Molecular Biology Testing for Non-Invasive Diagnosis of Allograft Rejection: HEART & LUNG

HEART

PICO 1: In heart transplant patients with stable graft function, is GEP a reliable surveillance tool for subclinical acute rejection monitoring, compared to endomyocardial biopsy?

Population: Heart transplant recipients – either adult or pediatric
Intervention: Donor-derived cell free DNA, Peripheral blood Gene Expression Profiling (Allomap), Cardiac biomarkers (such as NT-proBNP, BNP, Troponin)
Comparators: Endomyocardial biopsy for rejection surveillance, Coronary angiography for CAV
Outcome: Most of the studies are observational and have been designed to validate a diagnostic tool. In one case the study was randomised (IMAGE trial)

Supporting data:
Validated in a large multi-center US-based randomized clinical trial (IMAGE)9, a smaller single-center (CSMC, USA) randomized trial (eIMAGE)10, and 2 large prospective cohort studies (CARGO II1 which included 17 US and European centers, and OAR11 which included 35 US centers) as non-inferior to routine biopsies with respect to composite outcome (rejection, graft dysfunction, death or re-transplantation) and had similar overall survival at 2yrs.
Provides a high (>99%) negative predictive value for ruling out rejection (PPV in all studies is modest 4-7% reported likely due to low incidence of ACR)
Received a class IIa, level B recommendation by 2010 International Society of Heart and Lung Transplantation (ISHLT) guidelines for ACR surveillance
Timing of initiation of surveillance with Allomap
Per the IMAGE and eIMAGE studies, patients >55 days post HT on <20mg of prednisone daily and up to 5 years post HT9, 10
Suitable population to be applied in – stable, asymptomatic patients at low risk for ACR
HT recipients >15 years of age with stable graft function (LVEF>=50%) and asymptomatic11
No history of AMR (ever) or treated ACR Grade 2R or greater during the preceding 2 months10
Absence of DSAs10
On corticosteroid dose <20mg/d10
Have not received hematopoietic growth factors or blood transfusions during the previous 30 days10
Are not pregnant10
No history of severe CAV9
CMV infection (both asymptomatic viremia and CMV disease)12
Diagnostic cut-off value
Many transplant programs have since adopted the same GEP thresholds to prompt an EMB as used in IMAGE9 and eIMAGE10: ≥30 during 2-6 months post-HT and ≥34 after 6 months; the fact that this measurement yields a quantitative result means that the test can be custom-tailored to particular questions— threshold values can be chosen to maximize

sensitivity (at the expense of increasing false positives) or specificity (at the expense of sensitivity), as desired by the clinician's needs in managing patient care

Caveats

Not designed for monitoring of AMR
Tested in cohorts at low risk for ACR
Not validated in randomized clinical trials in European cohorts of HT recipients, primary validation occurred in US-based randomized control trials and large prospective cohorts
Cost and logistics are the major limitations to its use in the European context
Multiple factors affect its performance – per the manufacturer instructions, Allomap should not be used <30d after a blood transfusion that contains WBC; in patients treated with corticosteroid dosage >20mg/d or within 21 days following rejection therapy with steroids; Allomap has also been shown to be affected by race, CMV12 or other viral infections or systemic inflammatory conditions, multi-organ transplant and growth factors

Author: K. Khush/Co-author: A. Nikolova

STATEMENT: Peripheral blood GEP assay (Allomap) is a reliable non-invasive diagnostic tool to rule out acute cellular rejection in stable, low-risk heart transplant recipients>15 years of age who are >55 days post HT. Performance of this assay in Europe remains limited and is subject to cost considerations (Class IIa, Evidence level B)

PICO 2: In heart transplant patients with stable graft function, is ddcfDNA a reliable surveillance tool for subclinical acute rejection monitoring, compared to endomyocardial biopsy?

Population: Heart transplant recipients – either adult or pediatric
Intervention: Donor-derived cell free DNA, Peripheral blood Gene Expression Profiling (Allomap), Cardiac biomarkers (such as NT-proBNP, BNP, Troponin)
Comparators: Endomyocardial biopsy for rejection surveillance, Coronary angiography for CAV
Outcome: Most of the studies are observational and have been designed to validate a diagnostic tool. In one case the study was randomised (IMAGE trial)

Supporting Data

ddcfDNA assays manufactured by different vendors have been shown in multiple large prospective cohort studies to be a reliable method for detection of both AMR and ACR (D-OAR5 – Allosure, DEDUCE7 – Prospera, Stanford GTD3- research grade assay, GRAfT6 – research-grade assay). All the studies were conducted in the US. However, randomized clinical trials have never been performed to demonstrate its non-inferior performance compared to EMB; the upcoming DETECT and MOSAIC studies are the first multicenter, randomized controlled clinical trials which will determine whether dd-cfDNA based surveillance starting as early as 4 weeks after heart transplant is noninferior to EMB-based screening for rejection.
Provides a high NPV (>97%) for rejection rule-out5-7

Timing of initiation of surveillance with Allomap

All assays demonstrate ddcfDNA levels reach stable baseline by 28d post HT and levels have been shown to remain stable up to 2 years post HT (DEDUCE and D-OAR); DEDUCE study (which uses Prospera assay) demonstrated that ddcfDNA levels increase after 2 yrs post HT7

Suitable population to be applied in

All large cohort studies included stable, asymptomatic patients at low risk for rejection; the only study that included patients at elevated risk for AMR was the Cedars Sinai single center parallel arm study of D-OAR and it demonstrated that those patients with AMR0 had almost twice as high ddcfDNA levels (median 0.16%) compared to their counterparts at low AMR risk who had AMR0 on EMB (median 0.07%)5. Subsequent data from the SHORE registry demonstrated that ddcfDNA levels rise with development of de novo DSAs post-transplant, which may account for the higher ddcfDNA levels seen in this immunologically high-risk population13.

