Plasma mitochondrial DNA level as an early marker of systemic inflammatory response syndrome and organ failure in patients undergoing cardiac surgery

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Aim To assess whether plasma mitochondrial DNA (mtDNA) levels in the early postoperative period can predict the development of systemic inflammatory response syndrome and organ failure in patients undergoing cardiac surgery.

Methods This pilot, prospective, observational, cohort study included 85 patients undergoing cardiac surgery. Plasma mtDNA levels were determined immediately after the surgery, and the development of systemic inflammatory response syndrome, acute kidney injury, acute heart failure, and adult respiratory distress syndrome was assessed.

Results The mtDNA levels showed good potential for predicting the development of systemic inflammatory response syndrome within 1-2 days after the surgery (area under the curve = 0.74). Regarding the cut-off point, a mtDNA level of >0.54 ng/mL predicted the development of systemic inflammatory response syndrome in the early postoperative period with a sensitivity of 73.7% and a specificity of 66%. The odds ratio for the development of acute kidney injury with/without systemic inflammatory response syndrome was 3.4 [confidence interval (CI) = 1.27–9.08; p = 0.0149]; acute heart failure, 5.7 [CI = 2.20–14.84; p = 0.0003]; and adult respiratory distress syndrome, 3.6 [CI = 1.01–11.10; p = 0.047].

Conclusion The plasma mtDNA levels in the early postoperative period can be used as a predictive marker for the development of systemic inflammatory response syndrome and multiple organ failure in patients undergoing cardiac surgery. Moreover, systemic inflammatory response syndrome is associated with the development of acute kidney injury, acute heart failure, and adult respiratory distress syndrome.

Keywords mitochondrial DNA; acute kidney injury; postoperative complication; systemic inflammatory response syndrome

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Introduction

Every year about 7 million invasive surgical cardiac interventions are undertaken worldwide [1]. Despite the high efficacy and safety of general anaesthesia methods, the perioperative morbidity in cardiac surgery is rather high: according to F. Ji et al. [2], it can amount to 30%, and based on data of H. Cheng [3], it can even exceed 60%. The mortality rate associated with the development of perioperative complications in coronary artery bypass grafting is 2%–3% [4].

It is evident that prognostication and early diagnostics of perioperative complications can drastically ease the process of their prevention and treatment or, at least, alleviate the manifestation of the produced lesions. For this purpose, the expert community recommends assessment of troponin and NT-proBNP levels in the early post-operative period [5, 6]. However, these tests are effective only in prognostication of complications of the cardiovascular system and not in the risk assessment of systemic inflammatory response or acute kidney injury (AKI).

Theoretically, such a marker can become a substance whose emergence in the patients after the cardiac surgery initiates a sequence of events that can lead to multiple organ failure. Damage-associated molecular patterns and mitochondrial deoxyribonucleic acid (mtDNA) are considered as the most probable biologically active substances [7]. The increase in mtDNA concentration is seen in a number of pathological conditions: sepsis [8], pulmonary embolism [9], cancer [10] and atherosclerosis [11]. The increase in mtDNA concentration in the blood of trauma patients is a prognostic factor that correlates with trauma severity and lethality [12–16].

The hypothesis of the study: mtDNA is a marker of the development and activity of systemic inflammatory response.

The aim of the study: to examine the significance of determination of mtDNA content in the blood plasma of cardiac surgery patients in the early post-operative period for prognostication of the development of systemic inflammatory response and multiple organ failure.

Methods

General characteristics of the patients and methods of the study

A pilot prospective observational cohort study was undertaken to examine the association between mtDNA levels in the blood plasma and the incidence of systemic inflammatory response, AKI, acute respiratory distress syndrome and acute cardiac failure in cardiac surgery patients. The study was approved by the Ethics Committee of M.F. Vladimirskiy Moscow Regional Research And Clinical Institute (protocol No. 4, 19.01.2017).

A signed informed consent was obtained from 103 patients admitted to the Department of Cardiac Surgery Resuscitation of the SBHIMR, M.F. Vladimirskiy MRRCI to participate in the study; however, according to the criteria stated below, only 85 patients were included into the final protocol (Fig. 1).

