Tohoku J. Exp. Med., 2020, **250**, 87-93

# Elevated Plasma Levels of Mitochondria-Derived Damage-Associated Molecular Patterns during Liver Transplantation: Predictors for Postoperative Multi-Organ Dysfunction Syndrome

**Kantoku Nagakawa,[1] Akihiko Soyama,[1] Masaaki Hidaka,[1] Tomohiko Adachi,[1] Shinichiro Ono,[1] Takanobu Hara,[1] Mitsuhisa Takatsuki[1] and Susumu Eguchi[1]**

[1]Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Nagasaki, Japan

The systemic cytokine response during surgery has been reported to be stimulated by the molecules released from damaged cells, called damage-associated molecular patterns (DAMPs). The relationship between DAMPs and liver transplantation has not been reported. We aimed to clarify the relationship between the plasma levels of DAMPs and the short-term post-transplant outcomes, including mortality and postoperative multi-organ dysfunction syndrome (MODS). This retrospective cohort study enrolled 61 patients who underwent liver transplantation. Mitochondrial DNA fragments, as mitochondria-derived DAMPs (mtDAMPs), were isolated from frozen plasma obtained at the start and the end of transplantation and were quantified by polymerase chain reaction. The short-term post-transplant outcomes were compared among the groups categorized based on the median value of the intraoperative fluctuation of mtDAMPs levels. The mtDAMPs levels were increased from the start to the end of transplantation in 52 recipients (85.2%, n = 61). Regarding mortality, no significant differences were noted between the high group (n = 30) and the low group (n = 31). The higher plasma levels of mtDAMPs were correlated with the longer duration of postoperative vasopressor support ($P < 0.05$). Importantly, the rate of MODS on post-operative day 1 was significantly higher in the high group (high vs. low group: 21 patients [70%] vs. 11 patients [35.1%], $P < 0.01$). In conclusion, mtDAMPs were increased in plasma during liver transplantation in most recipients. This elevation was not associated with mortality, but associated with the post-transplant recovery. Measuring plasma mtDAMPs may be helpful for predicting posttransplant recovery among liver-transplant recipients.

**Keywords:** cytokines; damage-associated molecular patterns (DAMPs); liver transplantation; surgical stress; systemic inflammatory response syndrome (SIRS)
Tohoku J. Exp. Med., 2020 February, **250** (2), 87-93.

## Introduction

The systemic cytokine response to surgical trauma increases with the severity of the surgical insult. It was revealed that serum levels of cytokines such as interleukin (IL)-1 and IL-6 are elevated after surgery (Ayala et al. 1991; Baigrie et al. 1992; Sakamoto et al. 1994; Glaser et al. 1995) and that their responses were stimulated by damage-associated molecular patterns (DAMPs) (Zhang et al. 2010). DAMPs are molecules, such as nuclear or cytosolic proteins, that are actively excreted or passively released by dead, dying, injured, or stressed cells in response to trauma, ischemia, and tissue damage and further enhance inflammatory or cell-death signaling (Rubartelli and Lotze 2007; Hou et al. 2013). DAMPs include DNA, high-mobility group box 1 (HMGB1), and heat shock proteins (Nakahira et al. 2015). They play a number of roles in inflammation and immunity as inducers of the cytokine cascade and stimulators of systemic inflammatory response syndrome (SIRS) (Zhang et al. 2010).

Mitochondria can release mitochondria-associated molecules when cells are dying in response to cellular stress, including mechanical stress or infection; these molecules are called mitochondrial DAMPs (mtDAMPs) and

