

African Journal of Cardiology and Cardiovascular Medicine

Journal homepage: <https://www.afjccm.com/>



Research Article

Open Access

Circulating cell-free DNA as a biomarker of myocardial injury and rejection after heart transplantation

Ngozi Eze^{1*} and Brian Mwangi²

¹Faculty of Clinical Medicine, Eastern Medical Sciences Institute, Enugu, Nigeria.

²Department of Biochemistry, East African Medical Research University, Nairobi, Kenya.

Article Info

Volume 2, Issue 2, July 2026

Received : 25 March 2026

Accepted : 03 July 2026

Published: 25 July 2026

doi: [10.62587/AFJCCM.2.2.2026.28-36](https://doi.org/10.62587/AFJCCM.2.2.2026.28-36)

Abstract

Objective: To assess the usefulness of donor-derived cell-free DNA as a biomarker in the era of heart transplantation for myocardial injury and rejection. **Methods:** Serial plasma donor-derived cell-free DNA from heart-transplant recipients under surveillance were quantified by single-nucleotide-polymorphism next-generation DNA sequencing along with a contemporaneous endomyocardial biopsy scored for acute cellular and antibody-mediated rejection. Evaluated the utility of donor derived cell free DNA for the diagnosis of acute rejection by comparison to total cell free DNA, troponin, and area under a receiver operating curve analysis, and correlated the cell free DNA with the biopsy grade and with the course of treated acute rejection episodes. **Results:** Of 182 recipients with paired samples of 916, rejection was found in 9%. Cell-free DNA from donors was higher according to the rejection grade: 0.08% without rejection, 0.15% with cellular rejection and 0.28% with antibody-mediated rejection. It discriminated for all rejection means an area under the curve of 0.82 whereas for antibody-mediated rejection was 0.92, which was greater than both mean antibody level and mean troponin. The negative predictive value for the 0.2% level was 94%. Treated episodes had peaks of donor derived cell free DNA at diagnosis and below threshold after treatment. **Conclusion:** Donor-derived cfDNA increased in step with myocardial injury and Antibody-Mediated Rejection (AMR) in particular after heart transplantation discriminated and fell with successful therapy outcome with high negative predictive value. The findings suggest it could be used as a non-invasive tool to accompany surveillance biopsy, to rule out rejection.

Keywords: Heart transplantation, Donor-derived cell-free DNA, Allograft rejection, Liquid biopsy, Endomyocardial biopsy, Non-invasive monitoring

© 2026 Ngozi Eze and Brian Mwangi. This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

1. Introduction

Rejection remains a central threat to the transplanted heart. Despite immunosuppression, a substantial minority of recipients experience an episode of acute rejection in the first year, and rejection, together with its treatment,

* Corresponding author: Ngozi Eze, Faculty of Clinical Medicine, Eastern Medical Sciences Institute, Enugu, Nigeria.

contributes to the graft loss and the long-term morbidity that limit survival after transplantation (Khush *et al.*, 2019). Two forms are distinguished: acute cellular rejection, a T-cell-mediated infiltration of the myocardium, and antibody-mediated rejection, in which donor-specific antibodies injure the graft microvasculature; they differ in mechanism, in treatment and in prognosis, and a surveillance test that could detect them, and ideally distinguish them, would be of real clinical value (Stewart *et al.*, 2005; Berry *et al.*, 2013). It is against this need that non-invasive biomarkers of rejection have been sought.

For half a century the endomyocardial biopsy has been the arbiter of rejection after heart transplantation. It is the reference against which every other test is judged, and it remains indispensable, yet it is a demanding way to watch for a complication that most surveillance biopsies do not find. It is invasive, requiring catheterisation of the right heart; it carries a small but real risk of perforation, tricuspid injury and arrhythmia; it samples only a few fragments of a large organ and so can miss patchy rejection; and its grading is subject to observer variation (Pham *et al.*, 2010). As rejection has become less frequent under modern immunosuppression, the balance between the information a surveillance biopsy yields and the burden it imposes has shifted, and the search for a non-invasive alternative has intensified (Holzhauser *et al.*, 2023).