Inclusion criteria:

1. Age: 45–65 years;
2. Voluntary informed consent;
3. Types of operative interventions: Cardiac valve replacement, on-pump coronary artery bypass grafting and off-pump coronary artery bypass grafting.

Exclusion criteria:

1. Morbid obesity with body mass index of more than 35 kg/m^2;
2. Infarction or stroke in the previous 6 months;
3. Infectious disease 1 month before the surgery;
4. Renal insufficiency in the anamnesis (baseline blood creatinine or urea above reference values);
5. Left ventricle ejection fraction less than 30%.

Withdrawal criteria:

Repeated surgery or percutaneous coronary intervention in 30 days after the first operation.

The patients were divided into three groups: on-pump cardiac valve replacement, off-pump coronary artery bypass grafting and on-pump coronary artery bypass grafting.

Preparation and isolation of the deoxyribonucleic acid from blood plasma

In the perioperative period, approximately 3 mL of peripheral venous blood was drawn from the patients. The blood was mixed with ethylenediaminetetraacetic acid (1.5 mg/mL) and was centrifuged for 10 min at 3 000 g. The collected plasma was additionally centrifuged for 10 min at 10 000 g. The upper layer of the plasma was collected into a

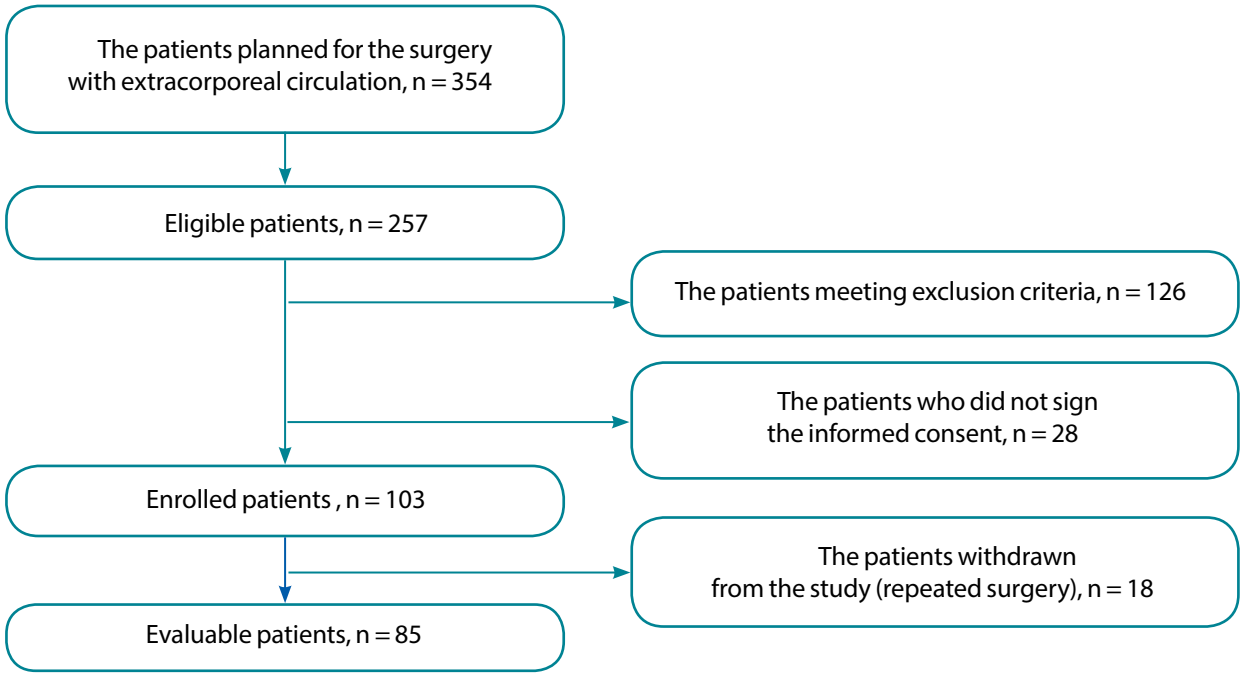

Fig. 1. Scheme of patients' selection

0.5 mL microcentrifuge tube and frozen at –20°C before DNA isolation. To control the efficiency of isolation in each 0.4 ml plasma sample, 10 µl (1.7 ng/µl) of the exogenous control DNA based on the 8 kb plasmid pBlueScriptSKII (-) is added before the isolation procedure.

