ISHLT JOURNAL WATCH
October 2023 | Pathology

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Early post-transplant reductions in club cell secretory protein associate with future risk for chronic allograft dysfunction in lung recipients: results from a multicenter study

Todd JL, et al. *J Heart Lung Transplant* 2023 | doi: 10.1016/j.healun.2023.02.1495

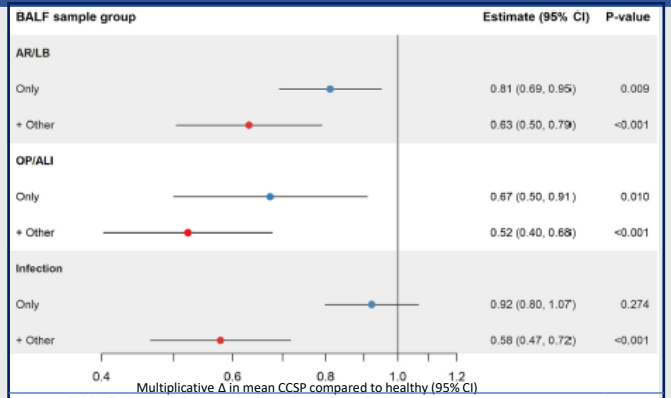
Study Highlights

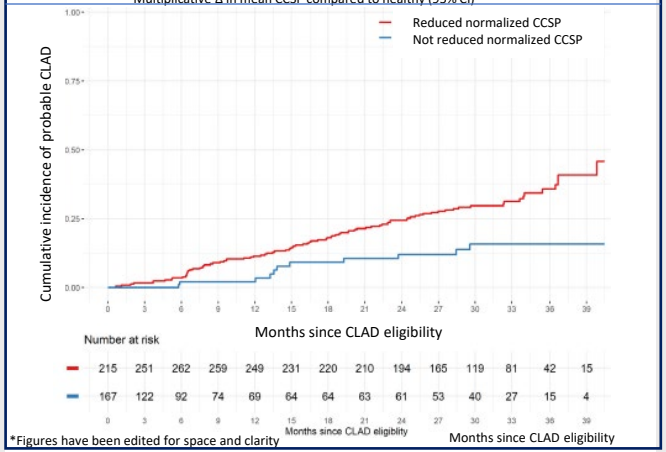
Objective: Chronic lung allograft dysfunction (CLAD) continues to challenge long-term survival transplantation. Club cells are known to play a role in regeneration and repair after lung injury. This study aims to 1) assess role of club cell secretory protein (CCSP) levels in bronchoalveolar lavage fluid (BALF) as an early biomarker for CLAD and 2) examine how CCSP levels change in association to various insults.

Methods: 392 newly transplanted adults from CTOT-20 cohort underwent 1,606 paired BALF and transbronchial biopsies in first post-transplant year. BALF microbiology studies and CCSP ELISA were performed. Rejection was graded according to ISHLT criteria. Expert consensus was used for non-rejection pathology.

Results: Subjects were most frequently transplanted for restrictive lung disease (54%). CCSP levels were significantly lower in rejection and injury versus healthy; there was no significant difference with infection only. In multivariate model, risk of probable and definite CLAD increased significantly with CCSP levels at or below the median, the latter with decreased significance.

Conclusions: CCSP levels below a defined threshold portends risk of CLAD. This supports the role of club cells and CCSP in the development of CLAD and provides a rationale to evaluate CCSP replacement as a therapeutic strategy.





*Figures have been edited for space and clarity

Reviewer's Comments

- Reliable early biomarkers for CLAD are needed to allow for early intervention and prevention.
- CCSP is conceptually and practically a promising biomarker. However, loss of significance for definite CLAD after multivariate analysis highlights complexity of disease and a need for better understanding of its pathophysiology.
- There is a need for less aggressive and more reliable monitoring of graft health, particularly in the pediatric population. As such, biomarkers for concomitant acute cellular rejection and lung injury are necessary.

Limitations

- Pediatric patients not included in this study.
- Considerable overlap in confidence intervals limits value of CCSP in distinguishing different patterns of lung injury and therefore applicability to short-term management decisions.
- Relatively short follow-up period.