Received July 5, 2019; revised and accepted January 23, 2020. Published online February 14, 2020; doi: 10.1620/tjem.250.87.
Correspondence: Susumu Eguchi, Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki, Nagasaki 852-8501, Japan.
e-mail: sueguchi@nagasaki-u.ac.jp

induce various immune responses of immune cells (Nakahira et al. 2015). MtDAMPs have been identified as important mediators of the innate immune response and implicated in various conditions, such as trauma, sepsis, and autoimmune disorders (Grazioli and Pugin 2018). Simmons et al. (2013) revealed that plasma mtDAMPs are associated with the development of SIRS, multi-organ dysfunction syndrome (MODS), and mortality in severely injured human subjects. Severe surgical stress is expected to occur via a similar mechanism to trauma. Liver transplantation is a highly invasive surgical procedure and is considered to be associated with severe surgical stress. However, surgical stress is multifactorial, with causative factors including wound size, hemorrhaging amount, and operation time, and can thus be difficult to express. Furthermore, the effect of surgical stress on post-transplant outcomes remains unclear, as the post-transplant course is affected by a number of factors, such as infection, rejection, and the graft function. We hypothesized that DAMPs express their multifactorial phenomena as an indicator and affect the outcomes of recipients.

In order to evaluate the effect of surgical stress on post-transplant outcomes, we investigated the relationship between the plasma levels of mitochondrial DNA fragments and the outcomes of liver transplantation. Mitochondrial DNA fragments were selected as mtDAMPs due to their sensitivity to oxidative damage. Oxidative stress is a key pathophysiological component of severe trauma and illness, DNA in the mitochondrial genome is considerably more sensitive to oxidative damage than nuclear DNA (Yakes and Van Houten 1997; Mikhed et al. 2015). We hypothesized that plasma mtDAMPs would be increased in the operation and that the increase would be associated with the post-transplant outcome.

## Patients and Methods

This retrospective cohort study enrolled 61 patients who underwent liver transplantation at Nagasaki University Hospital between September 2011 and May 2015. The recipients were > 18 years old with various types of liver disease, such as liver cirrhosis associated with hepatitis B virus, hepatitis C virus or alcohol, primary biliary cholangitis, primary sclerosing cholangitis, hepatic cell carcinoma, Wilson's disease, and drug-induced liver injury. Two recipients underwent deceased donor liver transplantation; the others underwent living donor liver transplantation. Blood samples were taken just before laparotomy and at the time of closure of the abdomen and placed into blood collection tubes containing anticoagulants.

The samples were centrifuged at 1,200 relative centrifugal force for 10 minutes at 21°C, and 200 $\mu$L of the plasma fraction was decanted and frozen at –80°C. The frozen plasma samples were defrosted at room temperature, and DNA was isolated using NucleoSpin® Plasma (TAKARA BIO INC., Shiga, Japan). Real-time polymerase chain reaction (PCR) was performed to quantify the sequences of mtDNA, nicotinamide adenine dinucleotide (NADH) dehydrogenase 1 (ND1), and NADH dehydrogenase 6 (ND6) (TaqMan® Gene Expression Assay, MT-ND1, and MT-ND6, respectively; [Applied Biosystems, Massachusetts, USA]) using the primers described in previous articles (Zhang et al. 2010; Simmons et al. 2013). These have a lower molecular weight than many other mitochondrial DNA fragments and were considered suitable for quantifying the mitochondrial injury because of their characteristically low risk of sequence damage.

Real-time PCR was performed using an Applied Biosystems® StepOnePlusTM Real-Time PCR System and Taqman® Fast Universal PCR Master Mix. The quantity of plasma mitochondrial DNA fragments was expressed as the cycle threshold (CT), which was defined as the number of cycles required for the fluorescent signal to reach the level of detection in real-time PCR. The CT values are inverse to the amount of target nucleic acid in the sample and are correlated with the number of target copies in the sample. Hence, lower CT values indicate high amounts of the target sequence and higher CT values mean lower amounts of the target nucleic acid.

We evaluated the outcomes and perioperative factors according to the increase of mitochondrial DNA fragments. The primary outcome was 90-day mortality, and the secondary outcomes were the development of multi-organ dysfunction syndrome (MODS), length of intensive-care unit (ICU) stay, duration of vasopressor and ventilator support, and induction of continuous hemodiafiltration (CHDF). We used the sequential organ failure assessment (SOFA) score as a marker of MODS, and we defined MODS as a SOFA score of > 9 points based on the results of a previous report (Zhang et al. 2010). We also analyzed the relationship between mtDAMPs and the following perioperative factors: the MELD score, operative time, amount of blood loss, and the anhepatic time.