Diagnostic cut-off value

The 2 most validated commercial assays (Allosure and Prospera) propose different diagnostic cut-offs: Allosure uses 0.20%5 and Prospera uses 0.15%7 to achieve NPV >97%. The high NPV at these diagnostic cut-offs make them very suitable as a “rule-out of rejection tool”; the fact that this measurement yields a quantitative result means that the test can be custom-tailored to particular questions— threshold values can be chosen to maximize sensitivity (at the expense of increasing false positives) or specificity (at the expense of sensitivity), as desired by the clinician's needs in managing patient care

Caveats

Donor fraction vs absolute cfDNA levels - Some have suggested that dd-cfDNA quantity may be a better marker than dd-cfDNA fraction, as it is independent of changes in background cfDNA; a recent study in kidney transplantation incorporated recipient cfDNA levels for detecting rejection, which increased sensitivity, albeit in a small cohort. A “two-threshold” algorithm was employed, which combined a cutoff for dd-cfDNA fraction with a cutoff for absolute quantity of dd-cfDNA. In the DEDUCE study, a post-hoc analysis using dd-cfDNA quantity indicated that incorporation of this measure could increase the sensitivity of the assay7

Prognostic role of asymptomatic cfDNA elevation - ddcfDNA has been observed to rise up to 5 months prior to clinically significant events (graft dysfunction, pathological rejection diagnosis, etc) – de Vlaminck3 and GRAfT6 studies. This represents an opportunity for early diagnosis and treatment. However, no studies have been performed to-date to support immunosuppression modulation based on dd-cfDNA levels.

CfDNA for surveillance of rejection treatment response - small studies have shown reduction in cfDNA levels with rejection treatment; however, the assays have not been validated for therapeutic guidance

CfDNA assays are unable to discriminate AMR from ACR and hence, the need for EMB (+/- more advanced gene expression testing) to determine rejection type as this guides treatment approach. The GRAfT study, which uses a research-grade cfDNA assay, raises the possibility for differentiating types of rejection based on DNA fragment size and content

CfDNA assays are currently processed in central laboratories in the USA with relatively slow turn-around time of up to 72h (Allosure and Prospera); the adoption of this technology in Europe is limited by cost considerations, regulatory approval by local agencies and the availability of the appropriate equipment and technology at local centers

CfDNA levels have been shown to be elevated in patients with dnDSA13, raising the possibility of identifying pathological DSAs using these assays – these findings are hypothesis-generating and remain to be verified in large studies

CfDNA levels are effected by multi-organ transplants, active malignancy, prior bone marrow transplant, pregnancy, <24 hours following an EMB, sepsis

Author: K. Khush/Co-author: A. Nikolova

STATEMENT*:* ddcfDNA appears to be a reliable tool for subclinical rejection surveillance (ruling out both ACR and AMR) in HT recipients who are at low rejection risk and >28days

post HT. Performance of this assay in Europe needs to be validated in the local logistic and technical context. (Class 2B, level of evidence B-NR)

PICO 3: *In heart transplant patients, is ddcfDNA (or GEP) reliable surveillance strategy to monitor for cardiac allograft vasculopathy as compared with standard diagnostic methods?*

Population: Heart transplant recipients – either adult or pediatric
Intervention: Donor-derived cell free DNA, Peripheral blood Gene Expression Profiling (Allomap), Cardiac biomarkers (such as NT-proBNP, BNP, Troponin)
Comparators: Endomyocardial biopsy for rejection surveillance, Coronary angiography for CAV
Outcome: Most of the studies are observational and have been designed to validate a diagnostic tool. In one case the study was randomised (IMAGE trial)

Author: L. Potena/Co-author: A. Nikolova

REFERENCES

1. Crespo-Leiro MG, Stypmann J, Schulz U, Zuckermann A, Mohacsi P, Bara C, Ross H, Parameshwar J, Zakliczynski M, Fiocchi R, Hoefer D, Colvin M, Deng MC, Leprince P, Elashoff B, Yee JP and Vanhaecke J. Clinical usefulness of gene-expression profile to rule out acute rejection after heart transplantation: CARGO II. Eur Heart J. 2016;37:2591-601.
2. Deng MC, Eisen HJ, Mehra MR, Billingham M, Marboe CC, Berry G, Kobashigawa J, Johnson FL, Starling RC, Murali S, Pauly DF, Baron H, Wohlgemuth JG, Woodward RN, Klingler TM, Walther D, Lal PG, Rosenberg S, Hunt S and Investigators C. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. Am J Transplant. 2006;6:150-60.
3. De Vlaminck I, Valantine HA, Snyder TM, Strehl C, Cohen G, Luikart H, Neff NF, Okamoto J, Bernstein D, Weisshaar D, Quake SR and Khush KK. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. Sci Transl Med. 2014;6:241ra77.
4. Grskovic M, Hiller DJ, Eubank LA, Sninsky JJ, Christopherson C, Collins JP, Thompson K, Song M, Wang YS, Ross D, Nelles MJ, Yee JP, Wilber JC, Crespo-Leiro MG, Scott SL and Woodward RN. Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. J Mol Diagn. 2016;18:890-902.
5. Khush KK, Patel J, Pinney S, Kao A, Alharethi R, DePasquale E, Ewald G, Berman P, Kanwar M, Hiller D, Yee JP, Woodward RN, Hall S and Kobashigawa J. Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study. Am J Transplant. 2019;19:2889-2899.
6. Agbor-Enoh S, Shah P, Tunc I, Hsu S, Russell S, Feller E, Shah K, Rodrigo ME, Najjar SS, Kong H, Pirooznia M, Fideli U, Bikineyeva A, Marishta A, Bhatti K, Yang Y, Mutebi C, Yu K, Kyoo Jang M, Marboe C, Berry GJ, Valantine HA and Investigators GR. Cell-Free DNA to Detect Heart Allograft Acute Rejection. Circulation. 2021;143:1184-1197.
7. Kim PJ, Olymbios M, Siu A, Wever Pinzon O, Adler E, Liang N, Swenerton R, Sternberg J, Kaur N, Ahmed E, Chen YA, Fehringer G, Demko ZP, Billings PR and Stehlik J. A novel donor-derived cell-free DNA assay for the detection of acute rejection in heart transplantation. J Heart Lung Transplant. 2022;41:919-927.
8. Richmond ME, Zangwill SD, Kindel SJ, Deshpande SR, Schroder JN, Bichell DP, Knecht KR, Mahle WT, Wigger MA, Gaglianello NA, Pahl E, Simpson PM, Dasgupta M, North PE, Hidestrand M, Tomita-Mitchell A and Mitchell ME. Donor fraction cell-free DNA

