Author/s:
Yoshino, O;Wong, BKL;Cox, DRA;Lee, E;Hepworth, G;Christophi, C;Jones, R;Dobrovic, A;Muralidharan, V;Perini, MV

Title:
Elevated levels of circulating mitochondrial DNA predict early allograft dysfunction in patients following liver transplantation

Date:
2021-12-01

Citation:
Yoshino, O., Wong, B. K. L., Cox, D. R. A., Lee, E., Hepworth, G., Christophi, C., Jones, R., Dobrovic, A., Muralidharan, V. & Perini, M. V. (2021). Elevated levels of circulating mitochondrial DNA predict early allograft dysfunction in patients following liver transplantation. Journal of Gastroenterology and Hepatology Australia, 36 (12), pp.3500-3507. https://doi.org/10.1111/jgh.15670.

Persistent Link:
https://hdl.handle.net/11343/298940

Perini Marcos (Orcid ID: 0000-0002-0165-1564)

Elevated levels of circulating mitochondrial DNA predict early allograft dysfunction in patients following liver transplantation.

Short Title:
Mitochondrial DNA and Liver Transplant

Osamu Yoshino[1 *], Boris Ka Leong Wong[2,3 *], Daniel RA Cox[1,2], Eunice Lee[1,] Graham Hepworth[4], Christopher Christophi[1], Robert Jones[1], Alexander Dobrovic[1,2 #], Vijayaragavan Muralidharan[1 #], Marcos Vinicius Perini[1 #].

1. The University of Melbourne Department of Surgery, Austin Health
2. Translational Genomics and Epigenomics Laboratory, Department of Surgery – Austin Precinct, The University of Melbourne, Austin Hospital, Heidelberg, Victoria, Australia.
3. School of Cancer Medicine, LaTrobe University, Bundoora, Victoria, Australia
4. School of Mathematics and Statistics, The University of Melbourne, Melbourne, Victoria, Australia

* Joint first authors; # Joint senior authors

Corresponding Author:

A/Prof Marcos V. Perini MD, PhD, FRACS

The University of Melbourne Department of Surgery
Austin Health,
Studley Road, Heidelberg
Melbourne, Victoria, 3084
Australia
Tel: +613 9496 3670
Fax: +613 9458 1650
E-mail: marcos.perini@unimelb.edu.au

Abbreviations:

Area under the curve (AUC); Circulating mitochondrial DNA (cmtDNA); Droplet digital PCR (ddPCR); Early allograft dysfunction (EAD); Ethylenediaminetetraacetic acid (EDTA); Liver transplantation (LT); Postoperative days (POD); Receiver operating characteristic (ROC); Positive predictive value (PPV); Negative predictive value (NPV).

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jgh.15670

Abstract

The role of circulating mitochondrial DNA (cmtDNA) in transplantation remains to be elucidated. cmtDNA may be released into the circulation as a consequence of liver injury; yet recent work also suggests a causative role for cmtDNA leading to hepatocellular injury. We hypothesized that elevated cmtDNA would be associated with adverse events after liver transplantation (LT) and conducted an observational cohort study. Twenty-one patients were enrolled prospectively prior to LT. Postoperative complications were observed in 47.6 % (n=10). Seven patients (33.3%) had early allograft dysfunction (EAD) and six patients (28.5%) experienced acute cellular rejection within six months of LT. cmtDNA levels were significantly elevated in all recipients post-LT compared with healthy controls and pre-operative samples (1,361,937 copies/ml [IQR 586,781 – 3,399,687] post-LT; 545,531 copies/ml [IQR 238,562 - 1,381,015] pre-LT; 194,562 copies/ml [IQR 182,359 - 231,515] in healthy controls) and returned to normal levels by five days after transplantation. cmtDNA levels were particularly elevated in those who developed EAD in the early post-operative period ($p < 0.001$). In all patients there was initially a strong overall positive correlation between cmtDNA and plasma hepatocellular enzyme levels ($p < 0.05$). However, the patients with EAD demonstrated a second peak in cmtDNA at post-operative day seven, which did not correlate with liver function tests. The early release of plasma cmtDNA is strongly associated with hepatocellular damage; however, the late surge in cmtDNA in patients with EAD appeared to be independent of hepatocellular injury as measured by conventional tests.

Introduction

The analysis of circulating mitochondrial DNA (cmtDNA) has received attention due to its potential as a clinically useful indicator of tissue damage and disease burden in a number of conditions.[1,2] More recent work has suggested a role for cmtDNA as a potent immunomodulator associated with sepsis, myocardial ischemia, chronic inflammatory diseases, trauma, and organ transplantation.[3] However, its role and potential as a biomarker in liver transplantation (LT) remains to be elucidated.