The burden of biopsy-based surveillance is not trivial. In the first year after transplantation a recipient may undergo a dozen or more scheduled biopsies, each requiring a hospital visit, vascular access and a pathologist, and the cumulative cost, discomfort and small per-procedure risk are substantial for a test that, most of the time, finds no rejection. This asymmetry, much testing for little yield, is precisely the situation in which a cheap, non-invasive screen that reliably identifies the low-risk majority would be most valuable, reserving the biopsy for those in whom it is likely to change management. Donor-derived cell-free DNA, drawn from the same blood sample already taken at each visit for drug levels and chemistry, fits that role naturally.

Circulating cell-free DNA offers such an alternative on a simple principle. Cells that die release fragments of their DNA into the blood, and after transplantation a portion of the circulating cell-free DNA carries the donor genome, shed by the allograft; when the graft is injured, as in rejection, it sheds more, and the donor-derived fraction rises (Snyder *et al.*, 2011; De Vlaminck *et al.*, 2013). Because donor and recipient differ at thousands of single-nucleotide positions, the donor fraction can be measured from a tube of the recipient's blood by sequencing, without a biopsy and without even a separate sample from the donor (Agbor-Enoh *et al.*, 2017).

The approach, first shown in heart transplantation a decade ago, has since been developed into standardised assays and evaluated in multicentre studies, which have established that donor-derived cell-free DNA is higher in rejection and, at a threshold near 0.2%, has a high negative predictive value, making it attractive above all as a means of ruling rejection out (Khush *et al.*, 2019; Agbor-Enoh *et al.*, 2021). It has entered clinical use, in parallel with its established role in kidney transplantation, though questions remain about its performance for the different types and grades of rejection and about how best to use it alongside the biopsy (Bloom *et al.*, 2017; Knight *et al.*, 2019).

This study evaluated donor-derived cell-free DNA as a biomarker of myocardial injury and rejection in a cohort of heart transplant recipients under surveillance. It measured the biomarker serially, paired each measurement with a contemporaneous biopsy, and assessed how well it discriminated rejection overall and by type, how it varied with rejection grade, how it compared with total cell-free DNA and troponin, and how it behaved around treated rejection episodes.

2. Materials and methods

2.1. Study design and population

This was a prospective diagnostic investigation done in adult heart transplant recipients in the course of routine rejection surveillance. It was reported following the Standards for Reporting of Diagnostic Accuracy Studies (Bossuyt *et al.*, 2015). Donor samples included cell-free DNA (cfDNA) plasma measurements obtained over the course of post-transplant, with a matching simultaneous endomyocardial biopsy (EMB). There are 182 recipients who returned 916 paired samples.

2.2. Donor-derived cell-free DNA quantification

Cell-free DNA (cfDNA) preservation tubes were used for the collection of peripheral venous blood which was then subjected to two-step centrifugation to isolate cell-free plasma and extract cell-free DNA from it. The donor derived fraction was estimated using a Single Nucleotide Polymorphism (SNP) method: A set of polymorphic markers was amplified and sequenced, and the percentage of sequenced reads which contained genomic polymorphisms characteristic of the donor as opposed to the recipient was calculated as the Donor Derived Fraction (DDF) based on the pattern of allele-fraction distribution across the entire set of polymorphic markers. No donor specific genotyping needed. The concentration of total cell free DNA at the same sample was taken as well.

2.3. Endomyocardial biopsy and rejection grading

The T-Tx was performed and at least three pieces of myocardial tissue were studied. Acute cellular rejection was assigned a grade 0R, 1R, 2R or 3R, and Antibody Mediated Rejection (AMR) a stage pAMR 0 to 3 based on the IHAT criteria which included immunohistochemistry for complement deposition. Diagnostic analysis outcome of acute rejection was defined as acute cellular rejection grade 2R or above and antibody mediated rejection pAMR 1 or above.

2.4. Comparator biomarkers

For comparison with donor-derived cell-free DNA, the total cell-free DNA concentration, a non-specific marker of cell death, and the high-sensitivity cardiac troponin, a marker of myocardial injury, were measured in the same samples.

2.5. Endpoints

The primary analysis was the discrimination of acute rejection by donor-derived cell-free DNA. Secondary analyses were its discrimination of antibody-mediated and of cellular rejection separately, its performance at the 0.2% threshold, its relationship to biopsy grade, its comparison with the two comparator biomarkers, and its course around treated rejection episodes.