and rejection in adult and pediatric heart transplantation. J Heart Lung Transplant. 2020;39:454-463.
9. Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, Deng MC, Cappola TP, Kao A, Anderson AS, Cotts WG, Ewald GA, Baran DA, Bogaev RC, Elashoff B, Baron H, Yee J, Valantine HA and Group IS. Gene-expression profiling for rejection surveillance after cardiac transplantation. N Engl J Med. 2010;362:1890-900.
10. Kobashigawa J, Patel J, Azarbal B, Kittleson M, Chang D, Czer L, Daun T, Luu M, Trento A, Cheng R and Esmailian F. Randomized pilot trial of gene expression profiling versus heart biopsy in the first year after heart transplant: early invasive monitoring attenuation through gene expression trial. Circ Heart Fail. 2015;8:557-64.
11. Moayedi Y, Foroutan F, Miller RJH, Fan CS, Posada JGD, Alhussein M, Tremblay-Gravel M, Oro G, Luikart HI, Yee J, Shullo MA, Khush KK, Ross HJ and Teuteberg JJ. Risk evaluation using gene expression screening to monitor for acute cellular rejection in heart transplant recipients. J Heart Lung Transplant. 2019;38:51-58.
12. Kanwar MK, Khush KK, Pinney S, Sherman C, Hall S, Teuteberg J, Uriel N and Kobashigawa J. Impact of cytomegalovirus infection on gene expression profile in heart transplant recipients. J Heart Lung Transplant. 2021;40:101-107.
13. Teuteberg J, Kobashigawa J, Shah P, Ghosh S, Ross D, DePasquale E and Khush KK. Donor-Derived Cell-Free DNA Predicts De Novo DSA after Heart Transplantation [Abstract]. JHLT. 2021;40.
14. Henricksen EJ, Purewal S, Moayedi Y, Waddell K, Gordon J, Morales DP, Luikart H, Han J, Feng KY, Lee R, Shudo Y, Jimenez S, Khush KK and Teuteberg J. Combining Donor Derived Cell-Free DNA and Gene Expression Profiling for Non-Invasive Surveillance after Heart Transplantation [Abstract]. JHLT. 2021;40.
15. Loupy A, Duong Van Huyen JP, Hidalgo L, Reeve J, Racape M, Aubert O, Venner JM, Falmuski K, Bories MC, Beuscart T, Guillemain R, Francois A, Pattier S, Toquet C, Gay A, Rouvier P, Varnous S, Leprince P, Empana JP, Lefaucheur C, Bruneval P, Jouven X and Halloran PF. Gene Expression Profiling for the Identification and Classification of Antibody-Mediated Heart Rejection. Circulation. 2017;135:917-935.
16. Holzhauser L, Clerkin KJ, Fujino T, Alenghat FJ, Raikhelkar J, Kim G, Sayer G and Uriel N. Donor-derived cell-free DNA is associated with cardiac allograft vasculopathy. Clin Transplant. 2021;35:e14206.
17. Raval N, Ravichandran A, Ghosh S, Wolf-Doty T, Ross D, Hall S and Uriel N. Association of Donor Age and Plasma Donor-Derived Cell-Free DNA Levels with CAV Development after Heart Transplant: SHORE Preliminary Data [Abstract]. JHLT. 2021;40.
18. Jimenez-Blanco Bravo M, Perez-Gomez L, Hernandez-Perez FJ, Arellano-Serrano C, Torres-Sanabria M, Gomez-Bueno M, Oteo-Dominguez JF, Mingo-Santos S and Segovia-Cubero J. Lack of Usefulness of Donor-Derived Cell-Free DNA as a Biomarker for Cardiac Allograft Vasculopathy: A Prospective Study. Front Cardiovasc Med. 2022;9:856600.

STATEMENT: ddcfDNA and GEP (Allomap) are not recommended as surveillance strategy for cardiac allograft vasculopathy post HT (Class III)

PICO 4: In heart transplant patients with stable graft function, is dd-cfDNA (or GEP) a reliable marker to stratify prognosis (or monitor the efficacy of therapy) as compared to standard clinical classifiers

Population: Heart transplant recipients – either adult or pediatric
Intervention: Peripheral blood Gene Expression Profiling (such as AlloMap™), Donor-derived-cell free DNA (such as, AlloSure™ and Prospera™, not commercially available in Europe, and Allonext™, available in Europe for investigational purposes)
Comparators: For prognosis stratification, no comparators have been used. May complement other standard clinical classifiers in prognosis stratification.
For monitorization of the efficacy of therapy, endomyocardial biopsy (EMB) would be the comparator
Outcome: For prognosis stratification, there is a general agreement in the use of MACTE (Major Adverse Cardiac Transplant Events), a composite of: acute rejection with hemodynamic compromise, graft dysfunction, death or retransplantation. Most of the studies are observational and retrospective.

Author: J. Segovia/Co-author: A. Minervini

Supporting data (the question is split into its 2 aspects: prognosis and IS guidance)
A) Prognostic stratification: In general, this question is related to PICO-1. Most studies of these biomarkers have been focused on their usefulness for surveillance of acute rejection in stable heart transplant (HT) recipients.
No association has been found between GEP scores and mortality during follow-up in different studies.
Two sub studies of major trials (IMAGE[1] and CARGO II[2]) published by Deng et al, 2014[3] and Crespo-Leiro et al, 2015[4], have tested the performance of AlloMap as a predictor of MACTE. In both cases, intraindividual variability (standard deviation of ≥4 GEP scores) predicted incidence of MACTE in the next 2-3 years of follow-up, with a hazard ratio of 1.76 per unit increase in variability in one of the papers[3]. Other ways of measuring repeated individual GEP scores (ordinal score, scores ≥ a given threshold) did not show a similar predictive ability.
As shown in OAR study (Moayedi 2019)[5], no meaningful changes of GEP were seen in relation to specific HT complications such as cardiac allograft vasculopathy (CAV), cancer or non-CMV infections.
As for dd-cfDNA, a preliminary study (Zangwill, 2020)[6] centered in the first 10 days after HT in a small pediatric population showed that a blunted decline of initially elevated dd-cfDNA may be associated with early death.
Only one exploratory abstract (Crespo-Leiro, 2017)[7] has been directed to evaluate the prognostic value of dd-cfDNA in stable HT recipients. It included 48 patients and 166 samples from CARGO-II trial, and showed an association between the median of several individual dd-cfDNA values and incidence of MACTE (as defined above), p=0.02, AUCOR=0.77. Other cf-DNA measures, such as maximum value, individual measures o variability of intraindividual measures, did not predict MACTE.
Of note, several groups have found clear relationship between “Total or nuclear cfDNA” (derived both from recipient and donor tissues) and several near-term events, such as death, cardiac arrest, and need for mechanical circulatory support[8]. It seems to be a marker of more extensive tissue damage, and has shown prognostic value in different populations of patients in the ICU setting. Total cfDNA elevations have been also seen in patients with infections after HT[9]. The same is true for sepsis, inflammatory diseases and cancer in non-transplant populations.
B) Use of GEP and dd-cfDNA to monitor the efficacy of therapy
GEP: A preliminary analysis of a subset of 127 pts. from CARGO study (Mehra, 2008)[10] proposed risk stratification into three risk groups: HT recipients with AlloMap scores ≤20

