

# Cellular Biomarkers of Rejection in Lung Transplant: The Usefulness of Eosinophil and Lymphocyte Counts

Piedad Ussetti, MD\*, Silvia Aguado, MD, and Rosalía Laporta, MD

*Servicio de Neumología, Hospital Puerta de Hierro, Madrid, Spain*

## ABSTRACT

There is increasing interest in the development of biomarkers of acute cellular rejection (ACR) and chronic allograft dysfunction (CLAD) in lung transplant recipients. However, few studies have focused on the predictive value of cellular biomarkers usually available in routine clinical practice such as eosinophils (EOS) and lymphocytes.

Peripheral blood eosinophilia has been associated with ACR in kidney, heart and liver transplant recipients. There are few studies in relation to EOS counts in lung transplants. In our experience, the increase in EOS in peripheral blood may be a warning sign of rejection that advises to expand the assessment with a bronchoscopy (FBS), transbronchial biopsy (TBB) and bronchoalveolar lavage (BAL).

Lymphocytes are essential cells in the development of ACR. The increase in lymphocytes in the BAL fluid (BALF) may be indicative of ACR, especially if it is associated with a simultaneous increase in the EOS count in the peripheral blood.

**Keywords:** Acute rejection. Bronchoalveolar lavage (BAL). Eosinophils. Lymphocytes. Peripheral blood.

**\*Correspondence to:**

Piedad Ussetti

Email: [pied2152@separ.es](mailto:pied2152@separ.es)

Received: 22-07-2023

Accepted: 21-08-2023

DOI: 10.23866/BRNRev:2023-M0094

[www.brnreviews.com](http://www.brnreviews.com)

## INTRODUCTION

The long-term survival of lung transplant recipients is limited by the development of chronic allograft dysfunction (CLAD). According to the International Heart and Lung Transplant Registry, CLAD is the main cause of mortality and affects more than 50% of recipients at five years post-transplant<sup>1</sup>. The risk factors related to the development of CLAD are repeated episodes of acute cellular rejection (ACR), lymphocytic bronchiolitis, infections, gastro-oesophageal reflux and environmental pollution<sup>2-6</sup>.

Currently, the diagnosis of CLAD is established when an irreversible and progressive reduction of lung function is detected<sup>7</sup>. However, in recent years there has been a growing interest in the development of biomarkers for early detection of patients at risk<sup>8-11</sup>.

An ideal biomarker should be specifically associated with the disease we are trying to identify, be present in either peripheral blood or the target tissue, and also be easily detectable and quantifiable. Different biomarkers have been associated with CLAD, but none of them have been shown to be sufficiently sensitive and/or specific to justify their use in medical routines. The clinical complexity of CLAD, which involves both immune and non-immune biological mechanisms, makes it improbable that a single biomarker could be used for early detection of the disease<sup>6,12-14</sup>.

While waiting for the “ideal biomarker” we must base our clinical decisions on the tools that are currently available. The goal of this article is to review our experience and the available literature regarding the usefulness

of eosinophil (EOS) counts in peripheral blood and lymphocyte counts in bronchioloalveolar lavage fluid (BALF) to predict acute and chronic lung allograft dysfunction.

## BIOMARKERS IN PERIPHERAL BLOOD

Peripheral blood is an easily accessible biological sample frequently used in the search for the “ideal biomarker”. Multiple studies have tried to identify biomarkers of rejection in peripheral blood for the early diagnosis of ACR and CLAD<sup>15-16</sup>. In this regard, we can highlight, amongst others, the determination of several cytokines and chemokines, KL6, exosomes, or donor-derived cell-free DNA. However, the cost and complexity of many of these techniques have limited their implementation in routine clinical practice<sup>17-21</sup>.

Blood counts are performed in the usual monitoring process of lung transplant recipients and some authors have analysed the predictive value of ACR and CLAD of different cell type counts.

### Eosinophils in peripheral blood

Eosinophils have been implicated in different biological processes, such as atopy, hypersensitivity, asthma, and fungal infections. However, their role in the development of ACR and CLAD is still to be fully understood.

Classic rejection mechanisms include direct cytotoxicity mediated by CD8<sup>+</sup>T lymphocytes and delayed-type hypersensitivity dependent on CD4<sup>+</sup>T lymphocytes. When the classic

activation pathways are blocked as a result of standard immunosuppressive treatment, the alternative alloimmune response could be Th2 type-mediated through EOS and interleukin (IL)-5 pathways<sup>22-25</sup>. Several studies have observed that EOS could be involved in graft rejection via a Th2-type immune response and that EOS counts could be an early and specific marker for ACR<sup>8,26</sup>.

