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Letter to the editor: Elevated Plasma Levels of Cell-Free DNA During Liver Transplantation Are Associated With Activation of Coagulation

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Cell-free DNA (cfDNA) is emerging as an immuno-modulator in numerous medical conditions including in malignancy, chronic inflammatory diseases, trauma, sepsis, and organ transplantation (1, 2). Significant evidence exists supporting a link between cfDNA and the presence of neutrophil extracellular traps (NETs). NETs, comprising an extra-cellular mesh of chromatin and neutrophil granular proteins, enable extracellular pathogen entrapment and clearance. NETs have attracted attention as they play a critical role in the immune system. Furthermore, Early reports have demonstrated that the inhibition of NETs can modulate the immune response (3, 4) .

von Meijenfeldt et al. recently described that elevated plasma cfDNA is associated with the activation of coagulation and subsequent NETs formation in liver transplantation (5). We read this article with great interest and we agree that the authors have certainly uncovered avenues for potential targets for thrombotic complications and ischemic reperfusion injury. There were, however, several technical confounders that were recognised and acknowledging them may improve the interpretation of the results and refine the conclusions.

Consistent with the findings of the paper, we recently reported that donor-specific cell-free DNA rapidly declines following successful engraftment (6). We have also shown that the levels of cfDNA is affected by transfused blood products which may contain a significant amount of DNA that is derived from the blood donor (7). Meijenfeldt et al excluded recipients who have recently undergone recent transfusion of blood products up to 7 days prior transplantation. It was not clear if blood products such as fresh frozen plasma and cryoprecipitate were transfused perioperatively. Although cfDNA has a short half-life in the circulation, the close monitoring of cfDNA levels by von Meijenfelt et al could have been confounded by the transfusion of the aforementioned blood products. As such, a cautious interpretation of the cfDNA results is required.

The described methodology of quantifying cfDNA in unpurified plasma is associated with marked variability as the electrolyte constituents of plasma can affect fluorescent signals when the Quant-iT PicoGreen® assays are used. Furthermore, the use of Quant-iT PicoGreen to quantify cfDNA has been reported to yield lower quantitative values with fragmented DNA and can be influenced by salt-concentrations (8).

In addition, cfDNA is fragmented and has a variable length up to 160bp. The use of non-specialised kits may lead to substantially inaccurate representation of total cfDNA and it is thus imperative to use specialised kits for the processing of cfDNA kits to minimise pre-analytical variables and refine the quality of the reported results.

PCR based measurements coupled with purified cfDNA are much more reproducible and widely accepted in the cfDNA literature. (9) In the era of DNA counting methodologies, we would like to propose that absolute quantification by digital PCR which should be used to increase the precision and improve the reproducibility (10).

Overall, the concept that cell free DNA plays a critical role in numerous immune conditions and processes appears plausible. Further research into this area using a more nuanced approach to cfDNA measurement is encouraged.

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