from 55 to 180 days post HT have very low risk of rejection, and this may identify a subgroup of patients for less frequent EMB or more aggressive steroid weaning. Conversely, patients with scores ≥30 would be a high risk group for closer surveillance and more cautious immunosuppression (IS) reduction. Between these 2 groups, there is an intermediate-risk group accounting for 56% of the population.
Accordingly, in the small randomized eIMAGE trial (Kobashigawa 2015)[11] steroid withdrawal was equally successful in patients with GEP-based vs EMB-based surveillance (90-95% overall success rate in the 68% HT recipients in which it was attempted).
dd-cfDNA for guidance of IS: no studies have been directed to this specific point. Dd-cf DNA is potentially useful to guide personalized IS, to monitor response to AR therapy (Grskovic, 2016)[12] and allograft heath during IS changes due to its short half-life (30 min-2 hours) and its high sensitivity to detect early graft injury (Khush 2021)[13].
Ongoing MOSAIC trial (ClinicalTrials.gov Identifier: NCT05459181) will help to clarify the role of several biomarkers (including GEP and dd-cfDNA) in the future.

Caveats

a) Prognostic stratification: There are no studies specifically designed for exploring the prognostic role of either GEP or dd-cfDNA in HT. Major studies on these biomarkers were performed in stable low-risk patients, with very low mortality rates during their limited (up to 3-year) follow-up.
However, the existence of 2 sub-studies[3-4] of major GEP trials with reasonably-sized populations (369 and 91 pts, respectively) and differing characteristics (one USA-based, the second mainly European) with coincidental findings should not be dismissed. Another limitation of these studies is the need for ≥4 consecutive GEP scores to evaluate variability (standard devation of all scores), a less direct parameter than
b) GEP and cc-cfDNA for surveillance of rejection treatment response: Based on a few small studies not specifically directed to this aim. These experiences must be taken as preliminary or hypothesis-generating, since we don´t have yet results of prospective studies with proper design to confirm the ability of these biomarkers for IS adjustment.
Cost and logistics are major limitations to its use in the Europe: CfDNA assays (Allosure and Prospera) are currently processed in central laboratories in the USA with turn-around time of up to 72h, but the adoption of this technology in Europe is limited by cost considerations, regulatory approval by local agencies and the availability of the appropriate equipment and technology at local centers.
Multiple factors affect its performance: Allomap should not be used <30 d after a blood transfusion that contains WBC; in patients treated with corticosteroid dosage >20mg/d or within 21 days following rejection therapy with steroids; Allomap has also been shown to be affected by race, CMV or other viral infections, systemic inflammatory conditions, multi-organ transplant.
CfDNA levels are affected by obtention of blood sample shortly after an EMB[14], multi-organ transplants, active malignancy, prior bone marrow transplant, pregnancy, sepsis, etc.

References:

1. Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, Deng MC, Cappola TP, Kao A, Anderson AS, Cotts WG, Ewald GA, Baran DA, Bogaev RC, Elashoff B, Baron H, Yee J, Valantine HA and Group IS. Gene-expression profiling for rejection surveillance after cardiac transplantation. N Engl J Med. 2010;362:1890-900.

2. Crespo-Leiro MG, Stypmann J, Schulz U, Zuckermann A, Mohacsi P, Bara C, Ross H, Parameshwar J, Zakliczynski M, Fiocchi R, Hoefer D, Colvin M, Deng MC, Leprince P, Elashoff B, Yee JP and Vanhaecke J. Clinical usefulness of gene-expression profile to rule out acute rejection after heart transplantation: CARGO II. Eur Heart J. 2016;37:2591-601.

3. Deng MC, Elashoff B, Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, Cappola TP, Kao A, Anderson AS, Cotts WG, Ewald GA, Baran DA, Bogaev RC, Shahzad K, Hiller D, Yee J, Valantine HA, IMAGE Study Group. Utility of gene expression profiling score variability to predict clinical events in heart transplant recipients. Transplantation 2014;97:708–714.

4. Crespo-Leiro MG, Stypmann J, Schulz U, Zuckermann A, Mohacsi P, Bara C, Ross H, Parameshwar J, Zakliczynski M, Fiocchi R, Hoefer D, Deng MC, Leprince P, Hiller D, Eubank L, Deljkich E, Yee JP and Vanhaecke J. Performance of gene-expression profile test score variability to predict clinical events in heart transplant recipients. BMC Cardiovascular Disorders 2015; 15:120-8.

5. Moayedi Y, Foroutan F, Miller RJH, Fan CS, Posada JGD, Alhussein M, Tremblay-Gravel M, Oro G, Luikart HI, Yee J, Shullo MA, Khush KK, Ross HJ and Teuteberg JJ. Risk evaluation using gene expression screening to monitor for acute cellular rejection in heart transplant recipients. J Heart Lung Transplant. 2019;38:51-58.

6. Zangwill SD, Kindel SJ, Ragalie WS, North PE, Pollow A, Hidestrand M, Tomita-Mitchell A, Stamm KD, Mitchell ME. Early changes in cell-free DNA levels in newly transplanted heart transplant patients. Pediatr Transplant. 2020;24:e13622.

7. Crespo-Leiro M, Hiller D, Woodward R, Grskovic M, Marchis C, Song M, Collins J, Zuckermann A. Analysis of donor-derived cell-free DNA with 3 year outcomes in heart transplant recipient. J Heart Lung Transplant 2017; 36:S69-70 (abstract)

8. Zangwill SD, Deshpande SR, Simpson PM, Liang HL, Zhang L, Dasgupta M, Richmond ME, Kindel SJ, Bichell DP, Mahle WT, Wigger MA, Schroder JN, Knecht KR, Pahl E, Gaglianello NA, North PE, Tomita-Mitchell A, Mitchell ME. Increase in nuclear cell-free DNA is associated with major adverse events in adult and pediatric heart transplant recipients. Clin Transplant. 2022;36:e14509.
9. Scott JP, Ragalie WS, Stamm KD, Mahnke DK, Liang HL, Simpson PM, Dasgupta M, Katz R, North PE, Tomita-Mitchell A, Zangwill SD, Kindel SJ, Mitchell ME. Total Cell-Free DNA Predicts Death and Infection Following Pediatric and Adult Heart Transplantation. Ann Thorac Surg. 2021;112:1282-9.