The relationship between EOS and graft rejection was published more than 30 years ago for kidney transplant recipients. In these initial studies, eosinophilia was invariably associated with ACR and could be detected prior to clinical suspicion<sup>27</sup>. Moreover, eosinophilic infiltration of the kidney allograft was associated with greater severity of the rejection<sup>28</sup>. More recently, Colas et al.<sup>29</sup>, in a prospective study of 1013 kidney recipients with stable renal function at three months post-transplant observed that an EOS count > 0.3 G/L was associated with a three-fold increased risk of rejection independent of immunosuppressive protocol.

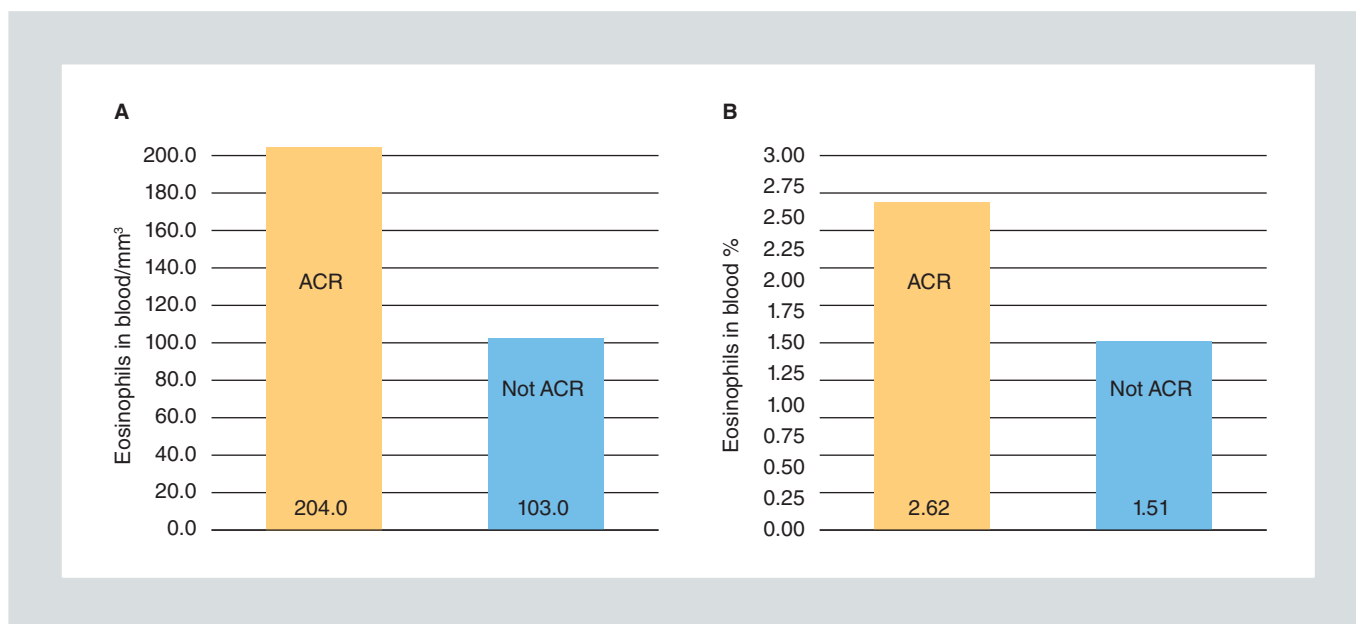
Similar results were later published regarding liver transplant recipients. In these patients, the increase in EOS counts was observed prior to the onset of liver function abnormalities. In this respect, some authors consider that liver dysfunction associated with an increase in EOS counts are unequivocal signs of ACR<sup>30-33</sup>.

In lung transplant recipients, eosinophilic infiltration of the graft has been considered a sign of severe rejection and risk of bad outcomes<sup>34,35</sup>. However, few studies have focused on the predictive role of rejection of EOS counts in peripheral blood. Trull et al.<sup>36</sup> published in 1998 a retrospective study of 54 heart

