the long term (whatever its pattern over time) not only remains the single major cause of death following lung transplantation but has continued largely unabated since the beginning of the modern era in the 1980s [1–3]. As obliterative bronchiolitis (OB) was the most common histopathological finding on biopsy of the failing lung allograft, this feature became the unequivocal manifestation of chronic rejection in the lung allograft [3]. The logistical difficulties of obtaining frequent and numerous sampling biopsies to reliably and easily detect OB, however, led to another leap of faith where a syndromic definition (bronchiolitis obliterans syndrome; BOS) became the “working definition” of chronic rejection in the lung allograft [4]. BOS had both defining features that had to be present (irreversible loss in forced expiratory volume in 1 s (FEV₁) as a percentage of the best achieved post-transplant) and exclusionary ones (the irreversible loss in FEV₁ was “otherwise unexplained”; e.g. clinically relevant airways infection and aspiration had to be absent) [4, 5]. However, although the concept of BOS greatly increased the ease with which “chronic rejection” could be diagnosed after lung transplant, it also railroaded thinking for many years. In particular, a protocolised definition of chronic rejection through the diagnostic label of BOS hindered thoughts that infection (key non-alloimmune factor)- and alloreactivity-associated obliterative bronchiolitis may not necessarily be independent events [6, 7] and that chronic rejection could either have a reversible component or manifest as a restrictive pattern of lung allograft loss [8].

It was therefore only a matter of time before the concept of chronic lung allograft dysfunction (CLAD) came to the rescue by opening up thinking that immediately embraced a broader range of potential pathways leading to irreversible allograft loss following lung transplantation [8, 9]. In particular, the increased sensitivity of this diagnostic label created a new canvas of possibility for how lung allograft loss could be conceptualised and mapped onto the pathobiological mechanisms that were likely to be the key driving factors (alloreactivity-associated inflammation, infection, nonspecific injury–repair dysregulation). Within this framework, the complexity, or better, the duplicity of CLAD was underscored by the Toronto group who, in 2011, first proposed a further subset label for a distinct non-obstructive phenotype of CLAD: the restrictive allograft syndrome (RAS) [10]. RAS appears to complicate the post-transplant evolution of approximately 30% of all patients with CLAD and is associated with a worse prognosis. Its definition relies

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on restrictive functional changes, interstitial pulmonary infiltrates on computed tomography (CT) scan and, when available, interstitial fibrosis and pleuro-parenchymal fibroelastosis of the lung periphery on histology.

While the original report by SATO *et al.* [10] proposed a new clinical sub-entity which related peculiar functional alterations with specific radiological changes, eventually confirmed by histology, VERLEDEN *et al.* [11], in this issue of the *European Respiratory Journal*, are putting emphasis on the morphological correlate of clinically observed RAS and perform a comparative in-depth analysis of pulmonary changes in lungs from RAS and BOS patients. They attempt to establish a detailed cartography of specific and discriminating lesions in both settings, on the macroscopic, as well as on the microscopic level. The “test” series that is used for the present analysis is exceptional, systematically combining routine clinical physiological testing and imaging with experimental radiology tools, such as micro-CT and detailed histology, in order to achieve a global radio-morphometric survey of the CLAD-lung. In this way, more expansive ways of *thinking* about lung allograft loss are being combined with new ways of *seeing* lung allograft functional and structural derangement, thereby giving us a more sensitive and specific language and evidence base with which to better unravel and understand the development of CLAD in an individual lung transplant recipient.

Indeed, VERLEDEN *et al.* [11] describe a detailed cross-sectional interrogation of explanted lungs from lung transplant recipients undergoing a repeat lung transplant procedure akin to an in-depth analysis that is usually only possible from experimental animal studies in which the whole lung can be readily examined, with the added benefit of being immediately clinically relevant. However, there are several provisos.

First, in the current report by VERLEDEN *et al.* [11], the selection of characteristic BOS and RAS clinical phenotypes from a small group of lung transplant recipients undergoing a relatively rare re-transplantation procedure necessarily frames how the subsequent data patterns identified should be analysed and interpreted. Specifically, do the “typical” BOS and RAS clinical phenotypes chosen for study represent two extreme ends of the CLAD spectrum or two distinct processes with significant overlap? Arguably both scenarios may be at play as suggested by the normally distributed loss of airways data for both BOS and RAS [11]. The observed reduction in number of visible airways from bronchial generation six to 11 in RAS as compared to BOS, as well as the fact that substantial narrowing of bronchioles is present in RAS (even greater than in BOS when considering generations three to six), further questions whether these OB lesions are an extension of RAS type pathological processes, a separate phenomenon in their own right, or an acceleration of “typical” OB in a RAS microenvironment. Of additional interest here is that the usual risk factors associated with BOS [3–5] are relatively evenly represented in the highly selected BOS and RAS groups undergoing re-transplantation in this report. Although contrasting RAS to BOS as the dichotomy of a peripheral/interstitial *versus* a central/bronchial insult makes a lot of sense in physiological terms (*i.e.* restriction *versus* obstruction), this may not be so useful in the tissue or cellular dimensions. For example, at this level of resolution the presence of a broad alloreactivity may be manifest as differential pathology at various sites as a function of underlying differences in hypoxic injury that either occurred during an initial ischaemia–reperfusion insult or later secondary to the development of vascular rejection that may or may not parallel airway rejection processes. The possibility that CLAD phenotypes may be interlocking in complicated ways is particularly intriguing and immediately begs the question as to what is the minimum number of common processes at play that may explain most of the spectrum and variance in histopathology seen in the explanted lung allograft tissues studied. The authors propose an expanding fibrotic process beginning in the periphery and “overrunning” the terminal and pre-terminal bronchioles. Interestingly, this peripheral interstitial remodelling appears to predominate in the upper lobes and is associated with fibrous thickening of the visceral pleura, resembling idiopathic pleuro-parenchymal fibroelastosis (a rare pulmonary disease which shares the topography as well as the morphology of RAS [12]). In contrast, classic bronchiolitis obliterans does not show predilection for the upper lobes, but is observed homogeneously throughout the lung. The different distribution patterns as well as the authors’ observation that bronchial narrowing in RAS is commonly achieved by inclusion into the interstitial process, rather than through subepithelial deposition of collagen (as seen in BOS), appears to support a concept where RAS and BOS evolve at distinct sites from differentially weighted alloimmune and non-alloimmune aetiological factors but may converge in fatal synergy (figure 1).