10. Mehra MR, Kobashigawa JA, Deng MC, Fang KC, Klingler TM, Lal PG, Rosenberg S, Uber PA, Starling RC, Murali S, Pauly DF, Dedrick R, Walker MG, Zeevi A, Eisen HJ; CARGO Investigators. Clinical implications and longitudinal alteration of peripheral blood transcriptional signals indicative of future cardiac allograft rejection. J Heart Lung Transplant. 2008;27:297-301

11. Kobashigawa J, Patel J, Azarbal B, Kittleson M, Chang D, Czer L, Daun T, Luu M, Trento A, Cheng R and Esmailian F. Randomized pilot trial of gene expression profiling versus heart biopsy in the first year after heart transplant: early invasive monitoring attenuation through gene expression trial. Circ Heart Fail. 2015;8:557-64.

12. Grskovic M, Hiller DJ, Eubank LA, Sninsky JJ, Christopherson C, Collins JP, Thompson K, Song M, Wang YS, Ross D, Nelles MJ, Yee JP, Wilber JC, Crespo-Leiro MG, Scott SL, Woodward RN. Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. J Mol Diagn. 2016;18:890-902.

13. Khush KK. Clinical utility of donor-derived cell-free DNA testing in cardiac transplantation. J Heart Lung Transplant. 2021;40:397-404

14. Zangwill SD, Stamm KD, Hidestrand M, Tomita-Mitchell A, Mitchell ME. Effect of endomyocardial biopsy on levels of donor-specific cell-free DNA. J Heart Lung Transplant. 2019;38:1118-20

STATEMENT:

- Current available evidence does not support the direct use of the levels of dd-cfDNA or GEP from peripheral blood to stratify prognosis in patients after HT. (Class III, level of evidence C).
- GEP variability (calculated as standard deviation of ≥4 separate individual scores) might be of value in the prediction of incidence of MACTE* (Class Performance of this assay in Europe needs to be validated in the local logistic and technical context. (Class IIb, level of evidence B)
- Current available evidence does not support the use of dd-cfDNA or GEP from peripheral blood to guide immunosuppressive therapy in patients after HT. (Class III, level of evidence C).

**MACTE = Major Adverse Cardiac Transplant Events, composite of acute rejection with hemodynamic instability, graft failure, retransplant and death.*

PICO 5: In heart transplant patients with stable graft function, are cardiac biomarkers (NT-proBNP, BNP, Troponin) reliable surveillance tool for subclinical acute rejection monitoring, compared to endomyocardial biopsy?

Population: Heart transplant recipients – either adult or pediatric
Intervention: Cardiac biomarkers (such as NT-proBNP, BNP, Troponin)
Comparators: Endomyocardial biopsy for rejection surveillance
Outcome: Most of the studies are observational and have been designed to validate a diagnostic tool. In one case the study was randomised (IMAGE trial)

Author: M. Crespo/Co-author: A. Minervini

STATEMENT:

Troponin

1) Because of the contradictory results of the available studies and the insufficient diagnostic power, there is no enough evidence to support troponin routine use in the diagnostic pathway of AR in place of EMB. However, high sensitivity Troponin (hsTn) assays showed good sensitivity and negative predictive value, and this raise the possibility that they can be used to "rule-out" ACR and so to limit the use of surveillance EMB. (Class IIb, Evidence level: B-NR)
2) On the other hand, hsTn could be of help in decision making to add EMB information, complementing its limitations regarding sampling error or clinicopathological discordance. (Class IIb, Evidence level: B-NR)
3)Further research is needed to understand whether troponin might serve as ancillary parameter in a multifactorial approach, so that its addition to new biomarkers, such as cell-free DNA could lead to an enhanced "liquid biopsy" capable to replace EMB. (Class IIb, Evidence level: B-NR)

Supporting evidence

- Every damage to cardiac myocytes that leads to myocardial necrosis causes release of troponin into the circulation. Myocyte damage is the pathologic hallmark of moderate to severe acute cellular rejection so an elevated serum cardiac troponin (cTn) level would be expected during an episode of ACR. However, the results of several studies are discordant.
  Some of them (Battes, Mullen, Wahalander) didn't find a significant correlation between troponin plasma levels and acute rejection demonstrated by EMB. Ahn found a correlation between AR and only hs-cTnI ratio index. According to Gleissner rejection episodes are often associated with elevated TnT but his results didn't have enough sensitivity or specificity to replace EMB. Bladuini, Dyer, Patel e Munoz-Esparza found troponin plasma levels significantly higher in patients with acute rejection and Erbel correlated hsTnT serum levels with mortality in the first year post-transplant.
- A meta-analysis by Fitzimons et al demonstrated that cTn assays do not have sufficient specificity to diagnose ACR in place of EMB; however, hscTn assays may have sufficient sensitivity and negative predictive value to exclude ACR and limit the need for surveillance EMB. Hill and Zengyang found similar results.
- In addition, hsTnI levels increase in parallel with higher histological grades of rejection, decrease after immunosuppressive treatment (Dyer) and had demonstrated its usefulness in the diagnosis of antibody-mediated rejection (Patel).

Caveats:

1- Results of the studies are contradictory, and even some studies that showed a positive association between troponin and rejection did not found a sufficient diagnostic power to replace EMB.
2- There are neither randomized controlled trial nor large prospective studies, but only small single-center observational studies so their results are not generalized to the population.
3- In the early post-transplant period there is elevation of plasma troponin in all patients (due to ischemia-reperfusion injury during organ procurement and implantation), so during the first weeks (or even months) cTn levels will not be useful in excluding the presence of ACR

(the precise length of this time in uncertain; according to Zhengyang et al. this period is confined to 1 month).
4- There are no studies comparing troponin with dd-cf-DNA.