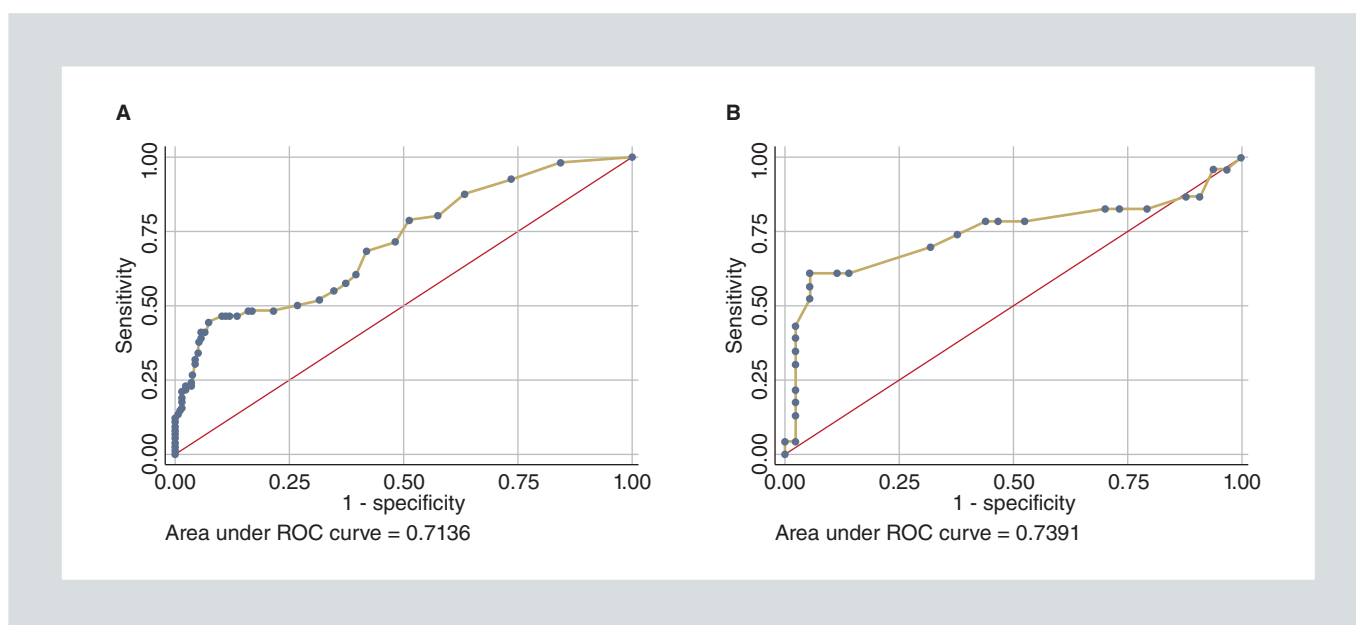
and lung transplant recipients in which they observed a correlation between EOS counts > 140/mm<sup>3</sup> and ACR. As previously described for liver and kidney transplant recipients, the increase in EOS counts could also be found prior to the clinical suspicion.

In our Lung Transplant Unit, we routinely monitor EOS counts during post-transplant follow-up. In this respect, we recently published a retrospective study of our experience with 583 consecutive fibre-optic bronchoscopies (FBS) with transbronchial biopsy (TBB) and bronchoalveolar lavage (BAL) performed on 256 recipients transplanted between 2012 and 2018<sup>37</sup>. Acute rejection was observed in 170 out of 583 TBB (29.2%). Prior to biopsy the median EOS count was 75 ± 57/mm<sup>3</sup> in the global population and no differences were observed between patients with or without ACR (84 ± 72/mm<sup>3</sup> versus 71 ± 50/mm<sup>3</sup>; NS). However, during ACR episodes we observed significantly higher absolute and relative EOS counts (absolute: 203.6 ± 248/mm<sup>3</sup> versus 103.1 ± 153/mm<sup>3</sup> p < 0.001; relative: 2.6 ± 2.7 % versus 1.5 ± 2.0% p < 0.001) (Fig. 1). Furthermore, higher peripheral EOS counts were also observed for higher grades of rejection, especially at the moderate-severe grade (OR 3.550; 95% CI, 3.00–4.099 and OR 3.563; 95% CI, 3.001–4.117, respectively). The relationship between EOS counts and ACR was independent of the underlying disease, the time after transplant and the corticosteroid dose.

Determining an optimal cut-off point to establish clinical suspicion of rejection can be complex. In our study, for the biopsies performed per protocol, we identified a cut-off point of EOS of 195/mm<sup>3</sup> (specificity 90%, sensitivity 46%) in the first 12 months after



**FIGURE 1. A:** median absolute (mm<sup>3</sup>) and **B:** relative (%) peripheral blood eosinophils counts measured on the day of transbronchial biopsy in patients with acute rejection (ACR) and patients without it.