Early allograft dysfunction (EAD) is a milder form of primary graft dysfunction and is associated with postoperative complications, higher mortality rates and decreased graft survival.[4-6] Liver ischemia and reperfusion injury have been hypothesized to cause hepatocellular injury leading to EAD. A number of donor and graft factors (e.g. marginal grafts, long ischemic times) and recipient immune reactions are also associated with graft dysfunction and failure.[7]

The roles and effects of cmtDNA after LT are not well understood. Early work has identified elevated levels of cmtDNA in blood samples from organ *donors* to be associated with higher complication and mortality rates following LT. [8] However, given that the graft often undergoes prolonged ischemia and subsequent reperfusion injury, we hypothesized that levels of cmtDNA will also be significantly elevated after liver transplantation, with the potential therefore to provoke a substantial inflammatory and immune reaction. The aim of this study was to provide the first description of longitudinally monitored cmtDNA levels in patients following LT, to interrogate any link between postoperative, recipient cmtDNA levels with measured donor cmtDNA and to investigate associated clinical outcomes in relation to post-operative levels of cmtDNA in LT recipients.

Methodology

This was a prospective, observational study conducted at Austin Hospital, Melbourne, Australia. Ethical approval was obtained from the Austin Health Human Research Ethics Committee (HREC/49951/Austin-2019). Consecutive adult patients undergoing LT between September 2019 and February 2020 were recruited. Cases where ex-vivo mechanical perfusion technology was utilized, were excluded. All clinically relevant data including donor and recipient information as well as laboratory values and clinical events, such as postoperative complications, were extracted from the institutional

electronic medical record. Following LT, patients were managed according to the local Victorian Liver Transplant Unit guidelines and were followed up for at least 6 months postoperatively. Informed consent for blood sampling from organ donors was waived after ethical approval from DonateLife, Victoria, Australia. Healthy volunteers were also recruited from our institution to establish control levels of cmtDNA.

Blood sampling, collection and processing

Blood samples were obtained from LT donors at the time of organ procurement and from recipients in the perioperative period. Recipient baseline blood samples were obtained prior to induction of anaesthesia for LT. Postoperative samples were collected daily for biochemical analyses including standard liver function tests (bilirubin, AST, ALT, GGT, ALP, INR) and cmtDNA quantification up to post-operative day 7 (POD7). Six healthy volunteers were recruited as a cmtDNA control group, providing a single blood sample. Blood samples were collected in EDTA-containing tubes, and the samples were centrifuged at 800 *g* for 10 minutes followed by 3,000 *g* for 10 minutes to obtain the plasma fraction. Plasma samples were then immediately stored at -80°C until analysis. To prepare plasma free of platelets and mitochondria, once thawed the plasma was further centrifuged at 15,000 *g* for 10 minutes before DNA extraction.

Definition of clinical variables

Donor risk index was calculated according to Feng's criteria.[9] If clinical suspicions of fatty infiltration of the graft were raised (through imaging or the operating surgeons), donor liver biopsy was performed and graded according to Brunt's criteria.[10] *In situ* split liver donation was done in all cases who received a right lobe. The definition of early allograft dysfunction (EAD) was based on the established proposal by Olthoff et al.[6] Vascular complications including hepatic artery thrombosis and portal vein thrombosis were defined as the lack of flow in the hepatic artery or portal vein on doppler-ultrasound confirmed by CT angiogram or surgical exploration. Biliary anastomotic stricture was defined as a stricture at the anastomotic site that required dilatation or stenting. All suspected cases of acute rejection underwent graft biopsy. Rejection was defined according to the histological findings.[11] The Third International Consensus definitions of sepsis and septic shock were used to define the sepsis and septic shock in the study.[12] Postoperative complications were graded according to Clavien-Dindo classification.[13]

Droplet digital polymerase chain reaction (ddPCR) analysis

Plasma samples were analyzed in batches. 4 mL of plasma from each sample was thawed to ambient temperature and plasma DNA was extracted using the QIAamp Circulating Nucleic Acid Kit (Qiagen) following the manufacturer's instructions. All DNA samples were eluted in 100 µL of AVE buffer (Qiagen) and stored at 4°C.