We analyzed the association of the mitochondrial DNA fragment levels, as a continuous variable, with the presence of death, MODS, and the induction of CHDF as categorical variables and the duration of ICU stay, vasopressor usage, ventilator support, and the liver function on post-operative day (POD) 7 as continuous variables. A relative risk analysis was performed to determine the association between a high increase in the mtDAMPs level (using the median CT value) and the post-transplant outcomes. Statistically significant differences ($P < 0.05$) were detected by nonparametric analyses. All statistical analyses were conducted using the JMP Statistical Software program, Version 11 (SAS Institute Inc., Cary, NC, USA).

This study was approved by the Ethical Committee of the Nagasaki University Hospital (decision number 18091019).

## Results

The CT values of mitochondrial DNA fragments are shown in Table 1. In 52 recipients (85%), the ND1 levels

Table 1. The CT values of mtDAMPs in 61 recipients.

| | Minimum | Median | Max |
|---|---|---|---|
| **ND1 (Pre-transplantation)** | 20.5725 | 28.4387 | 34.3864 |
| **ND1 (Post-transplantation)** | 19.6602 | 25.0453 | 29.9986 |
| **ND6 (Pre-transplantation)** | 18.8562 | 26.4175 | 32.0763 |
| **ND6 (Post-transplantation)** | 18.8130 | 23.2076 | 27.9616 |

The lower the CT level, the greater the plasma mtDAMP level.
CT, cycle threshold; ND, nicotinamide adenine dinucleotide dehydrogenase.

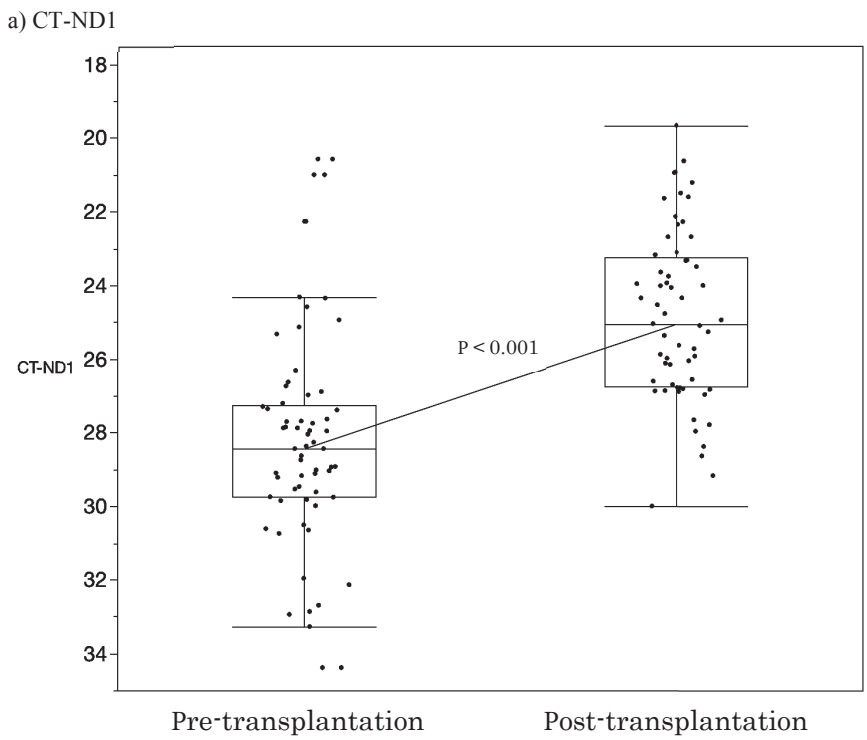
were increased after the operation. The changes in the mitochondrial DNA fragment levels (ND1 and ND6) before and after transplantation are shown in Fig. 1. We expressed the change as ΔCT, as follows:

ΔCT = CT (post-transplantation) − CT (pre-transplantation).

