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reduced LVEF in the statistical model to test for effect modification by reduced LVEF. The interaction term between donor LVEF and ischemic time (both continuous and categorical: ejection fraction ≥ 50%, 40%−49%, 30%−39%) was evaluated in the regression models. Both versions of the interaction terms were not statistically significant and as such were not included in the final models (continuous interaction term: $p = 0.920$; categorical interaction term: $p = 0.143$). This is not unexpected as interaction terms typically require substantially increased power. Our study may have been underpowered for such an analysis. However, qualitatively, we observed a difference in the treatment effect according to the stratified groups (ejection fraction ≥ 50%, 40%−49%, 30%−39%). Indeed, a similar approach was undertaken by Russo et al[2] and John et al[3] when evaluating a difference in the impact of ischemic time according to donor age. Such compromises are often necessary because of power constraints. We are grateful for the opportunity to address the concerns highlighted by Dr Cavillo-Argüelles et al.

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Careful clinical evaluation of donor fraction cell-free DNA in rejection surveillance after heart transplantation

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We read with interest the article of Richmond et al[1] evaluating in a multicenter, prospective, blinded study (enrolled 241 patients) the index of donor fraction (DF), which is defined as the ratio of cell-free DNA (cfDNA) specific to the transplanted organ to the total amount of cfDNA in a blood sample. In this important study, DF levels were high both in acute cellular rejection and in antibody-mediated rejection. The authors concluded that cfDNA DF maintained promise as a non-invasively diagnostic test to rule out acute rejection after heart transplantation (HTx). From a methodologic point of view, we appreciated that the study[1] used "A novel quality assurance protocol based on a cfDNA fragmentation analysis was developed to detect problematic post-collection leukocyte lysis, so that any samples with evidence of leukocyte lysis above a conservative cutoff level could be rejected from the study in an unbiased manner" (P North et al, unpublished data, 2019). Similarly, during the study[1] "Recipient and donor genomic DNA from buffy coats prepared from these samples were extracted using a ReliaPrep Large Volume gDNA Isolation System (Promega)" (P North et al, unpublished data, 2019). Although these protocols were not available in detail, the problem of a rigorous quality of DNA is mandatory in such type of studies[2,3] as well as in all liquid biopsy clinical protocols.[4,5] Remarkably, this was a multicenter study involving 7 transplant centers, but the cohort of patients ($n = 241$) and fully analyzable samples (636 samples from 174 patients) were relatively small (power of the study). From a cultural point of view, endomyocardial biopsy remains the current gold standard for rejection surveillance after HTx, and evaluation of cfDNA DF is still reserved for a minority of HTx centers around the world. Moreover, cfDNA mainly originates from programmed cell death or acute cellular injury detecting the presence of cellular damage, but low levels of cfDNA can also circulate in the plasma of healthy individuals before their clearance by the liver.[5] Because there is no mandatory consensus on the utility of both additional non-invasive examinations and screening tests during low-grade rejection (e.g., acute cellular rejection Grade 1R or *pAMR1*), the take-home message to divulge cfDNA DF could be pre-mature in the clinical setting of low-grade rejection after HTx. In fact, in the era of coronavirus disease 2019 pandemic, the economic resources should be carefully addressed to crucial emerging necessities in the clinical management of patient's health.

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Hemostasis during extracorporeal membrane oxygenation: More questions

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