The hidden link between plasma mitochondrial DNA level and cardiac dysfunction after cardiopulmonary bypass

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Research article

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Abstract

Background: To investigate the hidden link between mitochondrial DNA (mtDNA) and cardiac function after cardiopulmonary bypass (CPB), we compared the relationship of plasma mtDNA level and the prevalence of cardiac dysfunction in patients receiving cardiac surgery with CPB.

Methods: From September 1st, 2015 to December 1st, 2016, 962 patients who received cardiac surgery with CPB were prospectively comprised in this study. Routine blood test and examinations for blood biochemistry, NT-proBNP and mtDNA were arranged in half an hour when patients were transferred into intensive care unit (ICU). The clinical outcomes related to the cardiac function were determined by at least two senior doctors responsible for ICU. The data were inputted with double check and the inner logic of these data was set to establish the database. SAS 9.4, R 3.2.4 and SPSS 21.0 were used in data analysis.

Results: Of the 962 patients (mean [SD] age, 51.6 [12.5] years; 459 [47.7%] male) comprised in this study, 692 patients received cardiac valve surgery, 83 patients received coronary artery bypass grafting (CABG), 58 patients received CABG and cardiac valve surgery at the same time, 70 patients received surgery on ascending aorta, 35 patients received surgery for simple congenital heart diseases and 15 patients received other types of surgery. The prevalence of cardiac dysfunction in this study is 9.25%. The level of mtRNA among patients with cardiac dysfunction and patients without cardiac dysfunction has no statistical significance (t=-1.42, P=0.1565). There was an obvious positive liner correlation between the level of plasma mtDNA and NT-proBNP in patients receiving CPB (r=0.1291, P=0.0386). There was an obvious positive liner correlation between the level of plasma mtDNA and APACHE II score in patients receiving CPB (r=0.2133, P<0.0001).

Conclusions: The level of plasma mtDNA was closely related with the cardiac function and illness severity in patients receiving cardiac surgery with CPB.

Background

Cardiopulmonary bypass (CPB) is an essential part of open-heart surgery. However, CPB-related inflammatory reactions may cause damage to the corresponding target organ function, which significantly affects the prognosis of patients[(1)]. Mitochondrial DNA (mtDNA), an inflammatory factor, is currently the research focus in the field of inflammatory response[(2)]. However, few studies have focused on the relationship of plasma level of mtDNA and organ function after CPB surgery. Meanwhile, there is still a lack of relevant clinical studies involving large sample cases. This study intends to analyze the difference between plasma level of mtDNA in patients with cardiac dysfunction or not in ICU after CPB by large sample clinical cases. Subsequently, we aim to explore the relationship of plasma level of mtDNA and cardiac function after CPB.

Methods

Patients

From September 1st, 2015 to December 1st, 2016, a total of 962 patients who received cardiac surgery with CPB in the cardiovascular surgery department of West China Medical of Sichuan University were prospectively comprised in this study. Patients under 18 years of age and off-pump cardiac surgery were set as exclusion criteria. Informed written consent were provided to all patients. All kinds of participants information were collected in the whole process of study, including basic information, laboratory data and clinical outcomes. The onset of low cardiac output syndrome is determined by at least two senior doctors responsible for ICU according to the established criteria as below: (i) CI<2.0L/min/m^2; (ii)Using high dose of vasoactive drugs; (iii) IAPB is required; (iv) ECMO is needed and (v) Ventricular assist devices are required. This study was conducted following the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee.

Blood samples collection

Blood samples were collected in two ethylenediaminetetraacetic acid-coated blood collection tubes within half an hour after surgeries. One sample was used for NT-proBNP assay, and results were reported by the hospital division of clinical hematology. The second sample was centrifuged at 4℃ (1000 rpm/min, 15 mins), and the supernatant was collected as plasma without touching the pellet or the bottom of the tube. Samples of plasma were stored in -80℃ freezer for DNA isolation and enzyme-linked immunosorbent assay (ELISA).