2.6. Statistical analysis

Donor-derived cell-free DNA was compared across biopsy grades by the Kruskal-Wallis test and summarised as the median and interquartile range. Discrimination was quantified by the area under the receiver operating characteristic curve with a bootstrap 95% confidence interval, and the curves of the biomarkers were compared (DeLong *et al.*, 1988). At the 0.2% threshold the sensitivity, specificity and predictive values were computed. For treated rejection episodes, donor-derived cell-free DNA was aligned to the time of diagnosis and plotted over the surrounding weeks. Analyses were performed in Python.

3. Results

3.1. Recipients and samples

The 182 recipients, of mean age 52 years and predominantly male, contributed 916 paired samples over surveillance, as summarised in Table 1. Rejection meeting the diagnostic definition was present in 9.2% of samples, comprising 51 biopsies with cellular rejection of grade 2R or higher and 33 with antibody-mediated rejection, the remainder showing no or minimal rejection, the expected distribution under modern immunosuppression.

3.2. Donor-derived cfDNA by biopsy grade

Donor-derived cell-free DNA rose stepwise with the severity of rejection on biopsy, as shown in Figure 1 and given in Table 2. Its median was 0.08% in samples without rejection and rose through cellular rejection to 0.28% in antibody-mediated rejection, the highest of the categories, a gradient that was highly significant across grades ($p < 0.001$). The overlap between the categories was nonetheless considerable, so that no single value cleanly separated a rejecting from a non-rejecting graft, which is the central challenge in using the biomarker.

Table 1: Recipient and sample characteristics	
Characteristic	Value
Recipients, n	182
Paired samples, n	916
Age, years (mean)	52
Male, %	74
Samples with acute rejection, %	9.2
Cellular rejection (ACR ≥2R), n	51
Antibody-mediated rejection (pAMR ≥1), n	33