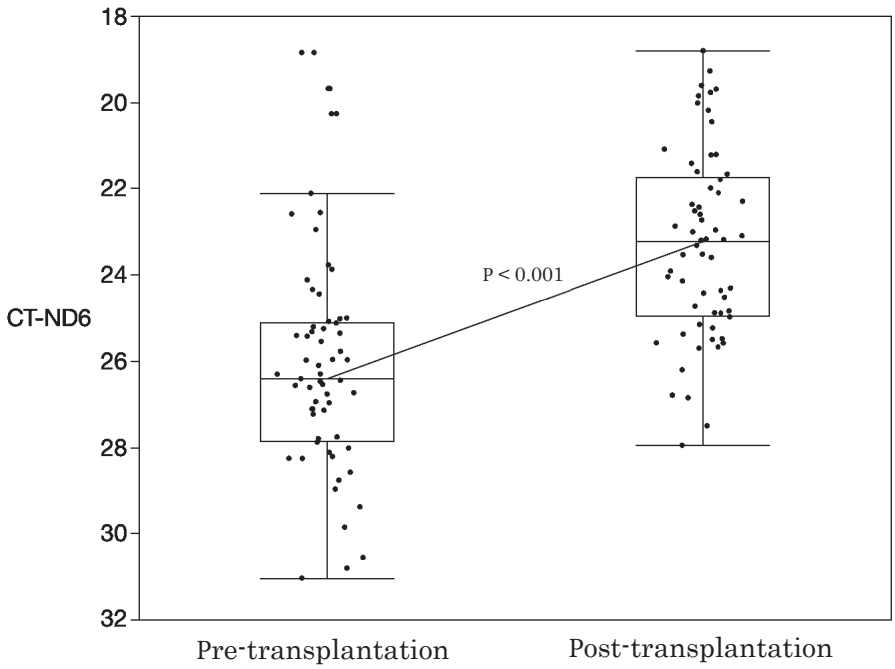
ΔCT of ND1 (ΔCT-ND1) was correlated with ΔCT of ND6 (ΔCT-ND6), and the Pearson's correlation coefficient was 0.98 (Fig. 1). This ensured the accuracy of the measurement of mitochondrial DNA fragments.

We divided the recipients into two groups (high and low) based on the median ΔCT-ND1 value (Fig. 2). No significant differences were noted in the characteristics; recipient sex, recipient age, donor age and the MELD score of the recipient, in the high group (n = 30; median: –6.313, range: –10.631 to –2.826) and the low group (n = 31; median: –0.917, range: –2.807 to 3.940) (Table 2). Regarding the perioperative factors, no significant differences between the two groups were noted in the operation time (high vs. low group: 770 minutes vs. 740 minutes, P = 0.27), blood loss (high vs. low group: 6,520 g vs. 6,600 g, P = 0.78), or postoperative lactate levels (high vs. low group: 2.65 mmol/L vs. 2.5 mmol/L, P = 0.24). By contrast, the anhepatic time in the high group was significantly longer than that in the low group (high vs. low group: 121 [78-423] minutes vs. 109 [24-274] minutes, P < 0.05) (Table 2).

With regard to the primary outcome (90-day mortality), there was no significant difference between the groups (high vs. low group: 5 patients [16.7%] vs. 4 patients [12.9%], P = 0.68). With regard to the secondary outcomes, the duration of vasopressor support in the high group was longer than that in the low group (high vs. low group: 2.5 [0-45] days vs. 0 [0-26] days, P < 0.05). Furthermore, the rate of MODS on post-operative day 1 (POD1) was significantly higher in the high group than in the low group (high vs. low group: 21 patients [70%] vs. 11 patients [35.1%], P



b) CT-ND6




c) The Pearson's correlation coefficient, Δ CT-ND1 and Δ CT-ND6

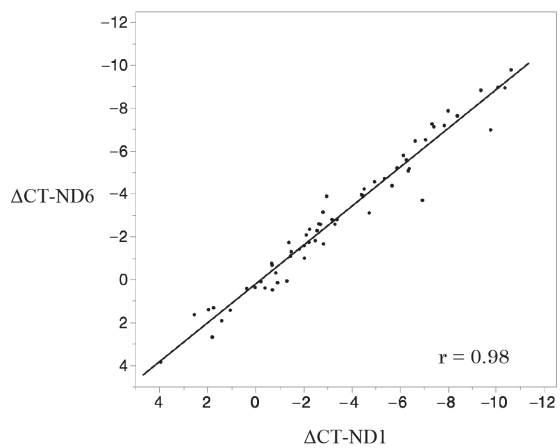



Fig. 1. Changes in mtDAMP levels and the relationship between ΔCT-ND1 and ΔCT-ND6.
The scale of the CT values is shown in an opposite manner to the amount of target nucleic acid, as the CT values are inverse to the amount of target nucleic acid in the sample.