Natriuretic peptides

Statement:

1) Natriuretic peptide levels (NPs) are not a reliable surveillance tool for subclinical AR monitoring, compared to EMB in stable HT patients. They may be helpful in long-term follow-up to detect subclinical graft dysfunction that should prompt further studies to rule out rejection or CAV.

   (Class IIb, Level of evidence B-NR)
2) Further studies is needed to understand if NPs might be used as a parameter in a multi-marker panel approach (maybe with troponin, cfDNA) to create a "liquid biopsy" able to replace EMB.
3) They may be helpful in long-term follow-up to detect subclinical graft dysfunction that should prompt further studies to rule out rejection or CAV.

Supporting data:

- Natriuretic peptides (NPs) are biomarkers that accurate predict heart failure and left ventricular dysfunction and, are predictive of prognosis in patients with advanced heart failure.
- Several studies found that NT-proBNP and BNP levels are elevated during episodes of acute allograft rejection, even when hemodynamic parameters are unchanged (Klingenberg, Wu) but the results are conflicting.
- Damodaran, Dyer, Kittleson, Wu, Rossano found a significantly correlation between BNP (or NT-proBNP) and acute cellular rejection and Martinez-Dolz, Klingernberg and Mehra (2 studies) found an association between NPs and poor outcomes after transplant and vascular injury (CAV).
- Arnau-Vives, Ambrosi, Almenar, Knetch, Lindblade, Hammerer-Lercher, Hervas and Cuppoletti correlated NPs to acute rejection and adverse outcomes but in their studies the biomarkers lack of diagnostic accuracy.
- Several studies suggest that they have a good negative predictive value to exclude acute allograft rejection and to predict adverse outcomes (this is particularly true in children cohorts)
- At last, Bader, Battes, Arora, Klingenberg and O'Neill didn't find any association between BNP and acute rejection.

Caveats

1- There are neither randomized controlled trial nor large prospective studies, but only small single-center observational studies, therefore their results are not generalized to the population.

2- BNP and NT-proBNP values are higher early in the post-transplant period, so they can't be used to predict rejection very early after transplant.

3- The main limitation of NPs is the marked heterogeneity of its values due to its biological variability among individuals; in fact lots of studies didn't find a correlation between absolute values of BNP and rejection but only between individual dynamic changes of NPs and AR (Kittleson, Knetch, Lindblade, Cuppoletti). Serial monitoring of BNP and NT-proBNP instead of their absolute values could be preferred to correlate them to AR.

REFERENCES:

1. Giarraputo, A., Barison, I., Fedrigo, M., Burrello, J., Castellani, C., Tona, F., Bottio, T., Gerosa, G., Barile, L., & Angelini, A. (2021). A Changing Paradigm in Heart Transplantation: An Integrative Approach for Invasive and Non-Invasive Allograft Rejection Monitoring. Biomolecules, 11(2).

2. Hill DA, Drazner MH, de Lemos JA. (2013). Do established biomarkers such as B-type natriuretic peptide and troponin predict rejection? Curr Opin Organ Transplant 18:581-8

3. Labarrere, C. A., & Jaeger, B. R. (2012). Biomarkers of heart transplant rejection: the good, the bad, and the ugly! [Review]. Translational Research: The Journal Of Laboratory & Clinical Medicine, 159(4), 238-251.

4. Ortiz-Bautista, C., Fernandez-Aviles, F., & Delgado Jimenez, J. F. (2021). Serum biomarkers of acute rejection: Towards precision medicine in heart transplant. J Heart Lung Transplant, 40(10), 1090-1097.

5. Fitzsimons, S., Evans, J., Parameshwar, J., & Pettit, S. J. (2018). Utility of troponin assays for exclusion of acute cellular rejection after heart transplantation: A systematic review. J Heart Lung Transplant, 37(5), 631-638.

6. Liu, Z, Perry, LA, Penny-Dimri, JC, Handscombe, M, Overmars, I, Plummer, M, et al. Donor Cardiac Troponin for Prognosis of Adverse Outcomes in Cardiac Transplantation Recipients: A Systematic Review and Meta-Analysis. Transpl Direct (2022) 8:e1261.

7. Liu, Z, Perry, LA, Penny-Dimri, JC et al. Elevated Cardiac Troponin to Detect Acute Cellular Rejection After Cardiac Transplantation: A Systematic Review and Meta-Analysis. Transpl Int, 08 June 2022

8. Balduini A, Campana C, Ceresa M, Arbustini E, Bosoni T, Serio A, et al. Utility of Biochemical Markers in the Follow-Up of Heart Transplant Recipients. Transplant Proc (2003) 35:3075–8. doi:10.1016/j.transproceed.2003.10.044

9. Madan S, Saeed O, Shin J, Sims D, Goldstein D, Piña I, et al. Donor Troponin and Survival after Cardiac Transplantation: An Analysis of the United Network of Organ Sharing Registry. Circ Heart Fail (2016) 9:e002909. doi:10.1161/circheartfailure.115.002909

10. Gleissner CA, Klingenberg R, Nottmeyer W, Zipfel S, Sack F-U, Schnabel PA, et al. Diagnostic Efficiency of Rejection Monitoring after Heart Transplantation with Cardiac Troponin T is Improved in Specific Patient Subgroups. Clin Transpl (2003) 17:284–91. doi:10.1034/j.1399-0012.2003.00050.x

11. Patel PC, Hill DA, Ayers CR, Lavingia B, Kaiser P, Dyer AK, et al. High sensitivity Cardiac Troponin I Assay to Screen for Acute Rejection in Patients with Heart Transplant. Circ Heart Fail (2014) 7:463–9. doi:10.1161/ circheartfailure.113.000697

12. Kittleson MM, Garg S. Solid Gold, or Liquid Gold?: Towards a New Diagnostic Standard for Heart Transplant Rejection. Circulation (2021) 143:1198–201. doi:10.1161/circulationaha.120.052925

13. Klingenberg R, Koch A, Gleissner C, Schnabel PA, Haass M, Remppis A, Katus HA, Dengler TJ. Determinants of B-type natriuretic peptide plasma levels in the chronic phase after heart transplantation. Transpl Int. 2005; 18:169 –176.

14. Mehra MR, Uber PA, Potluri S, Ventura HO, Scott RL, Park MH. Usefulness of an elevated b-type natriuretic peptide allograft failure, cardiac allograft vasculopathy and survival after heart transplantation. Am J Cardiol. 2004;94:454 – 458.