DNA isolation from 100 µL of blood plasma was performed with the kit Quick-gDNA Blood MiniPrep (Zymo Research, USA) according to the manufacturer's protocol. Real-time quantitative polymerase chain reaction was conducted in the thermal cycler iCycler (Bio-Rad, USA) using the following mixture: 5 µL of the analysed specimen; 10 µL of the mixture B Eva Green (Syntol, Russia); 0.5 µL of the mixture of each specific primer (10 µM) and 9.5 µL of deionized water. Each DNA specimen was used as a matrix in three identical polymerase chain reactions. Relative mtDNA content was quantified by real-time polymerase chain reaction [16].

Blood mtDNA concentration was determined at the following stages:

1. mtDNA1 (baseline)—blood mtDNA concentration after anaesthesia induction;
2. mtDNA2—blood mtDNA concentration after the end of the surgery;
3. mtDNA3—blood mtDNA concentration in 12 h after the surgery;
4. mtDNA4—blood mtDNA concentration in 72 h after the surgery.

Diagnostics of complications of interest

Constitutional inflammatory response is determined as the presence of two and more criteria, as proposed by The American College of Chest Physicians/The Society of Critical Care Medicine: temperature higher than 38°C or lower than 36°C; cardiac rate more than 90 beats per minute, respiratory rate more than 20 breaths per minute or pCO_2 less than 32 mm Hg; leucocytosis more than 12 000 or leucopenia less than 4 000 [17].

AKI according to the RIFLE criteria [18] is determined by increase in the serum creatinine content by 1.5 times and more, or reduction of glomerular filtration rate by 25% and more or reduction of urine output less than 0.5 mL/kg/h within 6 h and more.

Acute cardiac failure is determined as the need for infusion of inotropic agents (adrenaline, dobutamine) in a dose equivalent to 5 µg/kg/min of dobutamine or more over a period of not less than 6 h after the surgery.

Scale of vasopressor and inotropic support

The total dose of inotropic agents and vasopressors was calculated by the following formula: dobutamine (μg/kg/min) + dopamine (μg/kg/min) + 100 × adrenaline (μg/kg/min) [19]. Acute respiratory distress syndrome was determined as the presence of focal alterations in the pulmonary tissue that developed within a week after surgical intervention, was not associated with fluid retention and also according to the extent of oxygenation index decline (PaO_2/FiO_2) [20].

Statistical analysis

Lilliefors and Shapiro–Wilk tests were used for the preliminary analysis of qualitative parameters for normality of distribution. Mann–Whitney U-criterion was applied to compare quantitative values that do not follow a normal distribution.

Logistic regression and ROC analyses were applied in order to evaluate the quality of the predictors of surgical clinical outcomes with non-normal distribution. In logistic regression, chi-square statistics was used (the difference between the likelihood functions of the zero and the full models, indicating how significantly the independent model variables influence the dependent variable). In the ROC analysis, the area under the curve (AUC) parameter was assessed, and the cut-off point was determined by the ratio of criteria sensitivity (Se) and specificity (Sp): the requirement for a balance between sensiivity and specificity:

$$Se \sim Sp: Cut\ off = \min |Se - Sp|,$$

where Cut off—cut-off value,
Se—sensitivity, Sp—specificity.

To analyse the dynamics of the indices with non-normal distribution, Friedman rank analysis of variance with a posteriori analysis with application of non-parametric Wilcoxon test was used. The average values of normally distributed quantitative parameters were presented by arithmetic mean with standard deviation ($M \pm \sigma$), and non-normally distributed quantitative parameters were presented by interquartile interval median (Me [25%–75%]).