a) A dot and box plot of the CT values of ND1 (CT-ND1). The dots show the changes in the CT values of each 61 patients. The median CT value of ND1 in the post-transplant phase was significantly lower than that in the pre-transplant phase. This indicates that plasma ND1 levels were increased during transplantation.

b) A dot and box plot of the CT values of ND6 (CT-ND6). The dots show the change in the CT values for all of the 61 patients. The median CT value of ND6 in the post-transplant phase was significantly lower than that in the pre-transplant phase. This indicates that plasma ND6 levels were increased during transplantation.

c) The correlation between the change of the CT values of ND1 and ND6. The dots show the changes in the CT values of ND1 and ND6 for all of the 61 patients. Pearson's correlation coefficient was 0.98.

mtDAMPs, mitochondrial damage-associated molecular patterns; CT, cycle threshold; ND, nicotinamide adenine dinucleotide dehydrogenase.

< 0.01). There were no significant differences between the groups in the duration of ICU stay (high vs. low group: 5 [1-34] days vs. 4 [1-39] days, P = 0.31), ventilator support (high vs. low group: 2 [1-22] days vs. 1 [1-26] day, P = 0.08), or the liver function on POD7 (high vs. low group: AST 53.5 [22-878] IU/L vs. AST 45.0 [11-731] IU/L, P = 0.40; T-Bil 7.2 [2-17.8] mg/dL vs. T-Bil 7.8 [0.9-19.8] mg/dL, P = 0.79) (Table 2).

| | High group (n = 30) | Low group (n = 31) | P value |
|---|---|---|---|
| ΔCT-ND1 (median) | –6.313 (–10.631 to –2.826) | –0.917 (–2.807 to 3.940) | < 0.001 |

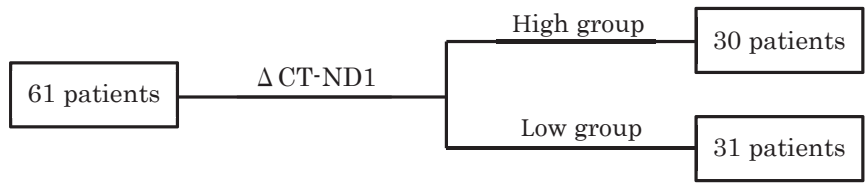



Fig. 2. Grouping according to the increase in the mtDAMP level.
We divided the recipients into two groups (high and low group) according to the median value of the change in the mtDAMPs level from pre-transplantation to post-transplantation.
mtDAMPs, mitochondrial damage-associated molecular patterns; CT, cycle threshold; ND, nicotinamide adenine dinucleotide dehydrogenase.

Table 2. The differences of the two groups.