PCR Primers for the human mitochondrial *cytochrome B* gene (*MT-CYB*) (forward 5′-CCGAAGTTTCATCATGCGGAGAT-3′ and reverse 5′-TTAATTAACCACTCATTCATCG-3′) were developed to target short sections of cmtDNA (cmtDNA fragments are noted to be generally shorter than nuclear-derived cfDNA fragments).[14] The ddPCR set-up comprised a 22 µL reaction; 11 µL of QX200 ddPCR EvaGreen Supermix (Bio-Rad), 100 nmol/L of each forward and reverse primer and 2 µL of plasma cmtDNA diluted to 1/100 with PCR-grade water. 20uL from each ddPCR reaction mixture was loaded onto a DG8 droplet generator cartridge (Bio-Rad) for droplet generation. After partitioning, the reactions were cycled on a C1000 Touch thermocycler (Bio-Rad) with the following conditions: one cycle of 95°C for 5 minutes; 40 cycles of 95°C for 30 seconds and 55.8°C for 60 seconds; one cycle of 4°C for 5 minutes; one cycle of 90°C for 5 minutes and a brief hold at 4°C. After the end-point PCR, the 96-well plate was analyzed using the QX200 Droplet Reader (Bio-Rad). Using QuantaSoft software (Bio-Rad), the derived concentration was calculated to determine the number of DNA copies per mL of recipient plasma (denoted copies/mL).

Statistics

Results are reported as median (interquartile range) for continuous variables and percentages for categorical variables. Demographic parameters between groups were compared with the Mann-Whitney U test. The Kruskal-Wallis test, followed by the Mann-Whitney U test, was used to compare plasma cmtDNA levels between the study groups and the control group. Changes in plasma cmtDNA levels between measurement periods were compared with the Friedman test, followed by post hoc pairwise comparisons using Wilcoxon tests with Bonferroni correction. Pearson's correlation coefficients were calculated to examine the association between recipient and donor plasma cmtDNA levels. After log transformation of plasma cmtDNA levels, linear mixed models were used to assess the correlations between plasma cmtDNA level and liver function test results.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic ability of cmtDNA in association with acute cellular rejection and EAD. The optimal sensitivity and specificity were obtained according to the Youden index.[15] With the assumption that the incidence of EAD is 30%, positive predictive values (PPV) and negative predictive values (NPV) were calculated. Statistical analyses were performed in SPSS version 26 and STATA version 15, and p <0.05 was considered statistically significant.

Results

A total of 21 adult LT patients were recruited. In four donors (19.0%), blood samples either were not available or did not arrive within six hours after collection. The most common indication for LT was alcoholic liver disease (Table 1). Overall, 19 (90%) patients received grafts from brain-dead donors and two (10.0%) from donors after circulatory death. Whole liver grafts were used in 18 (86.0%) cases. Right lobe grafts obtained from *in situ* split liver donor procedures were used in three cases (14.2%). Macroscopic steatosis was observed in five donors; all of the subsequent biopsies showed a mild degree of steatosis. EAD was observed in 7 (33.0%) patients. There were two patients with clinical episodes of sepsis or septic shock among the 21 patients. There were no instances of hyper-acute rejection or rejection episodes in the seven days after LT (Table 1). Six (28.5%) patients developed biopsy-proven T-cell mediated rejection within six months after LT (median 2.5 months [IQR 1.25-4.5]). All episodes of rejection were treated successfully with steroids. Three (14.2%) patients required a return to theatre due to postoperative haemorrhage (n=1), suspected hepatic artery thrombosis (n=1) or suspected portal vein thrombosis (n=1). Six (28.5%) biliary anastomotic strictures occurred and were dilated endoscopically within six months after LT (median 1.0 months [IQR 0.7-2.6]). No hepatic artery thrombosis occurred. None of the patients developed primary graft non-function requiring re-transplantation. There were no post-operative deaths within the six months following LT.

The median plasma cmtDNA level in the healthy control group was 194,562 copies/ml (IQR 182,359–231,515 copies/ml). The overall level of plasma cmtDNA among the donors and recipients were significantly elevated in comparison (Table 2). None of the clinical donor variables were associated with donor cmtDNA levels. Recipient and procedural variables, likewise, were not associated with cmtDNA levels on POD1 or POD7 - except the presence of steatosis in donors' liver (Table 3). On comparison of the patients with EAD (EAD group) and those without EAD (non-EAD group), we found no significant differences in demographics except the incidence of steatosis with four patients (57.1%) in the EAD group and one patient (7.1%) in the non-EAD group (p 0.006). There were no significant differences in rates of postoperative complications between groups (Table 4).