15. Ambrosi P, Oddoze C, Riberi A, Arques S, Portugal H, Metras D, Habib G . Usefulness of N- terminal-pro-brain natriuretic peptide levels in predicting survival in heart transplant recipients. Am J Cardiol. 2004;94: 1585–1587.

16. Rossano JW, Denfield SW, Kim JJ, et al. B-type natriuretic peptide is a sensitive screening test for acute rejection in pediatric heart transplant recipients. J Heart Lung Transplant 2008: 27:649–654.

17. Lindblade CL, Chun DS, Darragh RK, Caldwell RL, Murphy DJ, Schamberger MS. Value of plasma b-type natriuretic peptide as a marker for rejection in pediatric heart transplant recipients. Am J Cardiol 2005: 95: 909–911.

18. Park MH, Scott RL, Uber PA, Harris BC, Chambers R, Mehra MR. Usefulness of B-type natriuretic peptide levels in predicting hemodynamic perturbations after heart transplantation despite preserved left ventricular systolic function. Am J Cardiol 2002;90:1326 –9.

19. Almenar L, Hervas I, Martinez-Dolz L, et al. The value of brain natriuretic peptide for the diagnosis of heart transplant rejection. Transplant Proc 2002;34:174 –5

20. Muñoz-Esparza C, Garrido IP, Blanco R, Casas T, González-Cánovas C, Pastor-Pérez F, Peñafiel P, Minguela A, Valdés M, Pascual-Figal DA. Usefulness of high sensitivity troponin T assay in detecting acute allograft rejection after heart transplantation. Rev Esp Cardiol. 2011;64:1109–1113.

21. Cuppoletti A, Roig E, Perez-Villa F, et al. Value of NT-proBNP determinations in the follow-up of heart transplantation. Transplant Proc 2005;37:4033–5.

22. Arnau-Vives MA, Almenar L, Hervas I, et al. (2004). Predictive value of brain natriuretic peptide in the diagnosis of heart transplant rejection. J Heart Lung Transplant 23:850-6.

23. Bader FM, Rogers RK, Kfoury AG, Gilbert EM, Horne BD, Stehlik J, Renlund DG. (2009). Time- dependent changes in B-type natriuretic peptide after heart transplantation: correlation with allograft rejection and function. Congest Heart Fail. 2009 Mar-Apr;15(2):63-7.

24. Battes LC, Caliskan K, Rizopoulos D, et al. (2015). Repeated measurements of NT-pro-B-type natriuretic peptide, troponin T or C-reactive protein do not predict future allograft rejection in heart transplant recipients. Transplantation ;99:580-5.

25. Dyer AK, Barnes AP, Fixler DE, Shah TK, Sutcliffe DL, Hashim I, Drazner MH, de Lemos JA. (2012). Use of a highly sensitive assay for cardiac troponin T and N-terminal pro-brain natriuretic peptide to diagnose acute rejection in pediatric cardiac transplant recipients. Am Heart J ;163:595-600.

26. Erbel C, Taskin R, Doesch A, et al. (2012). High-sensitive troponin T measurements early after heart transplantation predict short- and long term survival. Transpl Int 26.

27. Gleissner CA, Zehelein J, Sack FU, et al.(2002). Extended experience and subgroup analysis using cardiac troponin T for rejection monitoring aftepater heart transplantation. Transplant Proc 34:2178-80

28. Hammerer-Lercher A, Mair J, Antretter H, et al. (2005). B-type natriuretic peptide as a marker of allograft rejection after heart transplantation. J Heart Lung Transplant ;24:1444.

29. Kittleson MM, Skojec DV, Wittstein IS, et al. (2009). The change in B-type natriuretic peptide levels over time predicts significant rejection in cardiac transplant recipients. J Heart Lung Transplant;28:704-9.

30. Mehra, M. R., Uber, P. A., Walther, D., Vesely, M., Wohlgemuth, J. G., Prentice, J., Tayama, D.,& Billingham, M. (2006). Gene expression profiles and B-type natriuretic peptide elevation in heart transplantation: more than a hemodynamic marker [Research Support, Non-U.S. Gov't]. Circulation, 114(1 Suppl), I21-26.

31. Mullen, J.C. (2002) Troponin T and I are not reliable markers of cardiac transplant rejection.European Journal of Cardio-Thoracic Surgery, Volume 22, Issue 2, August 2002, Pages 233–237

32. O'Neill JO, McRae AT 3rd, Troughton RW, Ng K, Taylor DO, Yamani MH, Young JB, Starling RC. (2005). Brain natriuretic peptide levels do not correlate with acute cellular rejection in De Novo orthotopic heart transplant recipients. J Heart Lung Transplant. 2005 Apr;24(4):416-20.

33. Shaw, S. M., Critchley, W. R., Puchalka, C. M., Williams, S. G., Yonan, N., & Fildes, J. E. (2012). Brain natriuretic peptide induces CD8+ T cell death via a caspase 3 associated pathway– implications following heart transplantation. Transplant Immunology, 26(2-3), 119-122.

34. Wahlander, H., Kjellstrom, C., & Holmgren, D. (2002). Sustained elevated concentrations of cardiac troponin T during acute allograft rejection after heart transplantation in children. Transplantation, 74(8), 1130-1135. Copyright??

35. Wu AH, Johnson ML, Aaronson KD, Gordon D, Dyke DB, Koelling TM. Brain natriuretic peptide predicts serious cardiac allograft rejection independent of hemodynamic measurements. J Heart Lung Transplant. 2005; 24:52–57.

36. Ahn, K. T., Choi, J. O., Lee, G. Y., Park, H. D., & Jeon, E. S. (2015). Usefulness of high-sensitivity troponin I for the monitoring of subclinical acute cellular rejection after cardiac transplantation. Transplantation Proceedings, 47(2), 504-510. Cross-sectional

37. Arora, S., Gullestad, L., Wergeland, R., Simonsen, S., Holm, T., Hognestad, A., Ueland, T., Geiran, O., & Andreassen, A. (2007). Probrain natriuretic peptide and C-reactive

protein as markers of acute rejection, allograft vasculopathy, and mortality in heart transplantation. Transplantation, 83(10), 1308-1315. Copyright???

38. Damodaran A, Dardas T, Wu AH, et al.(2012). Changes in serial B-type natriuretic peptide level independently predict cardiac allograft rejection. J Heart Lung Transplant. 31: 708.