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Clinical relevance of cell-free DNA quantification and qualification during the first month after lung transplantation

Pedini P, et al. *Front Immunol* 2023;14:1183949 | doi: 10.3389/fimmu.2023.1183949

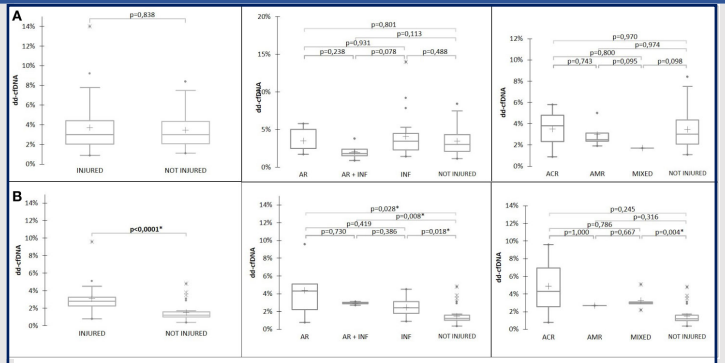
Study highlights

Objective: The study aimed to assess the clinical relevance of dd-cfDNA and cfDNA size profiles within the first month post-lung transplant (LTx), specifically in cases of acute rejection (AR) and infection (INF).

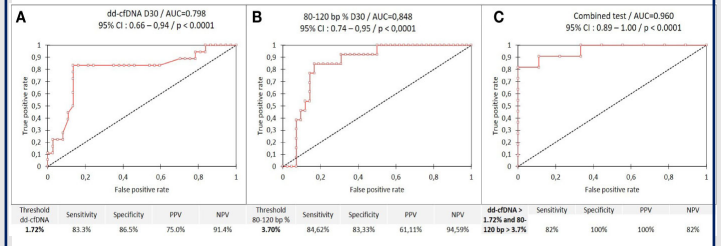
Methods: Sixty-two LTx recipients were enrolled in the study. Total cfDNA was quantified through fluorimetry and digital PCR, dd-cfDNA by using NGS analysis. Size profiles were assessed with BIABooster. At day 30, bronchoalveolar lavage and transbronchial biopsies categorized patients into uninjured grafts and injured grafts (AR, INF, or both).

Results: Total cfDNA quantification did not correlate with patient status at day 30. However, injured graft patients at day 30 had significantly higher dd-cfDNA levels (p=0.0004). A 1.72% dd-cfDNA threshold effectively identified patients with uninjured grafts (91.4% negative predictive value). Among recipients with dd-cfDNA >1.72%, quantifying small DNA fragments (80-120bp) >3.70% accurately detected infection, with 100% specificity and positive predictive value.

Conclusions: To utilize cfDNA as a versatile non-invasive transplant biomarker, combining dd-cfDNA quantification and small DNA fragment presence can effectively classify various allograft injuries.



%dd-cfDNA on Day 15 (A) and on Day 30 (B) in relation to the patient's condition.



ROC curves: (A) for %dd-cfDNA differentiation between graft patients who are not injured and those who are injured. (B) for %80-120bp size differentiation between graft patients who are not injured and those who are injured. (C) for the combined test distinguishing between infected and uninfected groups.

Reviewer's comments

- This study represents a valuable contribution for understanding the role of cfDNA as a non-invasive biomarker in LTx.
- The combination of bronchoalveolar lavage and transbronchial biopsies at day 30 for categorization strengthens the study's robustness.
- Future investigations should explore the impact of factors like ischemia time, preservation procedures, ECMO, and duration of mechanical ventilation.
- It would be valuable to examine the influence of donor-related factors, including donor age, in future studies.

Limitations

- Single center study and small sample size
- While the study identified thresholds, these findings should be externally validated in independent cohorts to confirm their reproducibility and reliability as transplant biomarkers for clinical decision-making.

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Unsupervised mRNA-seq classification of heart transplant endomyocardial biopsies

Romero E, et al. *Clin Clinical Transplantation, 2023* | doi: 10.1111/ctr.15011

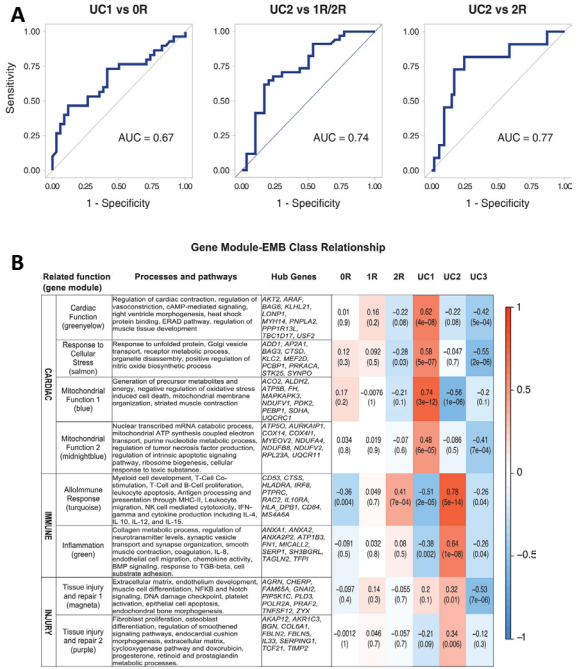
Study Highlights

Objective: Determine whether an unsupervised gene expression profiling approach can evaluate the endomyocardial biopsy (EMB) using only sequencing data, without relying on histology interpretation.