DNA isolation and RT-qPCR for mtDNA

Whole plasma DNA was isolated from plasma using DNeasy Blood and Tissue Kit (#69504; Qiagen, N.V., Hilden, Germany). Briefly, 50μl plasma samples were added to 50μl phosphate-buffered saline and centrifuged at 4℃ (16000g, 15 minutes). A total of 90μl of supernatant was collected. The subsequent procedures were carefully performed according to kit manufacturer's protocol. At the final step, 200μl elution buffer was added to resolve DNA. The level of mtDNA was measured with SYBR (Thermo Fisher Scientific, Waltham, MA, USA) green dye-based real-time poly-merase chain reaction (RT-qPCR) assay using ABI PRISM 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA). Primer sequence was human NADH dehydrogenase 1 gene(mtDNA): forward 5'-CGAGCAGTAGCCCAAACAAT-3' and reverse 5'-TGTGATAAGGGTGGAGAGGTT-3'. Plasmid DNA with complementary DNA sequence for human mtDNA was obtained from OriGene Technologies (SC101172; Rockville, MD, USA). Concentrations of plasma mtDNA were converted to copy number via DNA copy number calculator (http://cels.uri.edu/gsc/cndna. html; University of Rhode Island Genomics and Sequencing Center, Kingston, RI, USA). Plasmid DNA were diluted in 10-fold serial dilutions and measured as standard curve. All the samples were measured with standards at the same time. Plasma mtDNA level was recorded in copies per microliter of plasma according to the following formula: c=Q * V DNA / V PCR * 1 / V ext C represents the concentration of plasma mtDNA (copies/μl); Q means quantity of DNA measured by RT-PCR; V DNA is total volume of plasma DNA solution obtained from extraction, 200μl in our study. V PCR means volume of plasma DNA solution for RT-qPCR, 1μl in present study, and V ext is volume of plasma used for extraction, 50μl in this study.

Statistical analysis

The SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA) was employed in this study for statistical analysis. Continuous variables are expressed as mean (SDs). An independent sample t-test was used for validating the statistical significance of RT-qPCR results. Equivalent normal deviate was used to deal with the quantitative data that did not obey normal distribution. The rank-sum test was used for data that still did not obey normal distribution after transformation. Normality of distribution of continuous variables was assessed using Kolmogorov-Smirnov test. Comparisons between groups were carried out using Student's t-test according to normality of distribution. Multiple comparisons were analyzed with two-way analysis of variance followed by Bonferroni's test. Pearson's correlation coefficient test (two-tailed) was conducted. A $P<0.05$ was considered to indicate a statistically significant difference.

Results

Baseline information

962 patients were comprised in this study, with 459 male patients and 503 female patients, and the mean age was 51.6±12.5 years. Patients' basic information and the surgery types were presented in Table 1. All the surgeries were supported with CPB.

Table 1. Baseline information

Variable	Range	Mean (SD)
Age (years)	18–75.8	51.6±12.5
BMI (kg/m^2)	14.8–34.9	22.9±3.29
Gender(male/female)	459/503	
Time of MV(h)	2–232.5	30.27±36.99
ICU LOS(d)	1–12	2.88±1.79
Surgery type	**Number**	
MVR	198	
AVR	224	
DVR	261	
TVR	9	
CABG	83	
CABG+MVR/AVR	58	
Great artery surgery	79	
ASD	15	
VSD	20	
Others	15	
Total	962	

BMI: Body mass index, **LOS:** length of stay, **MV:** mechanical ventilation,

MVR: mitral valve replacement, **AVR:** mitral valve replacement, **DVR:** double valve replacement, **TVR:** tricuspid valve replacement, **CABG:** Coronary artery bypass grafting, **ASD:** Atrial septal defect, **VSD:** Ventricular septal defect.

Correlations between plasma level of mtDNA and cardiac function

T test was used to compare the difference of plasma mtDNA level in two groups of patients with cardiac dysfunction or not. The difference of plasma mtDNA level in two groups is not statistically significant. (t=-1.42，P＝0.1565). (Figure 1)

Correlations between NT-proBNP level and cardiac function

T test was used to compare the difference of NTproBNP level in two groups of patients with cardiac dysfunction or not. The difference in the natural logarithm of NT-proBNP level between two groups is statistically significant (t=-2.69, P=0.0074), and the NT-proBNP level in patients with cardiac dysfunction is higher than those without cardiac dysfunction. (**Figure 2**)

Correlations between plasma level of mtDNA and NT-proBNP after CPB

Bivariate correlation analysis was used to detect the correlation between plasma mtDNA and NT-proBNP after CPB. It was worth noting that positive correlation was identified (r=0.1291, P=0.0386). These interesting data showed that mtDNA levels were closely related to cardiac function in patients after CPB at the same time point. (**Figure 3**)

Correlations between plasma level of mtDNA and APACHE II Score after CPB

Bivariate correlation analysis was used to detect the correlation between plasma mtDNA and APACHE II Score after CPB. Interestingly, positive correlation was identified (r=0.2133, P<0.0001). These data showed that the level of mtDNA was closely related to the severity of illness of the post-operative patients. (**Figure 4**)

Discussion

Multiple factors, including basic cardiac function status and CPB-related inflammatory response, could contribute to cardiac dysfunction after CPB (1, 3). During the process of CPB, vital organ injuries, such as the myocardial ischemia-reperfusion injury, may occur due to the inflammatory cascade which was triggered by the overwhelming pro-inflammatory cytokines through different mechanisms (1).