Donor cmtDNA levels did not statistically differ between the EAD group and the non-EAD group (622,187 (313,465-1,358,328) copies/ml vs 851,812 (763,125-1,249,875) copies/ml, p 0.143). However, there was a positive correlation between donor cmtDNA concentrations and recipient POD1 cmtDNA level ($r = 0.458$, $p < 0.05$). Overall, levels of cmtDNA were elevated after the LT, and more significant postoperative elevations in cmtDNA were observed in both the EAD group when compared to the non-EAD group between POD1 and POD 3 (see Figure 1).

We also observed that cmtDNA concentrations in the EAD group began to rise again at POD 7 ($p <$ 0.005 vs. control group; p 0.004 vs. non-EAD). A substantial positive correlation was observed between cmtDNA concentrations and AST/ALT among patients in the non-EAD group in both early and late postoperative days. However, the correlation was not found in the late EAD group (Table 5).

The results of the cmtDNA ROC analysis to determine the association of EAD and the predictability of rejection are shown in Table 6. cmtDNA levels >4,812,500 copies/ml in POD1 were associated with EAD, with a sensitivity of 100%, specificity of 61.5% and NPV of 100% (AUC 0.769; 95% CI 0.508 - 1.000). ROC analysis of cmtDNA levels on POD3 and POD 7 yielded similar results (Table 6).

Discussion

Liver tissues are a rich in mitochondria and hence have the potential to form a significant source of cmtDNA. This renders cmtDNA quantification an attractive target as a biomarker for liver injury. Approximately 4000 mitochondria are present in each hepatocyte; these mitochondria or their

constituent parts (including the mitochondrial genome) may be released during cellular apoptosis and necrosis secondary to injury e.g. ischemic/reperfusion injury[16] or to hepatic parenchymal necrosis (e.g. acetaminophen overdose).[17] In contrast, cmtDNA can be released in an active manner, such as through the formation of neutrophil-extracellular traps (NETs), exosomes release and cell death-related autophagy through inflammation. NETs formation is observed in the liver sinusoids and potentially play a pivotal role in liver-intrinsic infections as well as liver dysfunction.[18]

We quantified cmtDNA from liver transplant recipients and their donors using ddPCR.[19] ddPCR is an exquisitely sensitive technology that does not require the use of external standards for calibration. Absolute quantification of a DNA target can be achieved by counting the positive fluorescence droplets in each of the 20,000 reaction partitions and applying Poisson statistics.[19] The size of cmtDNA fragments remains controversial; their length may be as short as 30 base pairs (bp) and as long as 170 bp. A recent study has demonstrated that the peak size of cmtDNA is 42 bp[14]. Therefore, we optimized the primer set to accommodate the shorter lengths of cmtDNA, to as little as 42 bp.

Plasma cmtDNA was significantly elevated in all liver transplant recipients. The quantity of cmtDNA after liver transplantation was extremely high, reaching 20 million copies/mL, approximately 100 times the plasma level in the control group and these values are similar to those reported in ICU patients with sepsis from various sources including severe intra-abdominal sepsis, necrotizing soft tissue infection, and pneumonia.[20] The cmtDNA assay employed quantified the *total* number of cmtDNA fragments in patients' blood post-transplantation and is not specific to donor or recipient-derived cmtDNA. Total levels of cmtDNA measured post-transplantation in this study will hence include contributions of cmtDNA released from a) the graft and b) as a consequence of surgical trauma and the immune sequelae of transplantation in the recipient's tissues.

This is an important strength of the study; cmtDNA released from *both* recipient tissues and the graft has the potential to act as a powerful immune modulator and influence patient outcomes. The interplay of these contributors of cmtDNA is likely to be important, neglect of graft or recipient-derived cmtDNA would provide an incomplete picture of the immune-modulatory activity of cmtDNA in the post-transplantation setting. At this stage there is no evidence to suggest that graft-derived or recipient-derived cmtDNA fragments have a more potent role in invoking post-operative pathological inflammatory responses, however this should form an area of further study.

This study is, to our knowledge, the first to describe the pattern of intense elevation of cmtDNA levels followed by subsequent normalisation over a number of days following LT. This mirrors observations in other periprocedural settings, including following trauma surgery, which are not complicated by the interplay of graft-host response. [21] Importantly, we found that cmtDNA levels in those patients who went on to develop EAD were significantly higher than those in the non-EAD group. This observation is consistent with the hypothesis that a greater degree of ischemia/reperfusion injury to graft leads more intense release of cmtDNA, the more significant injury leading to EAD. Overall, cmtDNA, AST, and ALT continued to decline in the non-EAD group after liver transplantation reflecting a positive correlation between cmtDNA and plasma liver enzymes and a lesser degree of ischaemic-reperfusion injury to graft.