Methods: A single center, retrospective analysis was conducted on 64 EMBs obtained from 47 adult heart transplant recipients. An unsupervised classification method was used to identify molecular signatures relative to EMBs. Gene modules and ontology were identified to understand the biological processes.

Results: The unsupervised method classified EMBs into three categories, based solely on clusters of gene expression. Unsupervised and histological classification resulted comparable. Gene ontology enrichment analysis revealed processes impacting on the regulation of cardiac and mitochondrial function, immune response, and tissue injury response.

Conclusion: The unsupervised methodology classified EMBs in three distinct categories highly correlated with mitochondrial, immune, and tissue injury response, as well as significant cytokine and natriuretic peptide levels.



Panel A: ROC curve for UC assignment probability against ISHLT 0R, 1/2R, or 2R. Panel B: Gene modules, processes, hub genes, and UC or ISHLT class correlations.

Reviewer's Comments

- The currently utilized unsupervised clustering methods can give a novel dimension of rejection pattern next to the histology assessment.
- The comparison with the histological assessment showed the opportunity of giving insight in the rejection patter detected.
- To characterize the biological processes involved, cell-typing assessment would have been useful, thanks to the power of RNA-seq data.
- The performance of the classification model could have been evaluated with comprehensive metrics next to the ROC curve showed.

Limitations

- Limited number of patients limited the representativity of the cohort.
- The rejection categories assessed (0R, 1R, 2R) are not balanced in the experimental design.
- Missingness of AMR and mixed cases to tackle the full spectrum of pathological diagnoses.
- Need of validation in a well characterized and multicenter prospective cohort.

ISHLT JOURNAL WATCH
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Comparison of two donor-derived cell-free DNA tests and a blood gene-expression profile test in heart transplantation

Rodgers N, et al. *Clin Transplant* Feb 2023 | doi: 10.1111/ctr.14984

Study Highlights

Objective: Donor-derived cell-free DNA (dd-cfDNA) testing is an emerging screening modality for non-invasive detection of acute rejection (AR). This study compared the testing accuracy for AR of two commercially available dd-cfDNA and gene-expression profiling (GEP) testing in heart transplant (HTx) recipients.

Methods: This was a retrospective, observational study of HTx patients who underwent standard and expanded single nucleotide polymorphism (SNP) dd-cfDNA. Assays were compared with GEP and each other for correlation, accurate classification, and prediction for AR.

Results: A total of 428 samples from 112 unique HTx patients were used for the study. A positive standard SNP correlated with the expanded SNP assay (p<.001). Both standard and expanded SNP tests showed low sensitivity (39%, p=1.0) but high specificity (82% and 84%, p=1.0) for AR. GEP did not improve sensitivity.

Conclusion: No significant difference were found between standard and expanded SNP assays in detecting AR. Improved specificity without change in sensitivity using dd-cfDNA in place of GEP testing was seen.

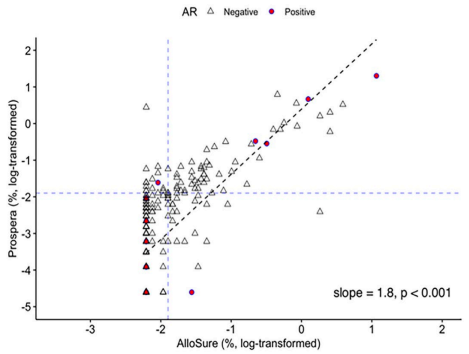

Figure 1: Scatter plot of standard and expanded SNP dd-cfDNA

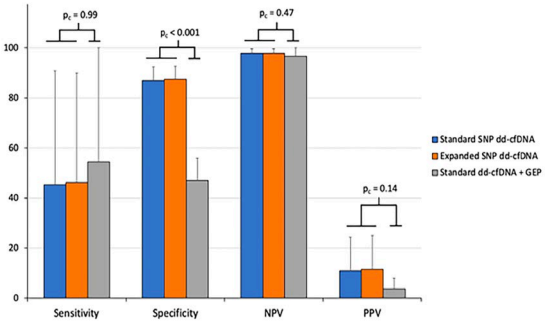

Figure 2: Barchart of sensitivity, specificity, NPV and PPV for standard and expanded SNP dd-cfDNA and standard dd-cfDNA +GEP tests.