The role of mtRNA as a specific inflammatory factor has emerged as the new research hot point in recent years (2). As sharing structural similarities with bacterial DNA, especially the unmethylated CpG sequence, mtDNA serves as damage-asociated molecular patterns (DAMPs) which can be recognized by the innate immune system when it was released into the cytosol and extracellular environment, triggering innate immune response, and promoting inflammation (4). According to previous studies, the significant increased level of circulating mtDNA can be observed in conditions like trauma or acute myocardial infraction (5-7). Meanwhile, the increased level of mtDNA in patients with system inflammatory reaction syndrome (SIRS) will further deteriorate the illness, leading to worse clinical outcomes (8, 9). However, the relationship between circuiting mtDNA and the cardiac function after CPB remains unclear, and clinical studies with large sample size of patients after CPB are also limited.

In this study, the cardiac dysfunction-related clinical outcomes are listed as below: (i) cardiac index (CI) <2.0L/min/m^2; (ii) Patients with hemodynamic instability and require large dose of vasoactive agents; (iii) Intra-aortic balloon pump (IABP) is required; (iv) Extracorporeal membrane oxygenation (ECMO) is required; (v) Ventricular assist device is required. Based on these criteria, we found the level of plasma mtDNA in patients with cardiac dysfunction is similar with that in patients without cardiac dysfunction.

Moreover, we found the correlation of the same time-point collected plasma mtDNA and NT-proBNP. NT-proBNP is a common indicator for cardiac dysfunction. As it is characterized with long half-life and good stability and can be detected in both serum and plasma, it is wildly referenced in clinical practice. The dynamic change of the plasma level of NT-proBNP is correlated with the clinical prognosis of patients with acute decompensated heart failure when they are discharged (10). Meanwhile, it is the independent risk factor for cardiovascular events (11). This study also found the correlation between the plasm level of mtDNA and NT-proBNP after CPB. That is another positive result.

It really matters how to explain these conflicting conclusions as both of them can reflect the cardiac function. Actually, clinical used criteria for cardiac dysfunction can only be positive when the patients with significant hemodynamic instability, missing out a part of patients with stable hemodynamics but suffer from cardiac dysfunction. While the result of NT-proBNP is much more valuable as it serves as a more sensitive biological index to reflect the status of cardiac function. We acknowledge the limitation of our study is the lack of the correlation analysis for plasma level of mtDNA and NT-proBNP at a series of time points. The dynamic monitoring and trend analysis for NT-proBNP will be much more valuable for clinical practice. The correlation of plasma level of mtDNA and NT-proBNP at one single time point can only reflect the cardiac function at that time point. While the clinical prognosis is the result of comprehensive effect of multiple factors which can affect the patients' cardiac function. To some extent, this may help to explain the reason why we can observe the correlation of the plasma of mtDNA and NT-proBNP at one single time point, but no correlation exists when we estimate it at a holistic level. Further efforts are still needed to explore the change of the plasma level of mtDNA and NT-proBNP and their correlation with clinical outcomes.

It's interesting to find that there is a fine linear correlation relation (r=0.2133, P<0.0001) between the plasma level of mtDNA and APACHE II scores after CPB. APACHE II scoring system, which is focus on the field of acute physiological status and chronic health condition, is one of the most wildly used scoring system to evaluate the illness severity and prognosis of the patients admitted to ICU (12, 13). Knaus et al (14). created the first APACHE scoring model at 1981, and subsequently provided APACHE II scoring system at 1985 to further evaluate the illness severity and prognosis of patients admitted to ICU (15). The whole scoring system is consisted by three parts: (i) acute physiological score; (ii) age score and (iii) chronic health condition score. The total score is the sum of the three parts and the theoretical total score is 71. The higher of the total score, the severer of the illness. Even though recent study revealed the significant correlation of the serum resistin level and the APACHE ii score in patients with celiac sepsis (16), few studies explored the correlation between inflammatory factors and the APACHE II score. The present study provides novel evidence for the correlation of mtDNA and APACHE II score, indicating the hidden link of the inflammatory response and illness severity.