**FIGURE 2.** ROC curves with a cut-off point of EOS in peripheral blood of 195/mm<sup>3</sup>. **A:** specificity 90%, sensitivity 46% in the first 12 months. **B:** 180/mm<sup>3</sup> (specificity 94%, sensitivity 60%) after the first year. EOS: eosinophils; ROC: receiver operating characteristic.

transplant, and of 180/mm<sup>3</sup> (specificity 94%, sensitivity 60%) after the first year (Fig. 2). Other authors have suggested that rejection should be suspected in patients with an EOS count > 400/mm<sup>3</sup> associated with a decrease

in lung function<sup>38-39</sup>. Such a high cut-off point seems excessive, since, as we observed in our study, lung transplant recipients usually have lower eosinophil counts than those described in the general population and for other types

of transplants. This fact is mainly due to the frequent use of corticosteroids in immunosuppression protocols of lung transplants. Therefore, to suspect rejection in clinical practice, it may be more useful to monitor the evolution of EOS counts in each individual case than to establish a universal cut-off point.

EOS counts in peripheral blood have also been associated to CLAD development due to their capacity to induce tissue damage and extracellular matrix remodelling. In this respect, Verleden et al.<sup>40</sup> observed an association between EOS counts  $> 240/\text{mm}^3$  with bad prognosis and mortality of patients with the restrictive phenotype of CLAD. Kaes et al.<sup>41</sup> have recently published that a relative EOS count  $> 8\%$  at any time during the post-transplant follow-up is associated with an increased risk of mortality and restrictive phenotype of CLAD. The 8% cut-off point was established after analysing the median of the maximum values of EOS observed in deceased patients (specificity 76.72%, sensitivity 47.89%).

## BIOMARKERS IN BALF

Blood provides information on systemic inflammatory changes, but blood biomarkers may not adequately reflect the local micro-environment of the lung. Fibrobronchoscopy (FBS) with TBB and BAL are frequently performed on lung transplant recipients. Indications for the study are follow-up protocols, the onset of respiratory symptoms, or a decrease in lung function. TBB is the gold standard for diagnosing ACR<sup>42-44</sup>. However, it is an invasive technique that is not without risk, with a diagnostic sensitivity that depends largely on the indication for performing the procedure

and the experience of the pathologist. BAL is a less invasive diagnostic alternative that may reflect the inflammatory microenvironment of the lung parenchyma and be useful to differentiate rejection from other frequent complications such as infections<sup>45,46</sup>. Several biomarkers for acute and chronic allograft rejection have been identified in BALF. However, in parallel with what has been described for peripheral blood biomarkers, no single BALF biomarker has been proved to be sufficiently sensitive or specific to justify its use in regular clinical practice<sup>10,47</sup>.

Cell counts are performed routinely in BALF samples. In healthy individuals, more than 80% of the cells found in BALF are alveolar macrophages, around 5%–15% are lymphocytes and a small percentage of cells are neutrophils, eosinophils or mast cells<sup>48</sup>. In patients with ACR, an increase in total cellularity of BALF has been described<sup>45</sup>. This increase is more pronounced in parallel to the severity of the ACR and occurs at the expense of a variable increase in the percentage of neutrophils, lymphocytes and EOS<sup>49</sup>.

## Neutrophils in BALF

The increase in neutrophils in BALF samples is not specific to acute rejection, having also been associated with infections, lymphocytic bronchiolitis and CLAD. In this regard, in a retrospective study that included 778 TBBs, Vos et al.<sup>50</sup> showed that neutrophil counts were associated with the development of lymphocytic bronchiolitis. These authors attributed the discrepancy between the results from BALF and TBB samples to the different cellular compartments explored by each technique.

## Eosinophils in BALF

Eosinophils represent < 1% of BALF cell counts in transplant recipients without complications. Several studies have observed an increase in EOS counts related to the presence and severity of ACR. Greenland et al.<sup>49</sup> observed that patients with an EOS count > 0% presented a four times higher risk of ACR. However, EOS are infrequently detected in BALF samples and in Greenland's study they were identified in only 10% of cases. EOS in the BALF could be related to rejection but other causes of this increase as viral, fungal, or bacterial infections, should always be ruled out.

EOS in BALF have also been associated with poor post-transplant evolution. Todd et al.<sup>51</sup> observed that BALF eosinophilia was an independent predictor of future CLAD risk across a multicentre lung recipient cohort. Moreover, in a retrospective study, Verleden et al.<sup>52</sup> concluded that patients with EOS counts in BALF > 2% had a higher risk of CLAD and mortality.