Secondly, how exactly do the lesions identified in the explanted lungs develop over time and how can we best intervene are critical questions that remain unanswered. With this in mind, the complete “test” package used in this study cannot be extrapolated backwards to enable us to “look” multi-dimensionally and in real-time at the temporal evolution of CLAD patterns, their differential dynamic profiles and their contextual settings – all of which are essential to a deeper understanding of this problem. Although this compares unfavourably to experimental animal studies where a cohort can be prospectively followed once a specific intervention has occurred and sequentially sacrificed to determine how lesions temporally develop, a solution is by no means not imaginable. Clinical phenotypes based on current imaging and

FIGURE 1 The many “forest fires” of chronic lung allograft dysfunction: where are they, when were they “lit” and how do they “burn”?

lung physiology measurement have already clearly taken us part of the way there. The use of micro-CT on the explanted lungs was very informative in this study; however, the technical difficulties preventing the use of this experimental imaging tool, or some modification of it, in the human *in vivo* setting are currently insurmountable. What is now required, therefore, is a “minimal” set of surrogate “bio”markers (either from the lung or blood compartments) for the histopathological lesions identified by VERLEDEN *et al.* [11] so that we can readily “see” the lung allograft in multiple dimensions simultaneously over time and in different clinical contexts [13, 14]. In this way, we can hope to gain meaningful information with prognostic power as early as possible and create a platform in which appropriately targeted specific therapeutic and experimental interventions have an ever-increasing chance of success [15].

Thirdly, the authors report of fibrotic involvement of small pulmonary arteries and veins in RAS, a phenomenon which has also been described in idiopathic pleuro-parenchymal fibroelastosis, raises many more questions than can be easily answered in a study where all the lung transplant recipients are exposed to key insults with no or minimal easily discernible variation. In particular, VERLEDEN *et al.* [11] speculate that initial hypoperfusion and ischaemia may be aetiological triggers for the pleuro-parenchymal fibroelastosis of RAS. If this were true then it would only take a small step more to consider that the sudden disruption of the bronchial arteries during lung transplantation may also create a micro-environment where an “at risk” bronchial tree may variably manifest features of hypoxic injury (leading to significant necrosis of the bronchial anastomosis and eventual airway stenosis and graft dysfunction in the worst of cases [16]). Since bronchial arteries directly supply the bronchial/bronchiolar mucosa, as well as the visceral pleura, a concept where the fibrotic process is a “fire” that has been patchily “lit” and variably “burns” at two pulmonary sites (broncho-vascular axis and periphery/interstitium) is appealing and fits well into the distribution pattern of RAS observed in the end-stage transplanted lungs studied. Although the mechanisms by which ischaemia and hypoxia trigger excessive fibrosis are not completely elucidated, the centrality of this key pathobiological process in a wide range of biological and

medical conditions, including skin wound healing and myocardial infarction [17, 18], would argue that at least some of the variance seen in the CLAD phenotypes may be due to variation in this key insult (notwithstanding that all lung transplant recipients are exposed to it). Extrapolating further, any insights derived from such considerations following lung transplantation may subsequently “return the favour” and have far-reaching consequences in fields as diverse as idiopathic pulmonary fibrosis, lung epithelial–mesenchymal tissue interaction biology and the science of injury–repair mechanisms [19–24].

An improved understanding of the heterogeneity of CLAD subtypes, the pathobiological mechanisms that predominate (including initial/repeat/persistent injuries as well as the potential negative impact of reduced epithelial repair endotypes and even secondary auto-immune tissue damage) and the high likelihood that multiple processes may be at play to varying degrees in an individual lung has major prognostic, immunomodulatory and non-immunomodulatory therapeutic implications. The extent to which this potential benefit can be realised is largely a function of how we continue to imagine the problem space relating to CLAD, how we apply our tools to efficiently see in real time what is going on in the lung allograft across several dimensions with the highest resolution possible, and how we systematically keep track of our progress as each intervention is specifically applied to individuals and cohorts of lung transplant recipients. Although there is still a long way to go, by linking phenotypes of CLAD to changes in lung structure in the way that they have done, VERLEDEN *et al.* [11] have pointed us in the right direction and helped us rework our “thinking”, “seeing” and “naming” [25–27] of lung allograft dysfunction and loss. New ways of interrogating and monitoring the lung allograft over time and in different clinical contexts are the critical next steps in generating and shaping a deeper understanding of CLAD pathobiology. Such a knowledge base could then be translated into specific therapeutic actions using innovative strategies so that lung transplantation can one day fulfil its promise of being the cure that we all hoped it would be for the vast majority of lung transplant recipients who had no other choice but to go down this path.

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