Study limitations

Although present study found some genuinely interesting results, it is still a preliminary study, and well-designed clinical investigations are needed to confirm the association between mtDNA and heart function

status in ICU patients after cardiac surgery, especially at multiple time points. Correlation between dynamic changes of mtDNA and heart function after CPB should be included in future studies to explore effects of mtDNA. In addition, our study provided the novel associations between plasma mtDNA and NT-proBNP after CPB surgery through bivariate correlation analysis. However, they could not be proven under clinical conditions, which calls for further animal work to study the detailed mechanism between plasma mtDNA and NT-proBNP.

Conclusions

Plasma mtDNA level was positively correlated with NT-proBNP level and APACHE II score in patients receiving cardiac surgery with CPB, indicating that mtDNA was closely related with the cardiac function in these patients, and may reflect the illness severity.

Abbreviations

mtDNA: mitochondrial DNA; CPB: cardiopulmonary bypass; ICU: intensive care unit; CABG: coronary artery bypass grafting; ELISA: enzyme-linked immunosorbent assay; BMI: body mass index; LOS: length of stay; MV: mechanical ventilation; MVR: mitral valve replacement; AVR: mitral valve replacement; DVR: double valves replacement; TVR: tricuspid valve replacement; ASD: atrial septal defect; VSD: ventricular septal defect; DAMPs: damage-associated molecular patterns; SIRS: system inflammatory reaction syndrome; IABP: intra-aortic balloon pump; ECMO: extracorporeal membrane oxygenation

Declarations

Ethics approval and consent to participate: Written informed consent was obtained from individual or guardian participants.

Consent for publication: All the authors read and approved the final manuscript.

Availability of data and materials: The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests”

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Authors' contributions: Jingxiu Fan collected the data, conceived and wrote the manuscript. The rest of the authors reviewed the manuscript.

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References

1. Yaghoubi A, Danaee S, Imani S, Sheikhalizadeh M, Ghojazadeh M. Effect of citrate phosphate dextrose solution on reperfusion injury in coronary artery bypass surgical patients undergoing cardiopulmonary bypass. Journal of cardiovascular and thoracic research. 2011;3:123-7.
2. Boyapati RK, Tamborska A, Dorward DA, Ho GT. Advances in the understanding of mitochondrial DNA as a pathogenic factor in inflammatory diseases. F1000Research. 2017;6:169.
3. Ohki S, Oshima K, Takeyoshi I, Matsumoto K, Morishita Y. Endotoxin removal with a polymyxin B-immobilized hemoperfusion cartridge improves cardiopulmonary function after cardiopulmonary bypass. The Journal of surgical research. 2008;145:74-9.
4. Boyapati RK, Rossi AG, Satsangi J, Ho GT. Gut mucosal DAMPs in IBD: from mechanisms to therapeutic implications. Mucosal immunology. 2016;9:567-82.
5. Bliksoen M, Mariero LH, Ohm IK, Haugen F, Yndestad A, Solheim S, et al. Increased circulating mitochondrial DNA after myocardial infarction. Int J Cardiol. 2012;158:132-4.
6. Wang L, Xie L, Zhang Q, Cai X, Tang Y, Wang L, et al. Plasma nuclear and mitochondrial DNA levels in acute myocardial infarction patients. Coronary artery disease. 2015;26:296-300.
7. Qin C, Gu J, Liu R, Xu F, Qian H, He Q, et al. Release of mitochondrial DNA correlates with peak inflammatory cytokines in patients with acute myocardial infarction. Anatolian journal of cardiology. 2017;17:224-8.
8. Lam NY, Rainer TH, Chiu RW, Joynt GM, Lo YM. Plasma mitochondrial DNA concentrations after trauma. Clinical chemistry. 2004;50:213-6.
9. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature. 2010;464:104-7.
10. Di Somma S, Magrini L, Mazzone M, De Leva R, Tabacco F, Marino R, et al. Decrease in NTproBNP plasma levels indicates clinical improvement of acute decompensated heart failure. The American journal of emergency medicine. 2007;25:335-9.
11. Bidadkosh A, Lambooy SPH, Heerspink HJ, Pena MJ, Henning RH, Buikema H, et al. Predictive Properties of Biomarkers GDF-15, NTproBNP, and hs-TnT for Morbidity and Mortality in Patients With Type 2 Diabetes With Nephropathy. Diabetes care. 2017;40:784-92.
12. Markgraf R, Deutschinoff G, Pientka L, Scholten T. Comparison of acute physiology and chronic health evaluations II and III and simplified acute physiology score II: a prospective cohort study evaluating these methods to predict outcome in a German interdisciplinary intensive care unit. Critical care medicine. 2000;28:26-33.
13. VijayGanapathy S, Karthikeyan VS, Sreenivas J, Mallya A, Keshavamurthy R. Validation of APACHE II scoring system at 24 hours after admission as a prognostic tool in urosepsis: A prospective observational study. Investigative and clinical urology. 2017;58:453-9.
14. Knaus WA, Zimmerman JE, Wagner DP, Draper EA, Lawrence DE. APACHE-acute physiology and chronic health evaluation: a physiologically based classification system. Critical care medicine. 1981;9:591-7.

15. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Critical care medicine. 1985;13:818-29.
16. Yilmaz TU, Kerem M, Demirtas CY, Pasaoglu O, Tascilar O, Sakrak O, et al. Increased Resistin Levels in Intra-abdominal Sepsis: Correlation with proinflammatory cytokines and Acute Physiology and Chronic Health Evaluation (APACHE) II scores. Sultan Qaboos University medical journal. 2014;14:e506-12.

Figures

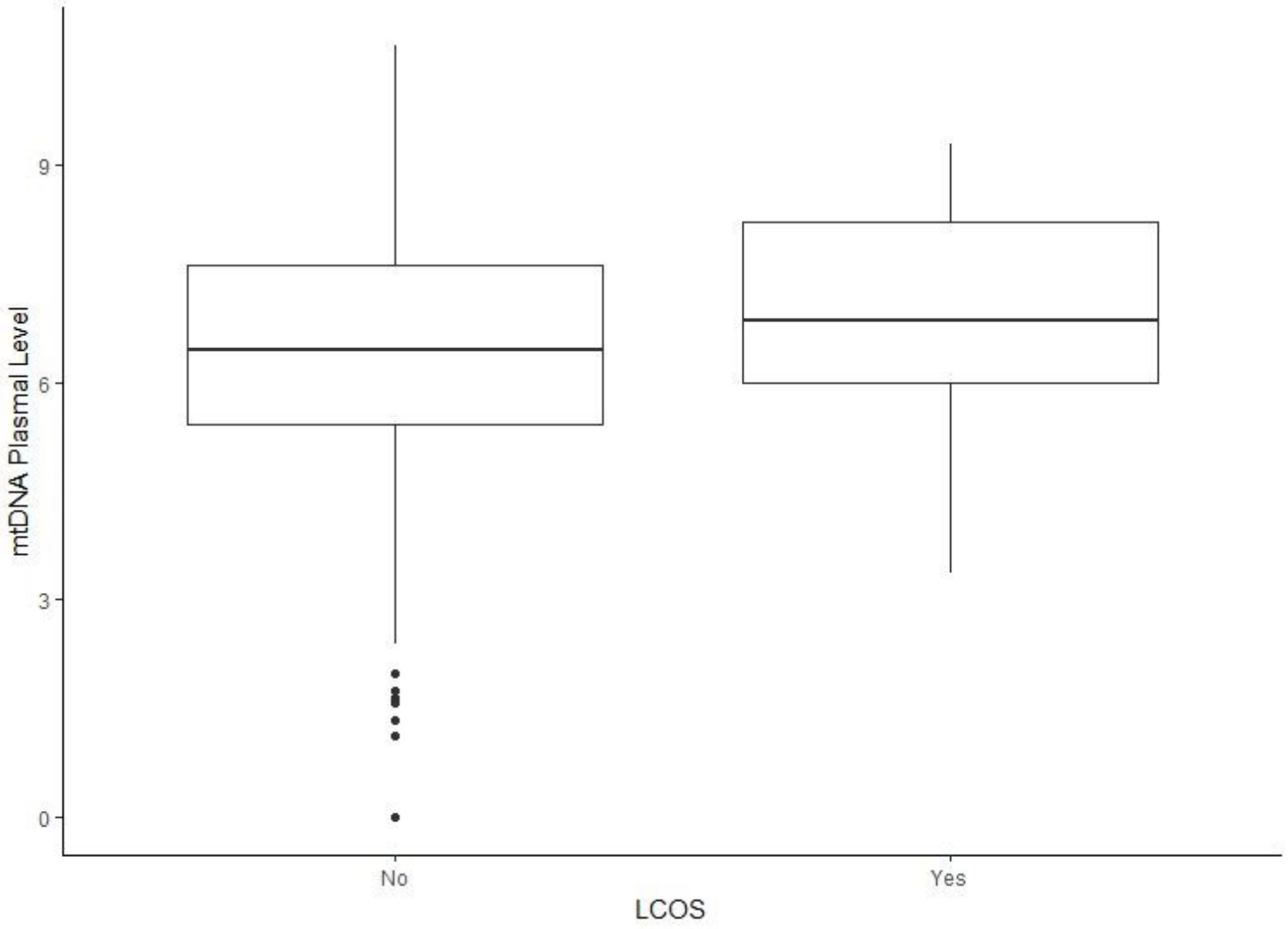

Figure 1

Comparison of mtDNA plasma levels in patients with LCOS and patients without LCOS after CPB. LCOS: low cardiac output syndrome, P□0.1565.