Reviewer's Comments

- Comparison of two current screening methods (standard and expanded SNP dd-cfDNA testing) showing no significant difference in testing accuracy. Interestingly, adding GEP to dd-cfDNA testing reduces sensitivity.
- Large cohort (428 patients analyzed) indicates potential to be applicable for general patient population.
- dd-cfDNA is a promising screening method, more in-depth comparison to the current gold-standard (EMB) in future studies needed.

Limitations

- Retrospective study from a single center experience.
- Almost all samples were obtained early post-HTx. Thus, conclusions for samples later after HTx cannot be confidently made.
- Study is underpowered to detect smaller differences between standard and expanded SNP testing that may exist.
- The sensitivity, specificity, PPV, and NPV calculated for standard dd-cfDNA + GEP were performed on a subset of the data, selection bias is a potential confounder in the analysis of the results.
- More nuanced cutoffs based on the clinical context of individual patients may be employed in the future.

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Prediction of heart transplant rejection from routine pathology slides with self-supervised deep learning

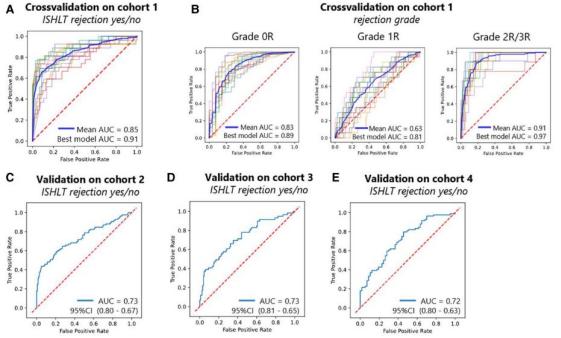
Seraphin TP, et al. *Eur Heart J-Digital Img* 2023 | doi: 10.1093/ehjdh/ztad016

Study Highlights

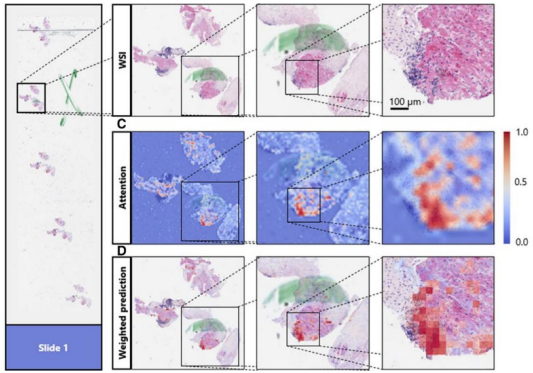
Objective: One of the most important diagnoses of heart transplantation is rejection on endomyocardial biopsies. Computer-based systems could assist in the diagnostic process and improve reproducibility. This study evaluated deep learning in diagnosing cellular rejection on pathology slides using ISLHT grading.

Methods and Results: The authors collected 1079 slides from 325 patients from 3 transplant centers in Germany divided into training and validation cohorts. They trained an attention-based deep neural network to predict rejection in the primary cohort and evaluated its performance using cross-validation and by deploying it to three cohorts. For presence or absence of rejection, the mean AUROC was 0.849 in the cross-validated experiment and 0.734, 0.729, and 0.716 in external validation cohorts. For a prediction of the ISHLT grade (0R, 1R, 2/3R), AUROCs were 0.835, 0.633, and 0.905 in the cross-validated experiment and 0.764, 0.597, and 0.913; 0.631, 0.633, and 0.682; and 0.722, 0.601, and 0.805 in the validation cohorts, respectively. The predictions of the artificial intelligence model were interpretable by human experts and highlighted plausible morphological patterns.

Conclusions: Artificial intelligence can detect patterns of cellular transplant rejection in routine pathology.



Cross validation and validation ROC for rejection (yes/no) and rejection grade



High attention regions contain inflammation, and the model ignores extraneous marks

Reviewer's Comments

- The neural network consistently performed most accurately in classifying biopsies into 0R
- The model performed as well or better than CRANE, the previous state of the art for machine learning in diagnosis of heart allograft rejection
- High attention regions overlap with areas of inflammation in specimens with rejection

Limitations

- There is large cohort to cohort variability in ROCs, which implies limited generalization of this neural network
- The neural network had much better cross-validation AUROC for 0R and 2R/3R ACR than 1R rejection, suggesting difficulty with fine distinctions
- While the training set was large for a transplant cohort (393 slides), this is small for a machine learning data set and larger cohorts are needed for accurate training.