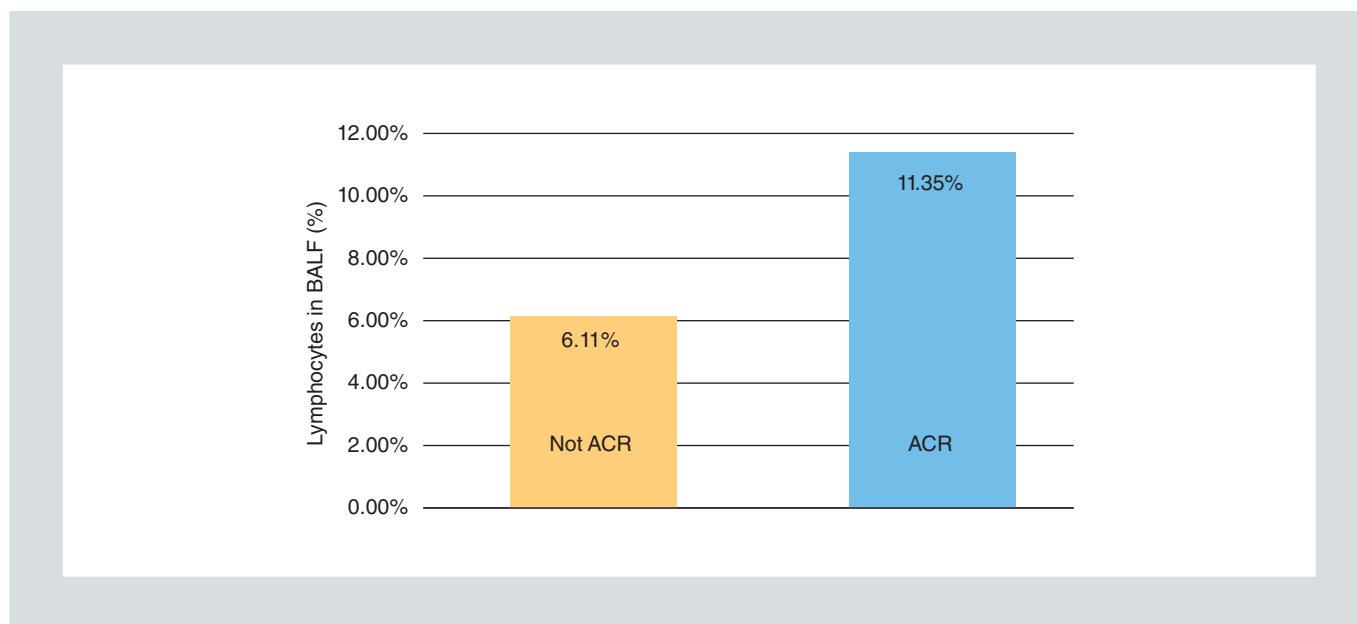
## Lymphocytes in BALF

Acute rejection is a process that is essentially related to the activation of lymphocytes. Therefore, different authors have associated the presence of lymphocytes in BALF samples with the onset of ACR. However, there is no consensus regarding its diagnostic usefulness or in the definition of a cut-off point. In this respect, the percentages of lymphocytes that have been associated with ACR cover a wide range between 1% and 60%<sup>53-55</sup>. In a retrospective study comparing cell counts in BALF

from recipients who were stable, and had ACR or CLAD, Slebos et al.<sup>45</sup>, observed that a percentage of lymphocytes > 1% was associated with the presence of ACR (specificity of 95.6%; sensitivity of 40.4%). The disparity in the results described may be due in part to the lack of a standard procedure for BAL, the handling of the cytology samples of BALF and the different techniques used for cell quantification.

In our unit, since the beginning of the program, the BAL has followed a standardised procedure that meets the recommendations published recently by the International Society for Heart and Lung Transplantation (ISHLT)<sup>56</sup>. Furthermore, cell counts in BALF are performed by flow cytometry, thus avoiding any variability associated to manual counts. With this methodology, we have retrospectively reviewed the usefulness of lymphocyte counts in BALF for diagnosing ACR in a sample of 887 TBB performed in 363 recipients transplanted between January 2014 and December 2020<sup>57</sup>. ACR was observed in 260 out of 887 TBB (29%). The lymphocyte counts in BALF were significantly higher in patients with ACR than in those without (11.35% versus 6.11%;  $p < 0.001$ ) (Fig. 3). The higher the percentage of lymphocytes, the higher the risk of ACR and the greater its severity. This relationship was independent of the underlying disease, the time post-transplant, the indication for the study and the corticosteroid dose (OR 1.10, 95% CI 1.080–1.131;  $p < 0.001$ ).