In the EAD group, cmtDNA concentrations showed a resurgence at POD 7 despite the continued decline of AST and ALT. This is an intriguing observation. When the post-operative time points were divided into early (from POD1 to POD4) and late (POD5 to POD7) periods, there was little correlation between cmtDNA and AST/ALT in the EAD group in the late postoperative period. These results were not related to analysis batch effects. We hypothesise that this lack of correlation may relate to alternative, non-hepatic, sources of cmtDNA in the later post-operative period. Inflammation secondary to other causes, rather than direct, surgical tissue damage or necrosis or ischaemic/reperfusion injury, may be a potential source for cmtDNA elevation. In another setting, orthopaedic trauma, it has been shown that tissue necrosis markers (AST, LDH, creatinine kinase) were independent of cmtDNA levels[21].

We considered whether this late surge (POD7) in cmtDNA might be related to those patients who received a split liver graft. However, our results did not show any association between split liver transplantation using the right lobe (three cases in this series) and postoperative elevation in the plasma cmtDNA. Indeed, we did not observe any difference in post-operative cmtDNA levels in patients who received a technically modified graft, in terms of initial elevation peaks or subsequent rates cmtDNA reduction. The effect of the ischemic segment 4 as a passive source of cmtDNA or the liver regeneration process as an active one is yet to be determined. Further studies are needed to elucidate the mechanisms and factors associated with the late increase in cmtDNA seen on POD7 in the EAD subset of patients.

A number of studies, outside of the transplantation setting, have implicated cmtDNA as having an important immunomodulatory role as a 'danger/damage associated molecular pattern' (DAMP).[22,23] cmtDNA release may be mediated by passive "leaking" from damaged cells or through active secretion, particularly via the formation of NETs. It is plausible that active release of cmtDNA as DAMP may have been observed in our cohort of liver transplant patients; this is indicated by the later elevation of cmtDNA post-operatively which was not associated with elevation in LFTs as would be expected in passive release from hepatic cellular injury. This late surge in cmtDNA also may represent the first signs of the immunological reaction or inflammation leading to EAD. Emerging evidence indicates that neutrophilia plays a pivotal role in the innate immune system together with cmtDNA leading to the formation of NETs.[24,25] Interestingly mtDNA can trigger NETs formation which further release mtDNA in the process of their formation, leading to self-perpetuating inflammation.[26,27] Toll-like receptor 9 (TLR9) recognizes specific cytosine-proceeding-guanine (CpG) motifs which are commonly found in bacterial DNA as well as cmtDNA. These CpG motifs therefore may be recognized in cmtDNA, thus stimulating the same inflammatory pathway as occurs in response to bacterial infections.[22] NETs formation via activated TLR9 by cmtDNA may be an important inflammatory pathway in graft damage or failure in liver transplantation.[28]

High levels of cmtDNA are associated with the onset of chronic graft-versus-host disease after stem cell transplantation and levels of cmtDNA positively correlate with B cell responsiveness to CpG-DNA.[29] In addition, mtDNA stimulation through TLR9 leads to B cell activation and appears to be associated with primary graft dysfunction in lung transplantation.[28] The association between elevated cmtDNA at POD7 and EAD in our study is consistent with recently published data indicating that mtDNA may have a major role in host immune reactions and graft dysfunction.[28,30]

The abundance of cmtDNA in comparison to nuclear-derived cfDNA makes cmtDNA an attractive prospect for use as a biomarker, however its analysis is complicated by a wider variety of potential sources than nuclear cfDNA. Platelets, for example, whilst anuclear could also be a significant source of measured cmtDNA.[31]However, we found little correlation between plasma cmtDNA levels and variation in postoperative platelet counts in this study. In this study we have quantified total circulating concentrations of mitochondrial-derived DNA (cmtDNA), further future study into the analysis of the variable contributors of measured cmtDNA is warranted.

