## Title

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**EDITORIAL COMMENTARY**

# Transcriptome-based diagnostics for chronic lung allograft dysfunction: A Socratic question revisited

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Current diagnostics in lung transplantation are not meeting our patients' needs. While we can identify phenotypes of acute rejection, such as perivascular (A-grade), bronchiolar (B-grade), and antibody-mediated rejection, the link between these pathologies and chronic lung allograft dysfunction (CLAD) is clear only for the more severe cases (1). Most lung transplant recipients go on to develop CLAD without clear warning (2). Moreover, interrater reliability for acute rejection pathologies is low (3). As the lack of clear predictors of imminent CLAD pose a challenge to preventative trials, novel diagnostics are needed to move the field forward (4).

While it had long been assumed that the limited prognostic utility of transbronchial biopsies for predicting CLAD reflected timing or sampling issues, recent molecular diagnostic data has suggested that the relevant changes may simply be invisible to light microscopy. In this issue of the *Journal of Heart and Lung Transplantation*, Halloran and colleagues apply a "molecular microscope" to determine if gene expression changes portend graft failure in lung transplant recipients (5). This manuscript examines RNA expression microarray data from 314 endobronchial mucosal biopsies and 457 transbronchial biopsies across 10 international centers using two distinct but related data reduction algorithms: Principal components and Archetypes. Both techniques allow the description of samples based on linear combinations of genes. In contrast with principal component analysis (PCA), where features are combined to maximize the dataset's variability in each orthogonal component, archetypal analysis postulates prototypical samples, from which most others can be described. The notion that what we observe is a derivative of a more fundamental form, either imprinted in human collective unconscious or beyond the sensible world, goes back to Platonic philosophy. However, this Archetype statistical algorithm was only described in 1994, where it was proposed, among other examples, as a novel approach to the problem of identifying a small set of best-fitting face masks from facial dimension data on 200 Swiss army soldiers (6). Archetypal analysis returns a 0–1 score matrix relating samples and archetypes, from which each sample is assigned to its best match (7). As shown in Figure 1, which illustrates the archetype classification algorithm on a dataset of animal characteristics (8), this approach has advantages and limitations in the insights it offers.

The application of archetypal analysis to RNA expression data has made an important contribution to transplant diagnostics (9). A key finding of this study was that assignment to the T-cell mediated rejection (TCMR) archetype based on a mucosal biopsy was associated with a statistically significant risk of subsequent graft loss. This archetype, which was labeled based on the similarity to transcripts associated with renal transplant TCMR, did not perform as well in transbronchial biopsies. Mucosal biopsies also demonstrated substantially higher