With respect to the cut-off point, a percentage of lymphocytes in BALF of 12% showed a diagnostic specificity for ACR of 71% (sensitivity 35%, positive predictive value 33%, negative predictive value 73%). This diagnostic



**FIGURE 3.** The average lymphocyte count in BALF was significantly higher in patients with ACR than in those without (11.35% versus 6.11%;  $p < 0.001$ ).

ACR: bronchiolo-alveolar lavage fluid; BALF: bronchiolo-alveolar lavage fluid.

specificity increased to 96% in the presence of simultaneous peripheral blood counts of  $\text{EOS} > 200/\text{mm}^3$  (sensitivity 17%, positive value 62%, negative predictive value 74%).

The high specificity of the combination of both determinations can allow us to start empirical treatment on patients who are at high risk when having the TBB procedure or in the presence of inconclusive histological samples. However, the low sensitivity of this combination does not allow us to rule out the presence of ACR, therefore they should always be interpreted carefully according to the patient's symptoms and in combination with other parameters.

## CONCLUSIONS

Until a sufficiently sensitive and specific biomarker is found, we must base our clinical

decisions on the tools currently available. Cellular blood and BALF counts are part of the routine follow-up of lung transplant recipients. Therefore, in a suitable clinical context, an increase of EOS count in blood can be a warning sign for acute or chronic allograft dysfunction. Moreover, in patients at risk for TBB or in the presence of inconclusive histological samples, the lymphocyte counts in BALF could be a diagnostic alternative. The consideration of both factors in combination significantly increases their diagnostic specificity of rejection, but lacks adequate sensitivity, and therefore must always be considered in conjunction with clinical symptoms and the evolution of lung function.

## DISCLOSURES

The authors declare no relevant conflicts of interest with respect to this work.