Moderate to severe steatosis is a well-known risk factor for EAD.[32] In our cohort, the degree of steatosis in all patients was mild (< 30%) due to a strict institutional graft selection protocol. Regardless, in those patients receiving a mildly steatotic graft, plasma cmtDNA levels were higher on POD7. This is in accordance with another study showing that subjects with non-alcoholic steatosis had higher plasma levels of cmtDNA.[26] Other potential risk factors (donor age, donation after circulatory death, cold and warm ischemic time) were not associated with recipient cmtDNA levels in our study. In addition to the donor's liver condition, the development of EAD will likely involve a multifactorial process influenced by levels of ischemia, the quality of the liver (fat content, prolonged ischemic time, donor age) and recipient factors (MELD score, sarcopenia, immunosuppression).[6,33-35]

This study was designed primarily to provide an initial description of cmtDNA dynamics post LT, to act as a foundation on which to build further investigations into this emerging field of translational research. However, we must acknowledge some of the study limitations, the most significant of which is the sample size. Secondly, to enhance feasibility, the study was designed to monitor levels of cmtDNA in participants during the first seven post-operative days. Although we clearly demonstrated plasma cmtDNA elevation in the setting of liver transplantation, subsequent inflammatory and immunological events were not investigated; further longitudinal studies with longer monitoring periods might add to the understanding of graft dysfunction and outcome.

Conclusion

This study is the first, to our knowledge to describe, the dynamics of cmtDNA release in patients following liver transplantation, examining cmtDNA levels against with subsequent clinical outcomes. In this study we have demonstrated a late surge of cmtDNA in the plasma of patients with EAD, which appears to be independent of direct liver injury as measured by conventional liver function tests. These novel findings warrant further research into the significance and roles of plasma cmtDNA in the pathophysiology and as a biomarker in liver transplantation.

Funding

Osamu Yoshino has received financial support from the Royal Australasian College of Surgeons, Small Project Grant 2020.

Disclosures

No competing interests to declare. None of the material has been published or is under consideration elsewhere, including online journals.

Figure Legend

Figure 1. Box plots showing changes in cmtDNA levels in three groups: 1. (PATTERN) non-EAD group (N=14); 2. (BLANK) EAD group (N=7); and 3. (RED) healthy control group (N=6). Time points (x axis) are as follows: 0, during anaesthetic induction; 1–7, post-operative days 1–7. The cmtDNA level in the non-EAD group was elevated from day 1 to day 3 when compared to their baseline levels (# $p < 0.05$). The EAD group showed significantly elevated plasma mtDNA levels at days 1, 2, 3, and 7 when compared with the preoperative base line level and the non-EAD group levels (## $p < .05$).