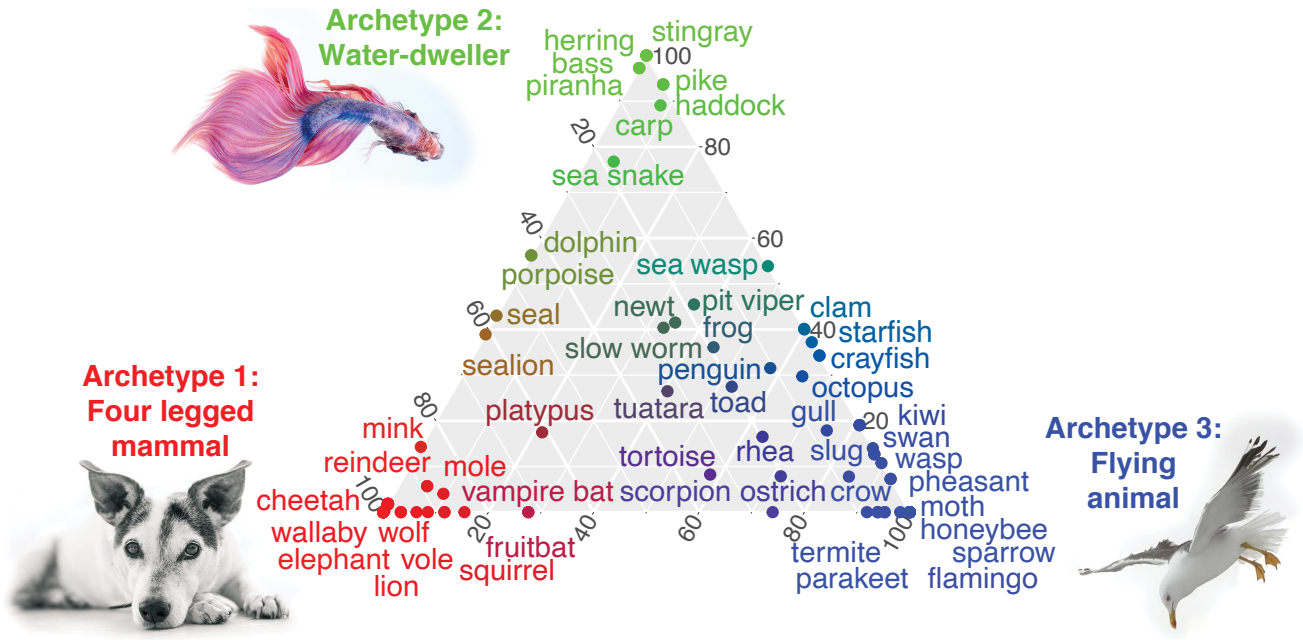


**Figure 1:** Archetypal analysis example as applied to animal classification. The zoo database (8) of 100 animals with parameters such as predator, toothed, number of legs, or lays eggs, was classified using the Archetype algorithm (7) with a target of 3 archetypes. A ternary plot shows each animal's score for each of the three archetypes. Animals are colored according to their archetype assignments (1: Red, 2: Green, and 3: Blue), and archetypes are labeled based on common characteristics.

reproducibility both for archetype assignments and scores on the first PCA component. While CLAD is predominantly a disease of small airway obliteration, the small and variable sampling of the airways in transbronchial biopsies may be one of the key issues limiting prognostication of CLAD from this biopsy type. As noted, interrater reliability on B-grade rejection shows only slight agreement (3). By contrast, large-airway inflammation is fairly uncommon but, when present, is associated with rapid CLAD onset (10). Further, the TCMR gene set is more strongly associated with large airway rejection pathology, as compared to lymphocytic bronchiolitis (11).

An alternate way to obtain transcriptomic data from small airways is through a transbronchial cytology brush. Brushings from CLAD airways have been shown to have substantial upregulation of type-1 immune genes (12). Similarly, a gene signature of lymphocytic bronchitis was associated with both CLAD and time to graft failure independent of airway infection (13). As gene expression from airway brushings outperformed transbronchial biopsies in that study, further analysis is needed to determine whether transbronchial brush or biopsy or airway biopsy is the optimal tissue type for molecular diagnostics. The answer may depend on the rejection phenotype: While constrictive bronchiolitis signatures may be evident in airway tissue, observing pleuroparenchymal fibroelastosis-type changes may require transbronchial biopsy.

Assembly of a large, international cohort targeting a clinically relevant outcome is a notable strength of this study. Even so, there were insufficient graft failure events to allow Cox proportional hazard modeling sufficiently robust to assess the TCMR archetype as an independent predictor of graft failure risk. These analyses looked at time to graft failure from biopsy, and so some of the observed associations with graft failure could be confounded by transcriptomic changes over time. Prior work showed that the first PCA component for mucosal biopsy gene expression was positively associated with post-transplant time (14). It also is unclear how long before CLAD onset molecular changes can be detected. Gene signatures from specific pathobiologies such as lymphocytic inflammation and fibrosis could peak sequentially (13). The hope is that serial measurements of gene expression changes might identify a window when CLAD is

treatable, and such findings might have important implications for when surveillance bronchoscopy is performed.

Another outstanding question is whether whole transcriptome profiling is necessary for a CLAD biomarker. Several studies on CLAD biomarkers using blood, BAL, and biopsies show that, although single genes are unreliable, a combination of as few as 5 gene transcripts can have reasonable performance in identifying CLAD versus stable subjects (15, 16). The finding here that the first principal component in transbronchial biopsies predicted graft failure and accounted for 48% of the total transcriptional variance suggests that the number of potential gene signatures could be vast.

Bringing this technology to the clinical bedside will not be simple, but other molecular diagnostics with non-transparent algorithms have made it to clinical practice (17). The requirement that all samples be processed and analyzed centrally, so that many samples can be pooled on one chip and the results compared against a proprietary database, is a challenge but may be essential for the economic viability of this approach. This Molecular Microscope Diagnostics System used here has yet to be approved by the European Union or Food and Drug Administration, but it is licensed to One Lambda and supported by an accredited laboratory in the US, which has the resources to address issues of clinical validity and utility that may be required for regulatory and third party payer approval (18).

How these gene signatures vary across phenotypes of rejection and CLAD is an important question. Interestingly, while antibody mediated rejection (ABMR) is an archetype of renal transplant rejection gene expression, no ABMR archetype was seen this cohort, despite one-third of subjects having donor-specific antibodies. The absence of an ABMR archetype suggests ABMR pathology may be less relevant for lung versus renal allografts. Such insights reflect the promise of this archetypal analysis: that gene signature-based endotypes may refine or replace clinical and histopathologic rejection endotypes, allowing novel trial designs of tailored therapies.

In Plato's Cratylus dialog, Socrates asks: "which would be the better and surer way of learning? To learn from the image whether it is itself a good imitation and also to learn the truth which it imitates, or to learn from the truth both the truth itself and whether the image is properly made?" (19). Perhaps archetypal analysis will help to elucidate the truth that CLAD imitates.

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