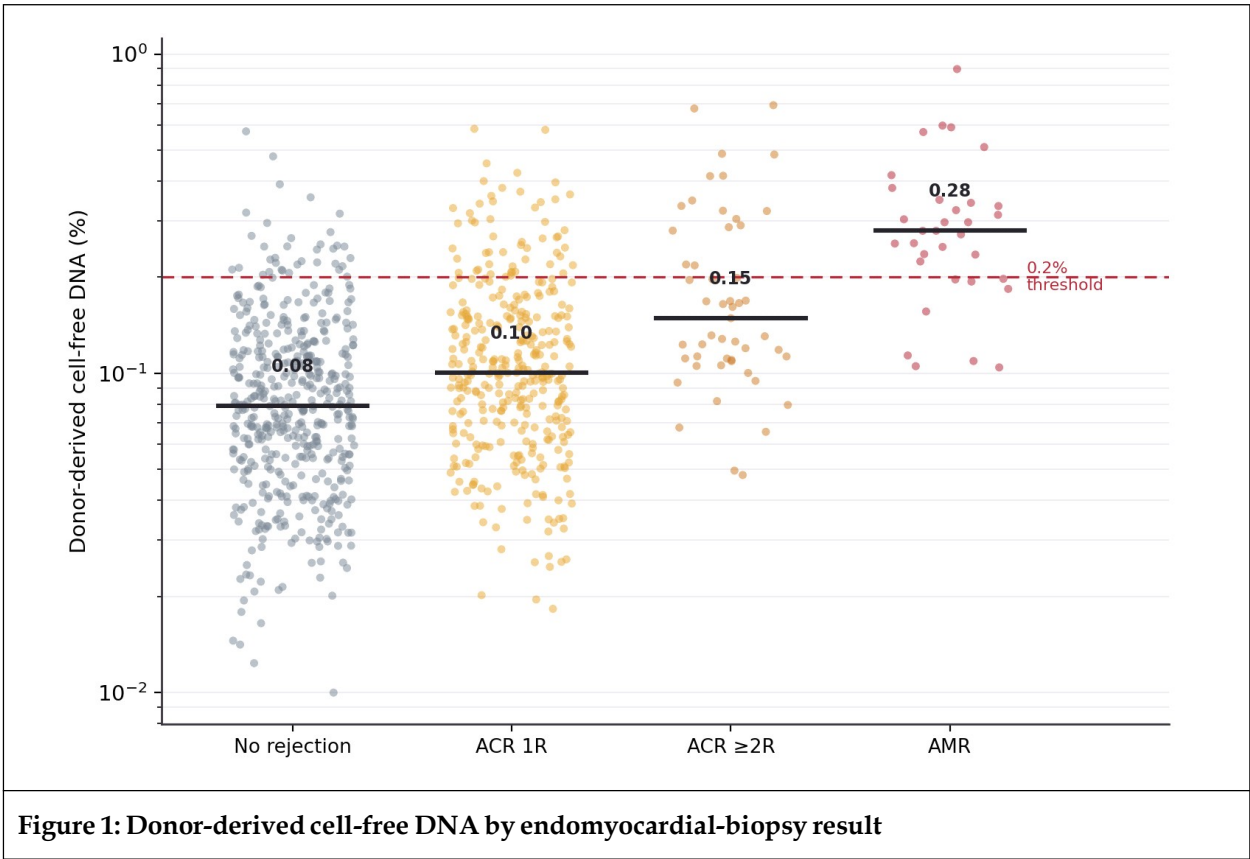


Table 2: Donor-derived cell-free DNA by biopsy category		
Biopsy category	Samples, n	dd-cfDNA %, median (IQR)
No rejection	478	0.08 (0.05–0.12)
ACR 1R	354	0.10 (0.07–0.15)
ACR ≥2R	51	0.15 (0.11–0.28)
AMR	33	0.28 (0.20–0.34)

3.3. Discrimination of rejection

Donor-derived cell-free DNA discriminated acute rejection with an area under the receiver operating characteristic curve of 0.82 (95% CI 0.77 to 0.86), shown in Figure 2. Its discrimination differed markedly by the type of rejection: it identified antibody-mediated rejection very well, with an area of 0.92, and cellular rejection less well, with an area of 0.75, consistent with the greater and more diffuse graft injury of the antibody-mediated process. Against the comparators, donor-derived cell-free DNA outperformed both total cell-free DNA (area 0.72) and troponin (area 0.68), as given in Table 3, confirming that it is the donor-specific fraction, and not merely the total burden of cell death or of myocardial injury, that carries the signal.