| | High group (n = 30) | Low group (n = 31) | P value |
|---|---|---|---|
| Characteristics | | | |
| ΔCT ND1 (median) | –6.313 (–10.631 to –2.826) | –0.917 (–2.807 to 3.940) | < 0.001 |
| ΔCT ND6 (median) | –5.179 (–9.772 to –2.787) | –0.663 (–3.131 to 3.852) | < 0.001 |
| Recipient sex (M:F) | 17:13 | 20:11 | N.S. |
| Recipient age (median) | 58 (47-69) | 59.5 (24-70) | N.S. |
| Donor age (median) | 39 (22-63) | 39 (24-62) | N.S. |
| MELD score (median) | 14 (8-37) | 18 (8-37) | N.S. |
| ICU admission (case) | 0 | 2 | N.S. |
| Perioperative factors | | | |
| Operation time (min) | 770 (510-1,057) | 740 (577-1,031) | N.S. |
| Blood loss (g) | 6,520 (2,050-16,123) | 6,600 (2,500-30,500) | N.S. |
| Graft size (g) | 472 (310-770) | 470 (298-814) | N.S. |
| GV/SLV | 44.7 (25.9-63.4) | 47.05(28.9-59.78) | N.S. |
| Anhepatic time (min) | 121 (78-423) | 109 (24-274) | < 0.05 |
| Cold ischemic time (min) | 101.5(49-465) | 96 (45-419) | N.S. |
| Warm ischemic time (min) | 49.5 (30-96) | 44 (32-129) | N.S. |
| Postoperative Lactate (mmol/l) | 2.65 (1-19) | 2.5 (1-8.6) | N.S. |
| Outcomes | | | |
| 90-day mortality (n [%]) | 5 (16.7%) | 4 (12.9%) | N.S. |
| ICU stay (days) | 5 (1-34) | 4 (1-39) | N.S. |
| Vasopressor support (days) | 2.5 (0-45) | 0 (0-26) | < 0.05 |
| Ventilator support (days) | 2 (1-22) | 1 (1-26) | 0.08 |
| Initiation of CHDF (n [%]) | 8 (26.7%) | 4 (12.9%) | N.S. |
| MODS POD1 (n [%]) | 21 (70.0%) | 11 (35.4%) | < 0.01 |
| MODS POD7 (n [%]) | 8 (26.7%) | 9 (29.0%) | N.S. |
| AST POD7 (IU/L) | 53.5 (22-878) | 45 (11-731) | N.S. |
| T-Bil POD7 (mg/dl) | 7.2 (2-17.8) | 7.8 (0.9-19.8) | N.S. |

There were significant differences between the two groups in the anhepatic time, the duration of postoperative vasopressor support and the development of MODS on POD1.
CT, cycle threshold; NADH, dehydrogenase; MELD score, The Model of End-stage Liver Disease score; ICU, intensive care unit; GV/SLV, Graft Volume/Standard Liver Volume; CHDF, continuous hemodiafiltration; MODS, multi-organ dysfunction syndrome; POD, postoperative day; AST, aspartate transaminase; T-Bil, total-bilirubin, N.S., not significant.

## Discussion

ND1 and ND6 in plasma are two types of mtDAMPs. This is the first study to show an intraoperative increase in the mtDAMPs levels in most patients during liver transplantation. The causes of increased mtDAMPs levels may be multifactorial, including the primary disease, surgical procedure, blood loss, blood transfusion, anesthesia, and the condition of the liver graft (Ferreira et al. 2001; Lee et al. 2014; Fucikova et al. 2015; Garcia-Martinez et al. 2016; Yasui et al. 2016). Although previous studies have suggested that increased blood loss and a lengthy operation would increase mtDAMPs levels (Sakamoto et al. 1994; Chaung et al. 2012), these operative factors were not found to be correlated with increased mtDAMPs levels in the present study.

Specifically, this study found that a long anhepatic time increased plasma mtDAMPs levels. The deleterious effects of a prolonged anhepatic phase have been attributed to hemodynamic changes resulting from the interruption of the portal venous system. The serum IL-6 levels in the recipient have been shown to offer a marker of hemodynamic performance during liver transplantation (Arranz et al. 2003). Previous studies of serum cytokine levels during liver transplantation have shown that the anhepatic phase to marks the start of a sharp increase in the recipient serum IL-6 level to over 100 times the levels measured in control patients (Faybik et al. 2003; Santiago et al. 2006; Eleftheriadis et al. 2014). IL-6 elevation has recently been reported to be associated with the production of mtDAMPs in a rat model (Ijtsma et al. 2009). The elevation of mtDAMPs in association with the elevation of IL-6 or other cytokines during the anhepatic phase might be a cause of the lengthy duration of vasopressor support and the development of MODS on POD1 in the present study.