The differences were considered as statistically significant at $p < 0.05$. Statistica 10.0 (StatSoft, Inc.) and MedCalc 12.5.0.0 (MedCalc Software bvba) were used for the calculation. The data (the number of positive and negative outcomes in each group (DNA concentration higher or lower than the selected criterion)) were entered into the program MedCalc that calculates odds ratios and 95% confidence intervals.

Results

General characteristics of the patients enrolled in the study.

Patient population, n	85
Age, years	61 ± 9
Ejection fraction, %	58 ± 10
Gender, male/female, n	60/25
Cardiac valve replacement, n	24
Off-pump coronary artery bypass grafting, n	29
On-pump coronary artery bypass grafting, n	32

The baseline mtDNA in the pooled patient population was 0.21 [0.15–0.31] ng/mL. By the end of the cardiac surgery, the studied parameter increased by 3.5 times, $p < 0.01$. In 12 h after the surgery, the growth continued and the mtDNA concentration exceeded baseline by 4.3 times ($p < 0.01$). By the end of the third day after the surgery, there was decline in the concentration of the studied parameter; however, mtDNA exceeded the baseline by almost two times, $p < 0.01$. The change in the mtDNA concentration with time supports the assumption that the massive cellular cytolysis, as a result of major and traumatic surgery, leads to the release of the substance of interest. The change in the blood mtDNA concentration with time during the perioperative period in the pooled cohort of the patients is presented below.

Mitochondrial deoxyribonucleic acid concentration in the blood	Median
1 ng/mL	0.21 [0.15–0.31]
2 ng/mL	0.73 [0.33–0.88]
3 ng/mL	0.91 [0.57–1.00]
4 ng/mL	0.40 [0.23–0.55]

Baseline mtDNA concentration did not have any statistical differences when compared in the subgroups of patients that received cardiac valve replacement and coronary artery bypass grafting, whether it was by on-pump or off-pump (Fig. 2).

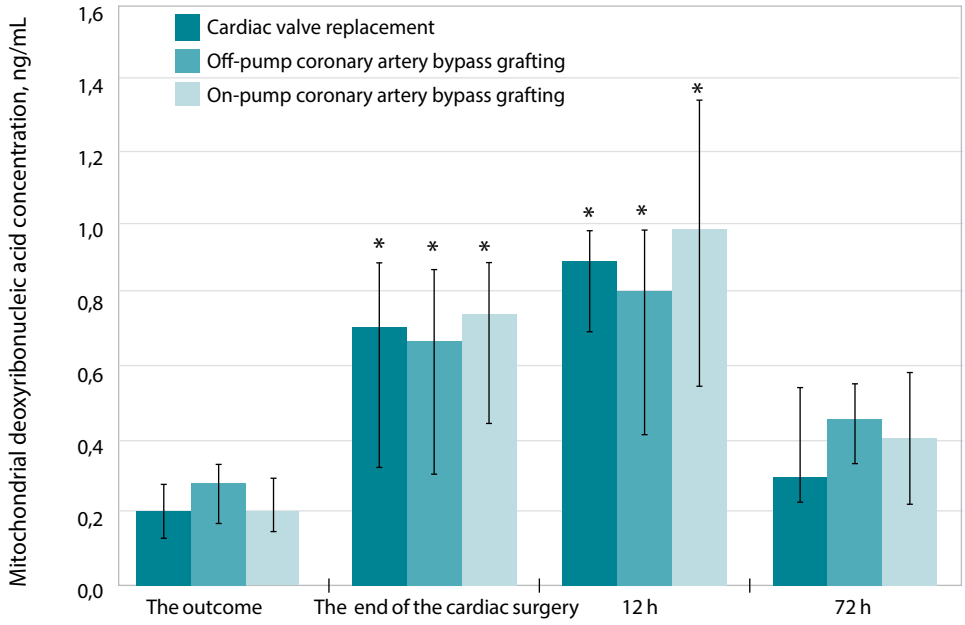

Fig. 2. Mitochondrial deoxyribonucleic acid concentration in the blood of patients at different stages of the study

I—interquartile interval; * significant difference regarding the outcome, $p < 0.05$

Further, in all the subgroups, mtDNA content over time was similar to that described for the total population of patients. A total absence of statistically significant differences between the groups at all specified study stages (Fig. 2) allowed us to provide further analysis without regard to the subgroup wherein each of the patients was included.