39. Knecht KR, Alexander ML, Swearingen CJ, Frazier EA. NTproBNP as a marker of rejection in pediatric heart transplant recipients. Pediatr Transplant. 2012; 16:335–339.

40. Martínez-Dolz L, Almenar L, Hervás I, Moro J, Agüero J, Sánchez-Lázaro I, Mateo A, Salvador A. Prognostic relationship between two serial determinations of B-type natriuretic peptide and medium-long-term events in heart transplantation. J Heart Lung Transplan. 2008 Jul;27(7):735-40.

41. Hervás I, Arnau MA, Almenar L, Pérez-Pastor JL, Chirivella M, Osca J, Bello P, Osa A, Martí JF, Vera F, Mateo A. Ventricular natriuretic peptide (BNP) in heart transplantation: BNP correlation with endomyocardial biopsy, laboratory and hemodynamic measures. Lab Invest. 2004 Jan;84(1):138-45.

LUNG

PICO 1: Is ddcfDNA a reliable marker to diagnose/monitor clinical and subclinical acute rejection or infection of the graft in lung transplant patients, compared with standard diagnostic methods?

Author: S. Agbor-Enoh/Co-author: R. Vos

STATEMENT: Beyond 6 weeks of transplantation, in addition to routine clinical care, ddcfDNA measurements can be used as a rule out test for clinical and subclinical infection and rejection, given its high NPV. (Class IIa, Evidence level B-NR)

PICO 2: Is ddcfDNA a reliable therapeutic marker to monitor treatment response for acute rejection or infection of the graft in lung transplant patients, compared with standard diagnostic methods?

Author: S. Agbor-Enoh/Co-author: R. Vos

STATEMENT: While ddcfDNA levels generally decline after treatment for acute rejection or infection is initiated, we currently make no recommendations to use ddcfDNA as an indicator of treatment response. (Class IIb, Evidence level C-LD)

PICO 3: Is ddcfDNA a reliable marker to stratify prognosis of lung transplant

recipients for chronic lung allograft dysfunction (CLAD), as compared to standard clinical classifiers?

Author: S. Agbor-Enoh/Co-author: R. Vos

STATEMENT: 1) ddcfDNA levels and trends in the early post-transplant period may be a predictive marker for death and/or CLAD in lung transplant patients. (Class IIb, Evidence level B-NR)

2) For patients with primary graft dysfunction (PGD), ddcfDNA levels may predict subsequent risk of CLAD. (Class IIb, Evidence level B-NR)

3) For patients with respiratory viral infections, ddcfDNA levels at time of infection may predict subsequent risk of CLAD and/or CLAD progression. (Class IIb, Evidence level B-NR)

References

1. Bhorade, S.M., et al., *Interobserver variability in grading transbronchial lung biopsy specimens after lung transplantation.* Chest, 2013. 143(6): p. 1717-1724.
2. Deng, M.C., et al., *Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling.* Am J Transplant, 2006. 6(1): p. 150-60.
3. De Vlaminck, I., et al., *Noninvasive monitoring of infection and rejection after lung transplantation.* Proc Natl Acad Sci U S A, 2015. 112(43): p. 13336-41.
4. Jang, M.K., et al., *Donor-derived cell-free DNA accurately detects acute rejection in lung transplant patients, a multicenter cohort study.* J Heart Lung Transplant, 2021. 40(8): p. 822-830.
5. Keller, M.B., et al., *Comparison of donor-derived cell-free DNA between single versus double lung transplant recipients.* Am J Transplant, 2022. 22(10): p. 2451-2457.
6. Khush, K.K., et al., *Donor-derived, cell-free DNA levels by next-generation targeted sequencing are elevated in allograft rejection after lung transplantation.* ERJ Open Res, 2021. 7(1).
7. Halloran, K.M., et al., *Molecular assessment of rejection and injury in lung transplant biopsies.* The Journal of Heart and Lung Transplantation, 2019. 38(5): p. 504-513.
8. Rosenheck, J.P., et al., *Clinical Validation of a Plasma Donor-derived Cell-free DNA Assay to Detect Allograft Rejection and Injury in Lung Transplant.* Transplant Direct, 2022. 8(4): p. e1317.
9. Sorbini, M., et al., *Validation of a Simple, Rapid, and Cost-Effective Method for Acute Rejection Monitoring in Lung Transplant Recipients.* Transpl Int, 2022. 35: p. 10546.
10. Keller, M., et al., *Biological Variation of Donor-Derived Cell-Free DNA in Stable Lung Transplant Recipients.* J Appl Lab Med, 2022. 7(4): p. 901-909.
11. Sayah, D., et al., *Plasma Donor-derived Cell-free DNA Levels Are Increased During Acute Cellular Rejection After Lung Transplant: Pilot Data.* Transplant Direct, 2020. 6(10): p. e608.
12. Agbor-Enoh, S., et al., *Late manifestation of alloantibody-associated injury and clinical pulmonary antibody-mediated rejection: Evidence from cell-free DNA analysis.* J Heart Lung Transplant, 2018. 37(7): p. 925-932.

13. Keller, M., et al., *Donor-derived cell-free DNA as a composite marker of acute lung allograft dysfunction in clinical care.* J Heart Lung Transplant, 2022. 41(4): p. 458-466.
14. Agbor-Enoh, S., et al., *Donor-derived cell-free DNA predicts allograft failure and mortality after lung transplantation.* EBioMedicine, 2019. 40: p. 541-553.
15. Magnusson, J.M., et al., *Cell-free DNA as a biomarker after lung transplantation: A proof-of-concept study.* Immun Inflamm Dis, 2022. 10(5): p. e620.
16. Keller, M., et al., *Use of donor-derived-cell-free DNA as a marker of early allograft injury in primary graft dysfunction (PGD) to predict the risk of chronic lung allograft dysfunction (CLAD).* J Heart Lung Transplant, 2021. 40(6): p. 488-493.
17. Tanaka, S., et al., *Donor-derived cell-free DNA is associated with acute rejection and decreased oxygenation in primary graft dysfunction after living donor-lobar lung transplantation.* Sci Rep, 2018. 8(1): p. 15366.
18. Bazemore, K., et al., *Donor derived cell free DNA% is elevated with pathogens that are risk factors for acute and chronic lung allograft injury.* J Heart Lung Transplant, 2021. 40(11): p. 1454-1462.
19. Bazemore, K., et al., *Elevated cell-free DNA in respiratory viral infection and associated lung allograft dysfunction.* Am J Transplant, 2022.