## REFERENCES

1. Chambers DC, Perch M, Zuckermann A et al. International Society for Heart and Lung Transplantation. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-eighth adult lung transplantation report - 2021; Focus on recipient characteristics. *J Heart Lung Transplant.* 2021;40:1060-72.
2. Sharples LD, McNeil K, Stewart S et al. Risk factors for bronchiolitis obliterans: a systematic review of recent publications. *J Heart Lung Transplant.* 2002;21:271-81.
3. Burton CM, Iversen M, Carlsen J et al. Acute cellular rejection is a risk factor for bronchiolitis obliterans syndrome independent of post-transplant baseline FEV1. *J Heart Lung Transplant.* 2009;28:888-93.
4. Koutsovera A, Levy L, Pal P, Orchanian-Cheff A, Martinu T. Acute cellular rejection: is it still relevant? *Semin Respir Crit Care Med.* 2018;39:181-98.
5. Glanville AR, Aboyou CL, Havryk A, Plit M, Rainer S, Malouf MA. Severity of lymphocytic bronchiolitis predicts long-term outcome after lung transplantation. *Am J Respir Crit Care Med.* 2008;177:1033-40.
6. Girgis RE, Tu I, Berry GJ et al. Risk factors for the development of obliterative bronchiolitis after lung transplantation. *J Heart Lung Transplant.* 1996;15:1200-8.
7. Verleden GM, Glanville AR, Lease ED et al. Chronic lung allograft dysfunction: Definition, diagnostic criteria, and approaches to treatment-A consensus report from the Pulmonary Council of the ISHLT. *J Heart Lung Transplant.* 2019;38:493-503.
8. Tissot A, Danger R, Claustre J et al. Early Identification of Chronic Lung Allograft Dysfunction: The Need of Biomarkers. *Front Immunol.* 2019; 10:1681.
9. Shtraichman O, Diamond JM. Emerging biomarkers in chronic lung allograft dysfunction. *Expert Rev Mol Diagn.* 2020;20:467-75.
10. Berastegui C, Gómez-Ollés S, Sánchez-Vidaurre S et al. BALF cytokines in different phenotypes of chronic lung allograft dysfunction in lung transplant patients. *Clin Transplant.* 2017;31.
11. Verleden GM, Vos R, Vanaudenaerde B et al. Current views on chronic rejection after lung transplantation *Transplant International.* 2015;28:1131-9.
12. Joshua YC, Yang 1,2, Stijn E et al. Cell-Free DNA and CXCL10 Derived from Bronchoalveolar Lavage Predict Lung Transplant Survival. *J Clin Med.* 2019;8: 241.
13. de Silva T, Voisey J, Hopkins P et al. Markers of rejection of a lung allograft: state of the art. *Biomark.Med.* 2022;16:483-98.
14. Pison C, Tissot A, Bernasconi E et al. Results and perspectives from the Cohort of Lung Transplantation and Systems prediction of Chronic Lung Allograft Dysfunction cohorts. *Front Med (Lausanne).* 2023;9;10:1126697.
15. Berastegui C, Román J, Monforte V et al. Biomarkers of pulmonary rejection. *Transplant Proc.* 2013;45:3163-9.
16. Verleden SE, Hendriks JM, Lauwers P et al. Biomarkers for Chronic Lung Allograft Dysfunction: Ready for Prime Time? *Transplantation.* 2023;107: 341-50.
17. Oellerich M, Budde K, Osmanodja B et al. Donor-derived cell-free DNA as a diagnostic tool in transplantation. *Front Genet.* 2022;13:1031894.
18. Sanders YY. New Clue: Prediction from Cell-Free DNA. *J Clin Med.* 2020; 21;9:2307.
19. Gunasekaran M, Sharma M, Hachem R et al. Circulating Exosomes with Distinct Properties during Chronic Lung Allograft Rejection. *J Immunol.* 2018;200:2535-41.
20. Bansal S, Sharma M, Mohanakumar T. The role of exosomes in allograft immunity. *Cell Immunol.* 2018;331:85-92.
21. Gielis EM, Ledeganck KJ, De Winter BY et al. M. Cell-Free DNA: An Upcoming Biomarker in Transplantation. *Am J Transplant.* 2015;15:2541-51.
22. Goldman M, Le Moine A, Braun M et al. A role for eosinophils in transplant rejection. *Trends Immunol.* 2001;22:247-51.
23. Onyema OO, Guo Y, Hata A et al. Deciphering the role of eosinophils in solid organ transplantation. *Am J Transplant.* 2020;20:924-30.
24. Martinez OM, Ascher NL, Ferrell L et al. Evidence for a nonclassical pathway of graft rejection involving interleukin 5 and eosinophils. *Transplantation.* 1993;55: 909-18.
25. Braun MY, Desalle F, Le Moine A et al. IL-5 and eosinophils mediate the rejection of fully histoincompatible vascularized cardiac allografts: Regulatory role of alloreactive CD8+ T lymphocytes and IFN-gamma. *Eur J Immunol.* 2000;30:1290-6.
26. Long H, Liao W, Wang L et al. The Versatile Roles of Eosinophils in the Immune System. *Transfus Med Hemother.* 2016;43:96-108.
27. Lautenschlager I, von Willebrand E, Hayry P. Blood eosinophilia, steroids, and rejection. *Transplantation.* 1985;40:354-7.
28. Almirall J, Campistol JM, Sole M, Andreu J, Revert L. Blood and graft eosinophilia as a rejection index in kidney transplant. *Nephron.* 1993;65: 304-9.
29. Colas L, Bui L, Kerleau C et al. Time-dependent blood eosinophilia count increases the risk of kidney allograft rejection. *EBioMedicine.* 2021;73: 103645.
30. Wang G-Y, Li H, Liu W et al. Elevated blood eosinophil count is a valuable biomarker for predicting late acute cellular rejection after liver transplantation. *Transplant Proc.* 2013;45:1198-200.
31. Rodriguez-Peralvarez M, Germani G, Tsochatzis E et al. Predicting severity and clinical course of acute rejection after liver transplantation using blood eosinophil count: eosinophils and rejection after liver transplantation. *Transpl Intl.* 2012;25:555-63.
32. Manzarbeitia C, Rustgi VK, Jonsson J, Oyløe VK. Absolute peripheral blood eosinophilia. An early marker for rejection in clinical liver transplantation. *Transplantation.* 1995;59:1358-60.
33. Barnes EJ, Abdel-Rehim MM, Goulis Y et al. Applications and limitations of blood eosinophilia for the diagnosis of acute cellular rejection in liver transplantation. *Am J Transplant.* 2003;3:432-8.
34. Bewig B, Stewart S, Böttcher H et al. Eosinophilic alveolitis in BAL after lung transplantation. *Transpl Int.* 1999;12:266-72.
35. Darley DR, Ma J, Huszti E et al. Eosinophils in transbronchial biopsies: a predictor of chronic lung allograft dysfunction and reduced survival after lung transplantation—a retrospective single-center cohort study. *Transpl Int.* 2021;34:62-75.
36. Trull A, Steel L, Cornelissen J et al. Association between blood eosinophil counts and acute cardiac and pulmonary allograft rejection. *J Heart Lung Transplant.* 1998;17:517-24.
37. Aguado S, Pérez Aguilar M, Royuela A et al. Peripheral blood eosinophilia as a marker of acute cellular rejection in lung transplant recipients. *J Heart Lung Transplant.* 2022;41:501-7.
38. Speck NE, Schuurmans MM, Murer C, Benden C, Huber LC. Diagnostic value of plasma and bronchoalveolar lavage samples in acute lung allograft rejection: differential cytology. *Respir Res.* 2016;17:74.
39. Schuurmans MM, Raeber ME, Roeder M et al. Adaptive Immunosuppression in Lung Transplant Recipients Applying Complementary Biomarkers: The Zurich Protocol. *Medicina (Kaunas).* 2023;59:488.
40. Verleden SE, Ruttens D, Vandermeulen E et al. Predictors of survival in restrictive chronic lung allograft dysfunction after lung transplantation. *J Heart Lung Transplant.* 2016;35:1078-84.
41. Kaes J, Van der Borght E, Vanstapel A et al. Peripheral Blood Eosinophilia Is Associated with Poor Outcome Post-Lung Transplantation. *Cells.* 2020; 9:2516.
42. Stewart S, Fishbein MC, Snell GI et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. *J Heart Lung Transplant.* 2007;26:1229-42.
43. Hopkins PM, Aboyou CL, Chhajed PN et al. Prospective analysis of 1235 transbronchial lung biopsies in lung transplant recipients. *J Heart Lung Transplant.* 2002;21:1062-7.
44. Trulock EP, Ettinger NA, Brunt EM et al. The role of transbronchial lung biopsy in the treatment of lung transplant recipients. An analysis of 200 consecutive procedures. *Chest.* 1992;102:1049-54.