The systemic inflammatory response in the early post-operative period developed in 55% of the patients after cardiac valve replacement, in 38% of the patients after off-pump coronary artery bypass grafting and in 45% of the patients after on-pump coronary artery bypass grafting. The difference was statistically insignificant, $p > 0.1$. The average incidence of systemic inflammatory response in the cohort was 46%.

The mtDNA, measured after the end of the surgery, showed the potential of systemic inflammatory response prognostication in 1–2 days of the post-operative period ((AUC) = 0.74). For determining the cut-off point, it was found that the mtDNA concentration of more than 0.54 ng/mL determined the systemic inflammatory response in the early post-operative period with the sensitivity and specificity of 73.7% and 66%, respectively.

The mtDNA, measured 12 h after the surgery, demonstrated prognostic level (AUC = 0.86) regarding systemic inflammatory response risk in 1–2 days of the post-operative period. For determining the cut-off point, it was found that the mtDNA concentration of more than 0.98 ng/mL proved the development of systemic inflammatory response in the early post-operative period with the sensitivity and specificity of 67.6% and 94%, respectively (Fig. 3).

As expected, the sensitivity and specificity of the test with mtDNA determination for prognostication of systemic inflammatory response development increased in the third stage of the study. However, it was essential to determine that the analysis also, immediately determined after the end of the surgery, demonstrated the prognostication potential of the method.

The AKI developed in 37% of patients after cardiac valve replacement, in 21% of the patients after off-pump coronary artery bypass grafting and in 25% of the patients after on-pump coronary artery bypass grafting. The difference was statistically insignificant, $p > 0.1$. The average incidence of AKI in the cohort was 27%.

Based on when AKI developed, the patients were distributed as follows: immediately after the end of the

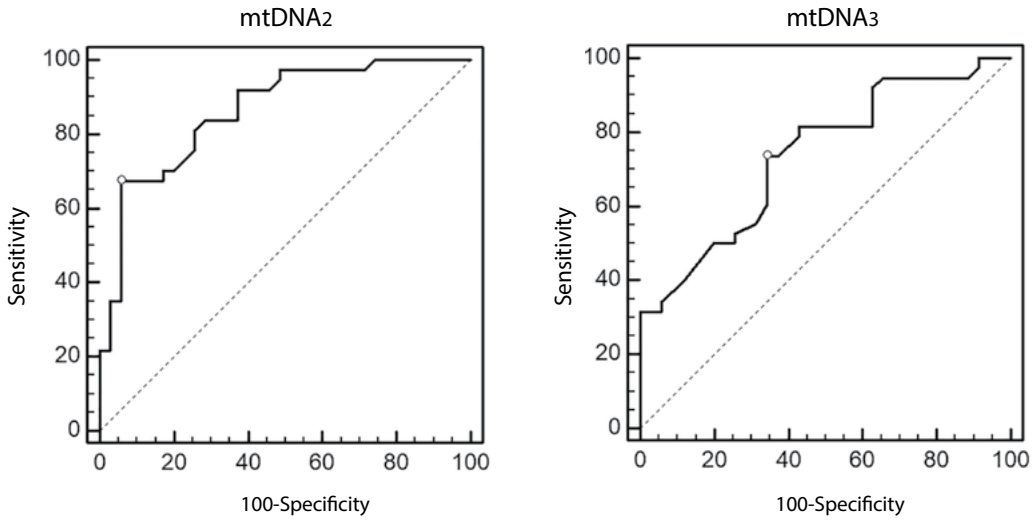

Fig. 3. ROC-curves for prognostication of a risk of the systemic inflammatory response depending on the mitochondrial deoxyribonucleic acid concentration in the blood

surgery in 2 (3%) patients; by the end of the first day in 8 (12%) patients; on the second day in8 (12%) patients and, finally, on the third day in 3 (4%) patients.

Odds ratio of the development of AKI in the patients with and without systemic inflammatory response was 3.4 [1.27–9.08], p = 0.0149. Therefore, the hypothesis about the association of systemic inflammatory response and AKI was confirmed.