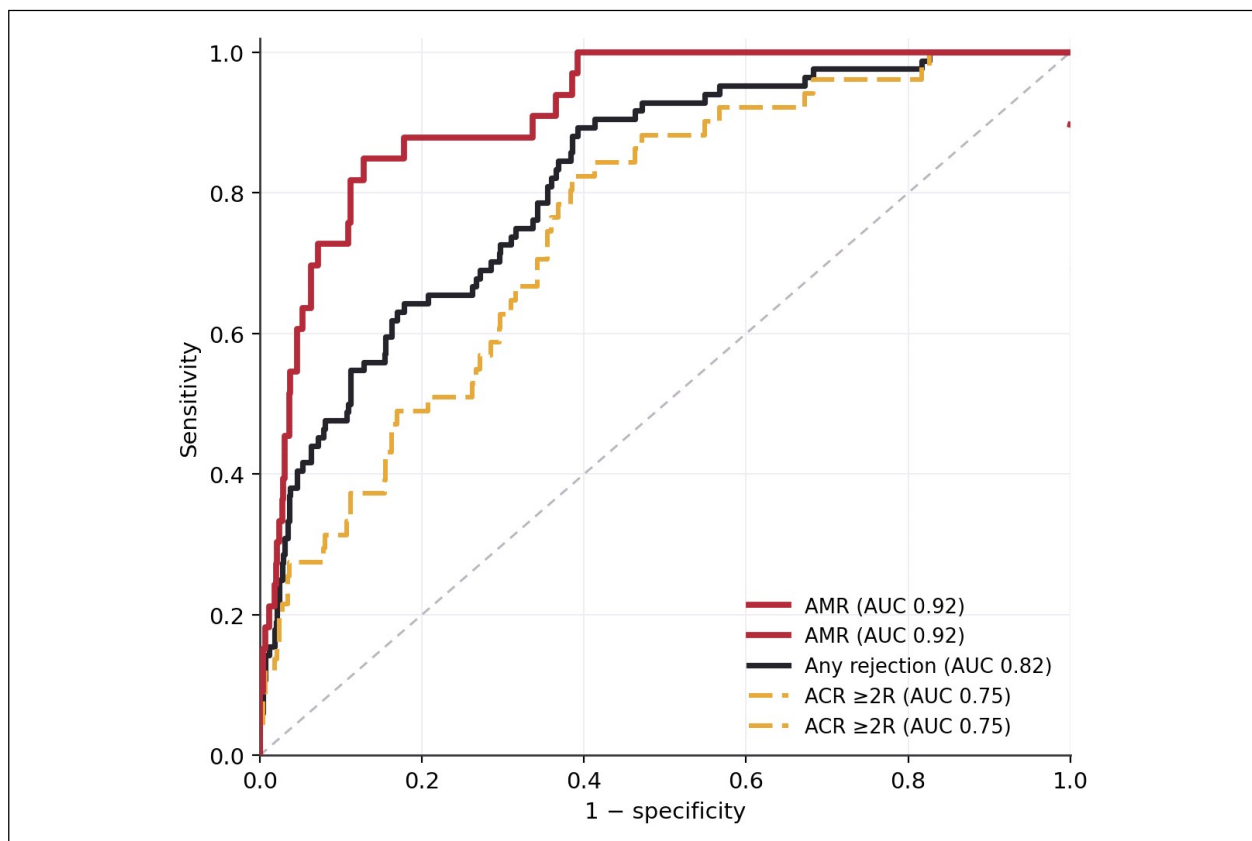


Figure 2: Discrimination of donor-derived cell-free DNA for rejection and its subtypes

Table 3: Discrimination of acute rejection by biomarker (area under the receiver operating characteristic curve)

Biomarker	AUROC	95% CI
Donor-derived cell-free DNA	0.82	0.77–0.86
For antibody-mediated rejection	0.92	n/a
For cellular rejection ($\geq 2R$)	0.75	n/a
Total cell-free DNA	0.72	n/a
High-sensitivity troponin	0.68	n/a