45. Slebos DJ, Postma DS, Koëter GH et al. Bronchoalveolar lavage fluid characteristics in acute and chronic lung transplant rejection. *J Heart Lung Transplant.* 2004;23:532-40.
46. Prop J, Wagenaar-Hilbers JP, Petersen AH et al. Characteristics of cells lavaged from rejecting lung allografts in rats. *Transplant Proc.* 1988;20:217-18.
47. Speck NE, Probst-Müller E, Haile SR et al. Bronchoalveolar lavage cytokines are of minor value to diagnose complications following lung transplantation. *Cytokine.* 2020;125:154794.
48. Tiroke AH, Bewig B, Haverich A. Bronchoalveolar lavage in lung transplantation. State of the art. *Clin Transplant.* 1999;13:131-57.
49. Greenland JR, Jewell NP, Gottschall M et al. Bronchoalveolar lavage cell immunophenotyping facilitates diagnosis of lung allograft rejection. *Am J Transplant.* 2014;14:831-40.
50. Vos R, Vanaudenaerde BM, Verleden SE et al. Bronchoalveolar lavage neutrophilia in acute lung allograft rejection and lymphocytic bronchiolitis. *J Heart Lung Transplant.* 2010;29:1259-69.
51. Todd JL, Weber JM, Kelly FL et al. BAL Fluid Eosinophilia Associates With Chronic Lung Allograft Dysfunction Risk: A Multicenter Study. *Chest.* 2023;S0012-3692(23)00461-0.
52. Verleden SE, Ruttens D, Vandermeulen E et al. Elevated bronchoalveolar lavage eosinophilia correlates with poor outcome after lung transplantation. *Transplantation.* 2014;97:83-9.
53. Zheng L, Orsida B, Whitford H et al. Longitudinal comparisons of lymphocytes and subtypes between airway wall and bronchoalveolar lavage after human lung transplantation. *Transplantation.* 2005;80:185-92.
54. Reynaud-Gaubert M, Thomas P, Gregoire R et al. Clinical utility of bronchoalveolar lavage cell phenotype analyses in the postoperative monitoring of lung transplant recipients. *Eur J Cardiothorac Surg.* 2002;21:60-6.
55. Vos R, Verleden SE, Ruttens D et al. Azithromycin and the treatment of lymphocytic airway inflammation after lung transplantation. *Am J Transplant.* 2014;14:2736-48.
56. Martinu T, Koutsokera A, Benden C et al. Bronchoalveolar lavage standardization workgroup. International Society for Heart and Lung Transplantation consensus statement for the standardization of bronchoalveolar lavage in lung transplantation. *J Heart Lung Transplant.* 2020;39:1171-90.
57. Aguado S, Laporta R, Aguilar M et al. A Combination of Cytological Biomarkers as a Guide in the Diagnosis of Acute Rejection in Lung Transplant Recipients. *Transplantation.* 2023;4:102-10.