Cardiac failure was registered in 58% of the patients after cardiac valve replacement, in 21% of the patients after off-pump coronary artery bypass grafting and in 43% of the patients after on-pump coronary artery bypass grafting, with p = 0.08. The average incidence of the cardiac failure in the cohort was 40%. Odds ratio of the development of the cardiac failure for the patients with and without systemic inflammatory response was 5.7 [2.20–14.84], p = 0.0003.

The acute respiratory distress syndrome was registered in 15% of the patients after cardiac valve replacement, in 16% of the patients after off-pump coronary artery bypass grafting and in 21% of the patients after on-pump coronary artery bypass grafting, with p > 0.05. The average incidence of acute respiratory distress syndrome in the cohort was 17%. Odds ratio of the development of the acute respiratory distress syndrome for the patients with and without systemic inflammatory response was 3.6 [1.01–11.10], p = 0.047.

Discussion

It was demonstrated for the first time that the determination of mtDNA concentration in the blood plasma specimens of cardiac surgery patients, immediately collected after the surgery, is a rather precise and reliable predictor of the systemic inflammatory response, acute cardiac failure, kidney injury and acute respiratory distress syndrome.

It was determined that mtDNA concentration immediately increased after the end of the surgery, reached peak concentration in 12 h after the intervention and declined by the end of day 4 of the post-operative period.

C. Qin et al. [21] had previously shown similar results. They found interrelation between the concentrations of mtDNA and inflammatory cytokines, such as tumor necrosis factor, interleukin-6 and interleukin 8, in cardiac surgery patients.

In our study, the mtDNA concentration was determined at every stage by the operative intervention itself and not by the type of operative intervention. Moreover, knowledge of whether extracorporeal circulation was used during the surgery was of little significance. Thus, our study findings show why significant differences in clinical outcomes are not observed after on-pump and off-pump coronary artery bypass grafting [22].

The test with mtDNA determination demonstrated the ability to prognosticate the development of systemic inflammatory response. By the ratio of sensitivity/specificity, the method is not inferior to the procalcitonin test [23]; however, it appears to be superior with respect to time characteristics. As far as it is known, the ability of procalcitonin to prognosticate systemic inflammatory response with the specimen, immediately collected after the surgery, has not been described [24].

Systemic inflammatory response plays a key role in AKI development after cardiac surgeries [25, 26]. This hypothesis was also confirmed during our study. Moreover, we demonstrated that systemic inflammatory response plays a key role also in the process of the development of acute cardiac failure, AKI and acute respiratory distress syndrome in the early period after cardiac surgery interventions.

International guidelines require assessment of troponin and NT-proBNP concentrations in the early post-operative period for prognostication of the potential cardiovascular complications [5, 6]. According to the guidelines, increase in the NT-proBNP concentration, determined in the blood of the patients immediately after the surgeries, increases the risk of acute cardiac failure by 3.5 times. According to our results, the test with mtDNA determination is more significant for prognostic purposes than NT-proBNP and troponin tests.

Earlier, it was demonstrated that the increase in mtDNA concentration in the blood of patients with trauma could be a prognostic marker, correlating with trauma severity and lethality [12–16]. Similar studies regarding the perioperative period of elective surgical interventions were not performed.

Limitations

This research was performed in the form of a pilot study with a limited number of patients. Presumably, this was the reason why it was not possible to determine the intergroup differences in mtDNA content in the blood of the patients after coronary artery bypass grafting and cardiac valve replacement.

In addition, the conclusion of advantage in time characteristics over the test with procalcitonin determination is hypothetical; the inability to conduct the quantitative procalcitonin analysis did not allow us to make a comparison in the frames of current study.

Conclusion

The interrelation between the mtDNA concentration, immediately collected after the surgery, and the incidence of systemic inflammatory response development was determined. Increased mtDNA concentration is an early predictor of acute cardiac and kidney failure and acute respiratory distress syndrome in the early post-operative period in cardiac surgery patients.

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Conflict of interest

Authors declare no conflict of interest.

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