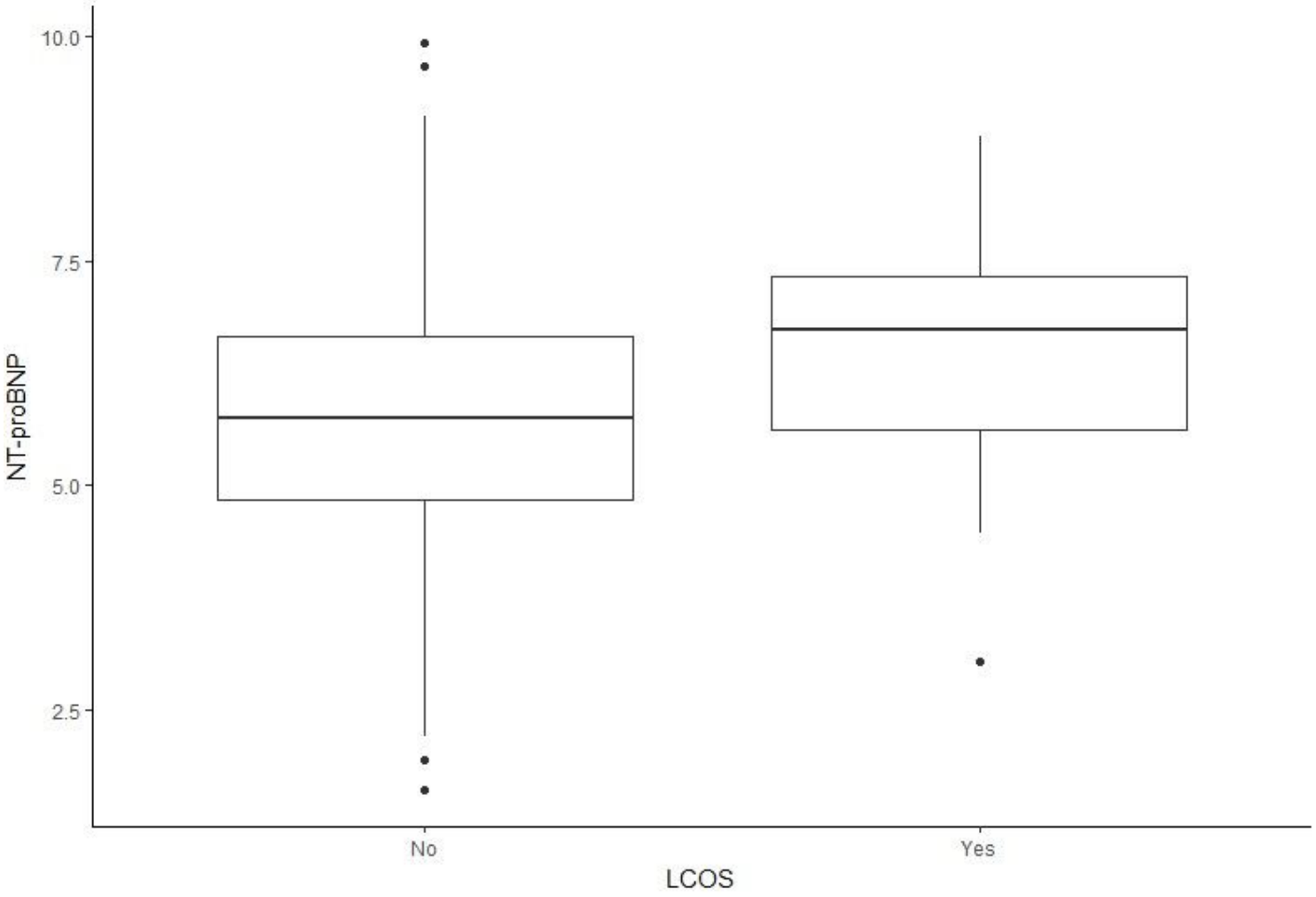

Figure 2

Comparison of NT-proBNP levels in patients with LCOS and patients without LCOS after CPB. NT-proBNP: N terminal pro BNP, P□0.0074.