Recent articles have indicated a relationship between mtDAMPs and the prognosis in trauma patients (Zhang et al. 2010; Simmons et al. 2013). However, no significant relationship between the mtDAMPs levels and 90-day mortality was identified in the present study. In comparison to trauma patients, the causes of mortality after transplantation are considered more multifactorial, involving factors such as rejection, infection, and an initial poor liver function. However, all recipients with $\Delta$CT-ND1 < –10 (3 recipients) died within 30 days after transplantation. In fact, the increase in mtDAMPs levels was significantly correlated with circulatory failure and MODS in this study. Taking into account the present results, including the longer duration of vasopressor support, tissue damage induced by markedly increased mtDAMPs levels may affect the postoperative course. However, whether the outcomes of liver transplantation are influenced by such increases in mtDAMPs—and if so, the degree to which precise levels of mtDAMPs exert such influences—should be carefully analyzed in future studies. Although some recipients did show decreased mtDAMP levels after transplantation, these patients had already shown high mtDAMPs, even before transplantation. This phenomenon may be related to pretransplantation conditions, such as severe liver disease or a very poor general condition. Further investigations are required to clarify the mechanisms under which mtDAMP levels are reduced during surgery.

The present study was associated with some limitations. First, this study was conducted at a single institution in a small number of patients. Second, normal levels of mtDAMPs remain unclear. We used the median difference between the pre- and post-transplantation levels as the cutoff value in the present study, but an appropriate cutoff level for mtDAMPs, in terms of the post-transplant outcomes, should be determined using a larger cohort.

In conclusion, the plasma levels of mtDAMPs were increased during liver transplantation in most cases. Although the elevation of plasma levels of mtDAMPs during surgery was not associated with short-term mortality in liver transplant recipients, measuring mtDAMPs may be helpful for predicting posttransplant recovery. Further study on mtDAMPs may clarify the possibility of mtDAMPs as a therapeutic target to achieve a better posttransplant recovery in patients undergoing liver transplantation.

## Acknowledgments

We did not receive substantial contributions from any non-authors.

## Conflict of Interest

The authors declare no conflict of interest.

## References

Arranz, J., Soriano, A., Garcia, I., Garcia, I., Concepcion, M.T., Navarro, J., Arteaga, A., Filella, X., Bravo, P., Barrera, M., Escribano, S., Jimenez, A., Moneva, E., Fernandez, J., Varona, A., et al. (2003) Effect of proinflammatory cytokines (IL-6, TNF-alpha, IL-1beta) on hemodynamic performance during orthotopic liver transplantation. *Transplant. Proc.*, **35**, 1884-1887.

Ayala, A., Wang, P., Ba, Z.F., Perrin, M.M., Ertel, W. & Chaudry, I.H. (1991) Differential alterations in plasma IL-6 and TNF levels after trauma and hemorrhage. *Am. J. Physiol.*, **260**, R167-171.

Baigrie, R.J., Lamont, P.M., Kwiatkowski, D., Dallman, M.J. & Morris, P.J. (1992) Systemic cytokine response after major surgery. *Br. J. Surg.*, **79**, 757-760.

Chaung, W.W., Wu, R., Ji, Y., Dong, W. & Wang, P. (2012) Mitochondrial transcription factor A is a proinflammatory mediator in hemorrhagic shock. *Int. J. Mol. Med.*, **30**, 199-203.

Eleftheriadis, T., Pissas, G., Antoniadi, G., Liakopoulos, V. & Stefanidis, I. (2014) Damage-associated molecular patterns derived from mitochondria may contribute to the hemodialysis-associated inflammation. *Int. Urol. Nephrol.*, **46**, 107-112.

Faybik, P., Hetz, H., Krenn, C.G., Baker, A., Berlakovich, G.A. & Steltzer, H. (2003) Perioperative cytokines during orthotopic liver transplantation without venovenous bypass. *Transplant. Proc.*, **35**, 3019-3021.