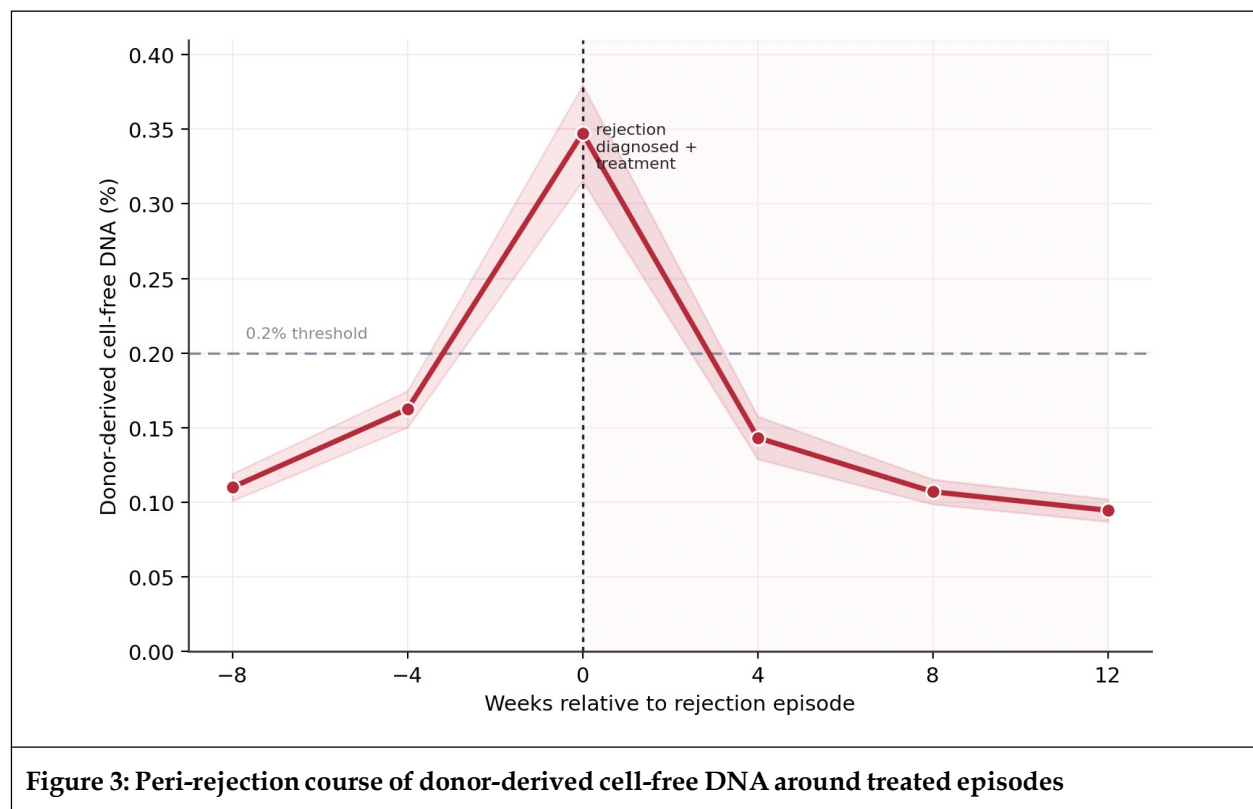
3.4. Performance at the 0.2% threshold

Donor-derived cell-free DNA (dd-cfDNA) correctly acted as a rule-out test at conventional threshold of 0.2%. The sensitivity for finding acute rejection was 48% and specificity was 89%, for a PPV of 31% and a NPV of 94%. The low prevalence of rejection leads to a high ‘negative predictive value’ – so most recipients whose

negative test result falls below the threshold are truly free of rejection, and a low ‘sensitivity’ – a negative test is not always able to rule rejection out with as high a level of confidence as is desired. It is important to make explicit the interplay of these test characteristics with the prevalence of rejection, because it specifies how the numbers should be interpreted. However, the negative predictive value is high because in large part rejection is not common: With about 1 out of 11 samples representing a case of rejection, even an imperfect test with modest sensitivity leaves few true cases in the group it calls negative. Again, the low prevalence leads to a low positive predictive value and most of the positive results would be false alarms because a confirmatory biopsy would show that it’s not. This is not a problem specific to this particular biomarker but to the mathematics of any screening for a rare disease; and it does indicate the proper use of the test – as a negative test result you will rely on; application of this test negative will result in the biopsy which will be the arbiter.

3.5. Peri-rejection course

Followed around treated rejection episodes, donor-derived cell-free DNA traced the expected rise and fall, shown in Figure 3. It was near the threshold in the weeks before diagnosis, rose to a peak of about 0.35% at the time rejection was diagnosed and treatment begun, and then fell over the following weeks to below the threshold, reaching 0.10% by three months, as the treated graft recovered. This dynamic behaviour, rising with active rejection and settling with its resolution, is what distinguishes a biomarker of disease activity from a fixed trait, and it suggests a role not only in detection but in confirming the response to anti-rejection treatment (Richmond *et al.*, 2020; Kim *et al.*, 2022).



4. Discussion

Total cfDNA and troponin did not correlate as well with myocardial injury and rejection as donor cfDNA in HCT recipients monitored by this approach and donor cfDNA had a high negative predictive value at the traditional cut-off and followed a pattern of increase and decrease following episodes of treated rejection, with antibody mediated rejection rising to 20%. It acted, in brief, as a dynamic marker (for the donor) which reflects graft injury.

The ability of the biomarker to detect antibody mediated more effectively than cellular rejection is not a disadvantage but a reflection of the biology of the biomarker – at high grade antibody mediated rejection there is diffuse injury to the microvasculature of the graft with donor DNA released in proportion, while at lower grade cellular rejection may also release donor DNA but is more focal, thus the biomarker reflects the amount

of donor graft injury, regardless of whether that is mediated by antibodies or cells. This has a pragmatic consequence; because donor-derived cell-free DNA is most likely to be the result of diffuse, higher injury processes, the two tests would not be redundant, but instead complementary, with this sampling best suited for testing in the context of a low likelihood of biopsy detecting the cause of the injury.

The high negative predictive value decisively influences the use of the test and the modest sensitivity that goes with it. Rejection is now a very rare occurrence during routine follow-up and a result below threshold is a good predictor of its absence, so the major impact of the biomarker is therefore to stop the large number of recipients with a low or normal test result going for biopsy; invasive testing is reserved for those with a raised result (Khush, 2021). Since this can be used as a rule-out test to avoid unnecessary biopsies – and it clearly should not be used in place of biopsy – donor-derived cell-free DNA has the potential to significantly cut down on the number of surveillance biopsies performed, an approach being adopted by non-invasive surveillance strategies.