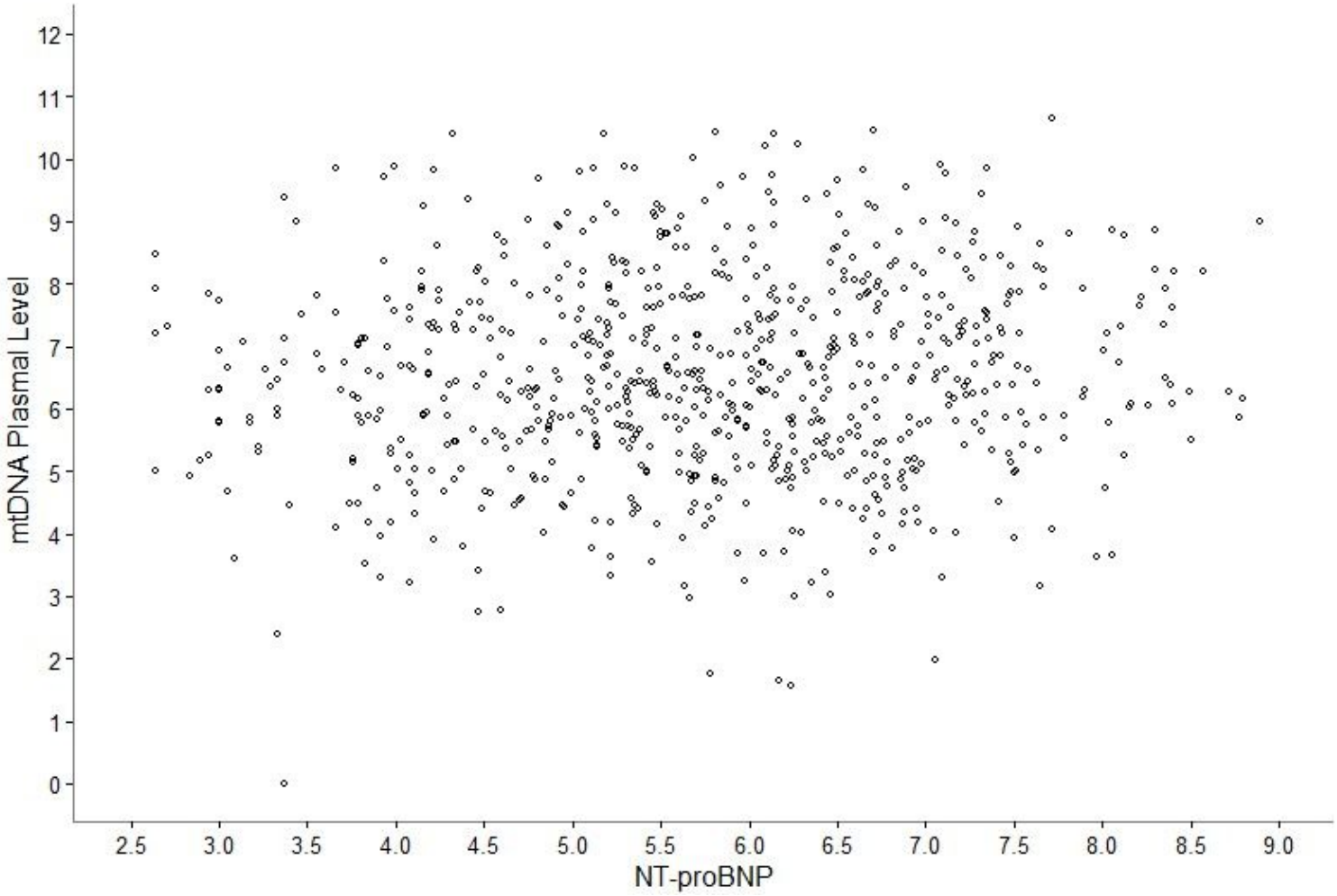

Figure 3

Scatter of mtDNA and NT-proBNP after CPB. r=0.1291, P=0.0386