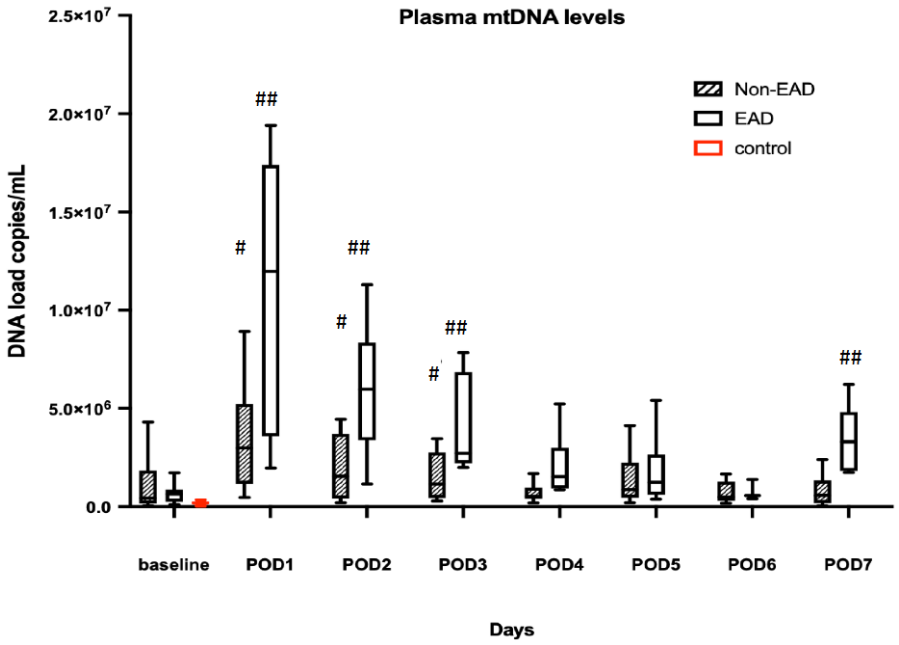
References

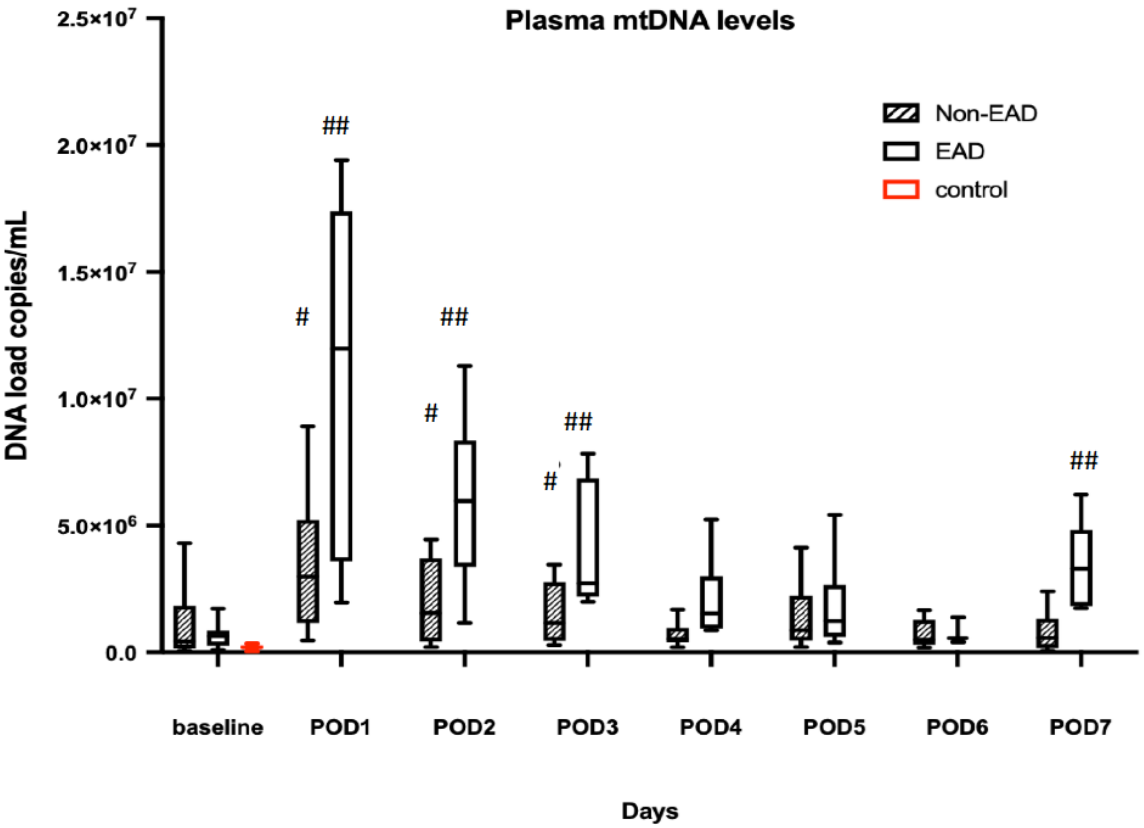
1. Simmons JD, Lee Y-L, Mulekar S, et al. Elevated Levels of Plasma Mitochondrial DNA DAMPs Are Linked to Clinical Outcome in Severely Injured Human Subjects. Annals of Surgery 2013.
2. Tsai NW, Lin TK, Chen SD, et al. The value of serial plasma nuclear and mitochondrial DNA levels in patients with acute ischemic stroke. Clin Chim Acta 2011;412:476-9.
3. Mills EL, Kelly B, O'Neill LAJ. Mitochondria are the powerhouses of immunity. Nat Immunol 2017;18:488-98.
4. Wadei HM, Lee DD, Croome KP, et al. Early Allograft Dysfunction After Liver Transplantation Is Associated With Short- and Long-Term Kidney Function Impairment. Am J Transplant 2016;16:850-9.
5. Song J-L, Yang J, Yan L-N, et al. A new index predicts early allograft dysfunction following living donor liver transplantation: A propensity score analysis. Digestive and Liver Disease 2017;49:1225-32.
6. Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl 2010;16:943-9.
7. Friedman BH, Wolf JH, Wang L, et al. Serum cytokine profiles associated with early allograft dysfunction in patients undergoing liver transplantation. Liver Transpl 2012;18:166-76.
8. Pollara J, Edwards RW, Lin L, Bendersky VA, Brennan TV. Circulating mitochondria in deceased organ donors are associated with immune activation and early allograft dysfunction. JCI Insight 2018;3.
9. Feng S, Goodrich NP, Bragg-Gresham JL, et al. Characteristics associated with liver graft failure: the concept of a donor risk index. Am J Transplant 2006;6:783-90.
10. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. The American journal of gastroenterology 1999;94:2467-74.

11. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997;25:658-63.
12. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315:801-10.
13. Clavien PA, Barkun J, de Oliveira ML, et al. The Clavien-Dindo classification of surgical complications: five-year experience. Ann Surg 2009;250:187-96.
14. Zhang R, Nakahira K, Guo X, Choi AM, Gu Z. Very Short Mitochondrial DNA Fragments and Heteroplasmy in Human Plasma. Sci Rep 2016;6:36097.
15. Youden WJ. Index for rating diagnostic tests. Cancer 1950;3:32-5.
16. Alberts B. Molecular biology of the cell. Sixth edition. ed. New York, NY: Garland Science, Taylor and Francis Group; 2015.
17. McGill MR, Staggs VS, Sharpe MR, Lee WM, Jaeschke H, Acute Liver Failure Study G. Serum mitochondrial biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome. Hepatology 2014;60:1336-45.
18. Heymann F, Tacke F. Immunology in the liver--from homeostasis to disease. Nat Rev Gastroenterol Hepatol 2016;13:88-110.
19. Huggett JF, Cowen S, Foy CA. Considerations for digital PCR as an accurate molecular diagnostic tool. Clin Chem 2015;61:79-88.
20. Hawkins RB, Stortz JA, Holden DC, et al. Persistently increased cell-free DNA concentrations only modestly contribute to outcome and host response in sepsis survivors with chronic critical illness. Surgery 2019.
21. McIlroy DJ, Bigland M, White AE, et al. Cell necrosis-independent sustained mitochondrial and nuclear DNA release following trauma surgery. J Trauma Acute Care Surg 2015;78:282-8.
22. Zhang Q, Raoof M, Chen Y, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 2010;464:104-7.
23. Itagaki K, Rica I, Konecna B, et al. Role of Mitochondria-Derived Danger Signals Released After Injury in Systemic Inflammation and Sepsis. Antioxid Redox Signal 2021.
24. Itagaki K, Kaczmarek E, Lee YT, et al. Mitochondrial DNA released by trauma induces neutrophil extracellular traps. PLoS One 2015;10:e0120549.
25. Almyroudis NG, Grimm MJ, Davidson BA, Röhm M, Urban CF, Segal BH. NETosis and NADPH oxidase: at the intersection of host defense, inflammation, and injury. Frontiers in Immunology 2013;4.
26. Garcia-Martinez I, Santoro N, Chen Y, et al. Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9. J Clin Invest 2016;126:859-64.
27. Lood C, Blanco LP, Purmalek MM, et al. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. Nat Med 2016;22:146-53.
28. Mallavia B, Liu F, Lefrancais E, et al. Mitochondrial DNA Stimulates TLR9-Dependent Neutrophil Extracellular Trap Formation in Primary Graft Dysfunction. Am J Respir Cell Mol Biol 2020;62:364-72.

29. Rozmus J, Ivison S, Kariminia A, et al. Higher levels of free plasma mitochondrial DNA are associated with the onset of chronic GvHD. Bone Marrow Transplant 2018;53:1263-9.
30. Zhang X, Wu X, Hu Q, et al. Mitochondrial DNA in liver inflammation and oxidative stress. Life Sci 2019;236:116464.
31. Boudreau LH, Duchez AC, Cloutier N, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. Blood 2014;124:2173-83.
32. Deroose JP, Kazemier G, Zondervan P, Ijzermans JN, Metselaar HJ, Alwayn IP. Hepatic steatosis is not always a contraindication for cadaveric liver transplantation. HPB (Oxford) 2011;13:417-25.
33. Peer V, Abu Hamad R, Berman S, Efrati S. Renoprotective Effects of DNAse-I Treatment in a Rat Model of Ischemia/Reperfusion-Induced Acute Kidney Injury. Am J Nephrol 2016;43:195-205.
34. Jimenez-Alcazar M, Rangaswamy C, Panda R, et al. Host DNases prevent vascular occlusion by neutrophil extracellular traps. Science 2017;358:1202-6.
35. Agopian VG, Harlander-Locke MP, Markovic D, et al. Evaluation of Early Allograft Function Using the Liver Graft Assessment Following Transplantation Risk Score Model. JAMA Surg 2018;153:436-44.

Figure 1





JGH_15670_Figure 1 Mithochondrial DNA and liver transplant.tif