But donor-derived cell free DNA is not the only non-invasive way to monitor rejection – and this can be understood in comparison to the other strategies. The first molecular test validated for this purpose and saving of biopsies in low-risk stable recipients is the gene-expression profiling of peripheral blood which reads the recipient immune response; however, the test does not reflect the cellular process of rejection and is less informative of the antibody-mediated process. Whereas the molecular assessment of the biopsy tissue itself has a power of resolving rejection that precision histology can't, it still depends on the biopsy (Halloran *et al.*, 2017). The unique benefit of donor-derived cell-free DNA is that it reports specifically on graft injury both derived from blood and not, and the very specific strength towards antibody-mediated rejection complements the cellular-immune focus of gene-expression profiling, potentially making the tests more powerful together than separately.

It is the dynamic behaviour after the occurrence of a treated episode that, in addition to instances in the diagnosis phase, opens the possibility for utilisation of the biomarker in the monitoring phase. The marker that rises when rejection occurs, and decreases after treatment for rejection, provides an objective repeatable measurement of the response to therapy, and one that, in theory, could inform treatment intensity and duration – criteria for which information is poorly provided by the biopsy, which is more static and intrusive. Kinetics as reported for both heart and kidney transplantation reflect this and the fact that the fall was down below the threshold by 3 months, summing the graft recovery.

The interpretation of the biomarker is influenced by a technical factor. The proportion of donated cell-free DNA is often expressed as a fraction of the total; it can be reduced for an irrelevant increase in the total DNA caused by graft background (infection or leucocyte turnover) or it could rise when the background is reduced caused by the failure of the graft recipient's cells to provide DNA during the process. Since the proportion of donor derived cell-free DNA is often expressed as a fraction of the total DNA, there can be a spurious decrease in the proportion of donor DNA when the total DNA increases (infection or leucocyte turnover) and vice versa. Reporting in addition to or in conjunction with the proportion of donor-derived cell free DNA, the total amount of donor-derived cfDNA, has also been found to increase accuracy and help to minimise the rate of false-positive results, and combining the proportion of cfDNA with the absolute quantity of cfDNA (from donors and non-donors combined) would be expected to further enhance the signal of injury in this context. Consequently, in the present study, the most common measure – the fraction – was employed, leading to a conservative estimate of the impact of using a combined metric. Finally, a moment of attention turns to the biology underpinning the signal, as it describes the advantages and the disadvantages of the biomarker. The donor DNA component of cfDNA is part of a pool of short DNA fragments which are released during cell death (apoptosis and necrosis); this donor DNA is rapidly cleared by the liver and kidneys, and therefore represents damage to the graft at or very near to the time of the sample. Most of the DNA in cfDNA is in the form of small DNA fragments shed by dead (apoptotic and necrotic) cells and this donor-derived DNA fraction is rapidly cleared by liver and kidney, therefore this DNA will most likely reflect graft cell death at or very soon after the sample was collected and not the cumulative result of cell death. This short half-life is what makes the biomarker dynamic – its rapid rise during the process of active rejection and its rapid fall during the process of recovery; it also leads to the fact that a single low value is reassuring only for the time that it is taken and a series of values are desirable. Finally and most importantly, the biomarker's proportionality with the amount

of graft injury explains why its rejection grade is as it is; with the biomass of antibody-mediated rejection, the grade is more representative of the diffuse injury of rejection compared with the focal injury of mild cellular rejection.

5. Conclusion

Most of the donor-derived C-DNA detectors of myocardial injury and allograft rejection increased with rejection grade, and distinguished rejection from total C-DNA and troponin, had a high negative predictive value at the commonly used threshold, and were increased and decreased around treated episodes. It would make a suitable rule-out test that gates the biopsy and is suitable as a monitoring test for response to anti-rejection therapy, complementing the biopsy without replacing it, and its introduction into clinical surveillance is an area that now merit evaluation in a management trial as it is non-invasive, donor specific, and dynamic, and might serve as a marker of graft injury that is not used.

Ethical approval

The study was approved by the Institutional Ethics Committee and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from every participant.

Conflict of interest

The authors declare that no conflict of interest exists in relation to this work.

Funding

This work received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability

The data supporting the findings of this study are available from the corresponding author on reasonable request.