Ferreira, F.L., Bota, D.P., Bross, A., Melot, C. & Vincent, J.L. (2001) Serial evaluation of the SOFA score to predict

outcome in critically ill patients. *JAMA*, **286**, 1754-1758.
Fucikova, J., Moserova, I., Urbanova, L., Bezu, L., Kepp, O., Cremer, I., Salek, C., Strnad, P., Kroemer, G., Galluzzi, L. & Spisek, R. (2015) Prognostic and predictive value of DAMPs and DAMP-associated processes in cancer. *Front. Immunol.*, **6**, 402.
Garcia-Martinez, I., Santoro, N., Chen, Y., Hoque, R., Ouyang, X., Caprio, S., Shlomchik, M.J., Coffman, R.L., Candia, A. & Mehal, W.Z. (2016) Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9. *J. Clin. Invest.*, **126**, 859-864.
Glaser, F., Sannwald, G.A., Buhr, H.J., Kuntz, C., Mayer, H., Klee, F. & Herfarth, C. (1995) General stress response to conventional and laparoscopic cholecystectomy. *Ann. Surg.*, **221**, 372-380.
Grazioli, S. & Pugin, J. (2018) Mitochondrial damage-associated molecular patterns: from inflammatory signaling to human diseases. *Front. Immunol.*, **9**, 832.
Hou, W., Zhang, Q., Yan, Z., Chen, R., Zeh Iii, H.J., Kang, R., Lotze, M.T. & Tang, D. (2013) Strange attractors: DAMPs and autophagy link tumor cell death and immunity. *Cell Death Dis.*, **4**, e966.
Ijtsma, A.J., van der Hilst, C.S., de Boer, M.T., de Jong, K.P., Peeters, P.M., Porte, R.J. & Slooff, M.J. (2009) The clinical relevance of the anhepatic phase during liver transplantation. *Liver Transpl.*, **15**, 1050-1055.
Lee, Y.L., King, M.B., Gonzalez, R.P., Brevard, S.B., Frotan, M.A., Gillespie, M.N. & Simmons, J.D. (2014) Blood transfusion products contain mitochondrial DNA damage-associated molecular patterns: a potential effector of transfusion-related acute lung injury. *J. Surg. Res.*, **191**, 286-289.
Mikhed, Y., Daiber, A. & Steven, S. (2015) Mitochondrial oxidative stress, mitochondrial DNA damage and their role in age-related vascular dysfunction. *Int. J. Mol. Sci.*, **16**, 15918-15953.
Nakahira, K., Hisata, S. & Choi, A.M. (2015) The roles of mitochondrial damage-associated molecular patterns in diseases. *Antioxid. Redox Signal.*, **23**, 1329-1350.
Rubartelli, A. & Lotze, M.T. (2007) Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol.*, **28**, 429-436.
Sakamoto, K., Arakawa, H., Mita, S., Ishiko, T., Ikei, S., Egami, H., Hisano, S. & Ogawa, M. (1994) Elevation of circulating interleukin 6 after surgery: factors influencing the serum level. *Cytokine*, **6**, 181-186.
Santiago, F., Bueno, P., Olmedo, C., Comino, A., Hassan, L., Ferron-Celma, I., Muffak, K., Serradilla, M., Mansilla, A., Ramia, J.M., Villar, J.M., Garrote, D., Ramirez, A. & Ferron, J.A. (2006) Time course of intraoperative cytokine levels in liver transplant recipients. *Transplant. Proc.*, **38**, 2492-2494.
Simmons, J.D., Lee, Y.L., Mulekar, S., Kuck, J.L., Brevard, S.B., Gonzalez, R.P., Gillespie, M.N. & Richards, W.O. (2013) Elevated levels of plasma mitochondrial DNA DAMPs are linked to clinical outcome in severely injured human subjects. *Ann. Surg.*, **258**, 591-596; discussion 596-598.
Yakes, F.M. & Van Houten, B. (1997) Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc. Natl. Acad. Sci. USA*, **94**, 514-519.
Yasui, K., Matsuyama, N., Kuroishi, A., Tani, Y., Furuta, R.A. & Hirayama, F. (2016) Mitochondrial damage-associated molecular patterns as potential proinflammatory mediators in post-platelet transfusion adverse effects. *Transfusion*, **56**, 1201-1212.
Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., Brohi, K., Itagaki, K. & Hauser, C.J. (2010) Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*, **464**, 104-107.