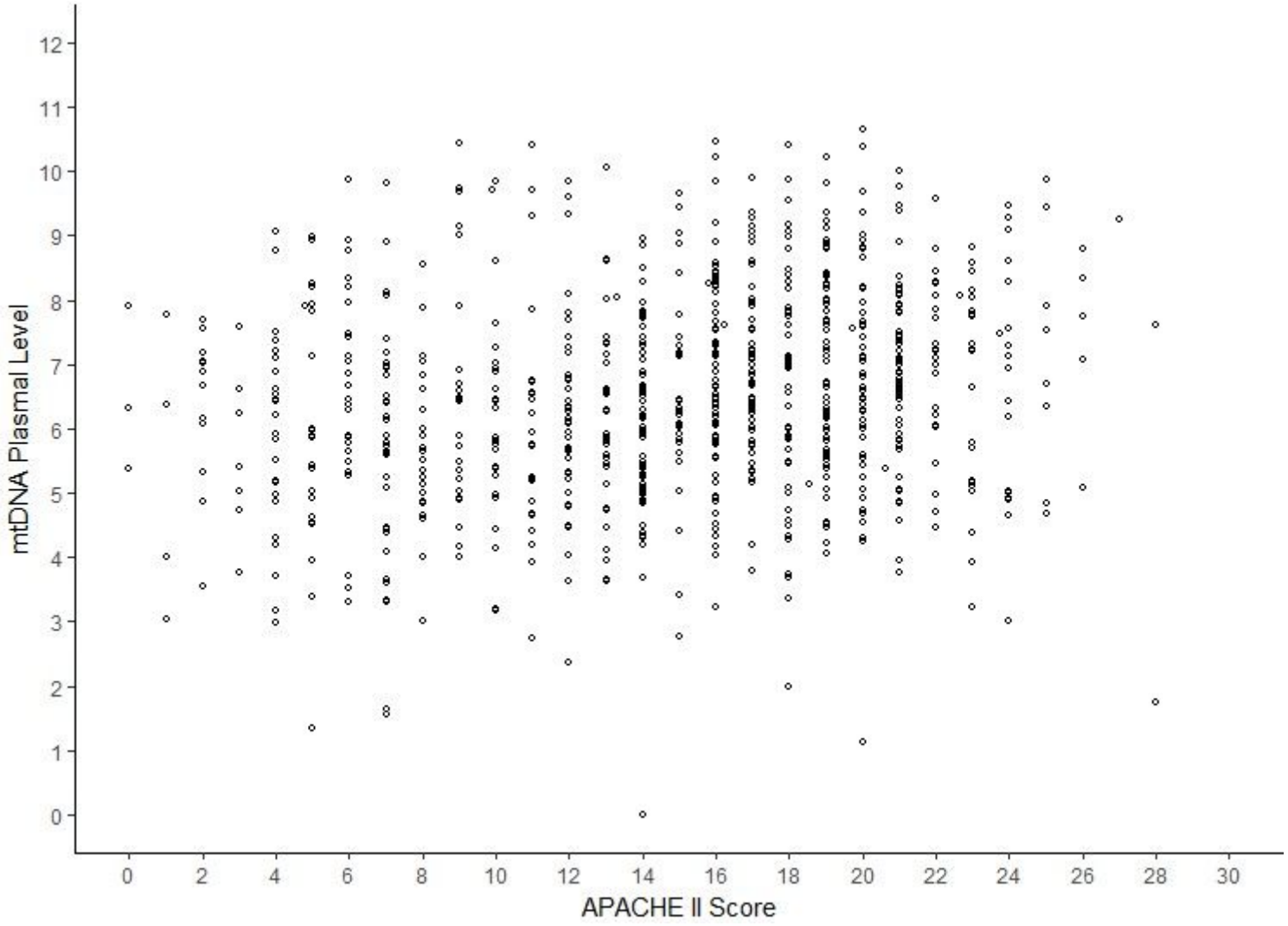

Figure 4

Scatter of mtDNA and APACHE II score after CPB. P<0.0001