References

- Agbor-Enoh, S., Shah, P., Tunc, I., Hsu, S., Russell, S., Feller, E. *et al.* (2021). [Cell-free DNA to detect heart allograft acute rejection.](#) *Circulation*, 143(12): 1184-97.
- Agbor-Enoh, S., Tunc, I., De Vlaminc, I., Fideli, U., Davis, A., Cuttin, K. *et al.* (2017). [Applying rigor and reproducibility standards to assay donor-derived cell-free DNA as a non-invasive method for detection of acute rejection and graft injury after heart transplantation.](#) *J Heart Lung Transplant*, 36(9): 1004-12.
- Berry, G.J., Burke, M.M., Andersen, C., Bruneval, P., Fedrigo, M., Fishbein, M.C. *et al.* (2013). [The 2013 International Society for Heart and Lung Transplantation working formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation.](#) *J Heart Lung Transplant*, 32(12): 1147-62.
- Bloom, R.D., Bromberg, J.S., Poggio, E.D., Bunnapradist, S., Langone, A.J., Sood, P. *et al.* (2017). [Cell-free DNA and active rejection in kidney allografts.](#) *J Am Soc Nephrol*, 28(7): 2221-32.
- Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P., Irwig, L. *et al.* (2015). [STARD 2015: An updated list of essential items for reporting diagnostic accuracy studies.](#) *BMJ*, 351: h5527.
- De Vlaminc, I., Valantine, H.A., Snyder, T.M., Strehl, C., Cohen, G., Luikart, H. *et al.* (2014). [Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection.](#) *Sci Transl Med*, 6(241): 241ra77.
- DeLong, E.R., DeLong, D.M. and Clarke-Pearson, D.L. (1988). [Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach.](#) *Biometrics*, 44(3): 837-45.
- Halloran, P.F., Potena, L., Van Huyen, J.D., Bruneval, P., Leone, O., Kim, D.H. *et al.* (2017). [Building a tissue-based molecular diagnostic system in heart transplant rejection: The INTERHEART study.](#) *J Heart Lung Transplant*, 36(11): 1192-200.

- Holzhauser, L., DeFilippis, E.M., Nikolova, A., Byku, M., Contreras, J.P., De Marco, T. *et al.* (2023). [The end of endomyocardial biopsy? A practical guide for noninvasive heart transplant rejection surveillance.](#) *JACC Heart Fail*, 11(3): 263-76.
- Khush, K.K., Cherikh, W.S., Chambers, D.C., Harhay, M.O., Hayes, D. Jr, Hsich, E. *et al.* (2019). [The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult heart transplantation report.](#) *J Heart Lung Transplant*, 38(10): 1056-66.
- Khush, K.K., Patel, J., Pinney, S., Kao, A., Alharethi, R., DePasquale, E. *et al.* (2019). [Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study.](#) *Am J Transplant*, 19(10): 2889-99.
- Khush, K.K. (2021). [Clinical utility of donor-derived cell-free DNA testing in cardiac transplantation.](#) *J Heart Lung Transplant*, 40(6): 397-404.
- Kim, P.J., Olymbios, M., Siu, A., Wever Pinzon, O., Adler, E., Liang, N. *et al.* (2022). [A novel donor-derived cell-free DNA assay for the detection of acute rejection in heart transplantation.](#) *J Heart Lung Transplant*, 41(7): 919-27.
- Knight, S.R., Thorne, A. and Lo Faro, M.L. (2019). [Donor-specific cell-free DNA as a biomarker in solid organ transplantation: A systematic review.](#) *Transplantation*, 103(2): 273-83.
- Pham, M.X., Teuteberg, J.J., Kfoury, A.G., Starling, R.C., Deng, M.C., Cappola, T.P. *et al.* (2010). [Gene-expression profiling for rejection surveillance after cardiac transplantation.](#) *N Engl J Med*, 362(20): 1890-900.
- Richmond, M.E., Zangwill, S.D., Kindel, S.J., Deshpande, S.R., Schroder, J.N., Bichell, D.P. *et al.* (2020). [Donor fraction cell-free DNA and rejection in adult and pediatric heart transplantation.](#) *J Heart Lung Transplant*, 39(5): 454-63.
- Snyder, T.M., Khush, K.K., Valantine, H.A. and Quake, S.R. (2011). [Universal noninvasive detection of solid organ transplant rejection.](#) *Proc Natl Acad Sci USA*, 108(15): 6229-34.
- Stewart, S., Winters, G.L., Fishbein, M.C., Tazelaar, H.D., Kobashigawa, J., Abrams, J. *et al.* (2005). [Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection.](#) *J Heart Lung Transplant*, 24(11): 1710-20.