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Cell-Free DNA in Emergency Medical Care

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Abstract Defining molecules with high prognostic value for predicting the course and outcomes of life-threatening sepsis, severe injuries, vascular accidents remains an urgent problem in emergency medicine. One of the promising candidate biomarkers of emergency states and critical illness is the content of extracellular DNA (exDNA) in blood plasma. The purpose of this review is to identify the prospects for the introduction of cfDNA in clinical medicine and the severities arose along this way. The levels and altered dynamics of the concentration of circulating DNA fragments, including the organ-specific fraction of exDNA seem informative today for assessing the degree of damage to the organ of interest, the probability of a complicated course and the prognosis of outcomes of emergency/critical illness in Intensive Care Unit (ICU) patients. Sources of exDNA circulating in the bloodstream may include the nuclei of dying cells from organs and tissues, damaged mitochondria, the pool of which should be remodeled with mitophagy, as well as microorganisms. Similarly to pathogen-associated molecules (PAMP) represented by fragments of bacterial and viral DNA, native DNA molecules associated with damage (DAMP) bind to toll-like receptors (TLR9) and intracellular DNA sensors (cGAS-STING, NLRP3), initiating the inflammatory processes in tissues and hemostatic disorders. These processes represent natural adaptive responses protecting against microbes, as well as disadaptation responses potentiating cell damage in organs. The increasing expression of genes encoding proinflammatory signaling pathways associated with NF-kB transcription factor and interferon-regulating factors (IRF), in turn, contribute to production of cytokines and other factors enhancing the stress-responses that alter the functional activity of cells in various organs. The available literature data suggest that the quantitative determining plasma exDNA, which serves as PAMP and DAMP to significantly contribute to pathogenesis of emergency states and critical illness, might aid in predicting the outcome and justifying the in-time personalization of treatment of emergency and post-emergency patients.

Keywords: cell-free DNA, critical illness, inflammation, impaired hemostasis

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BBB — blood-brain barrier
BP — base pairs
cAMP — cyclic adenosine monophosphate
CC – critical condition
cGAMP — cyclic guanosine monophosphate – adenosine monophosphate
cGAS — cyclic guanosine monophosphate – adenosine monophosphate synthetase
cGMP — cyclic guanosine monophosphate
CpG — dinucleotides
CVD — cardiovascular diseases
DAMPs — damage-associated molecular patterns
DNA — deoxyribonucleic acid
exDNA— extracellular DNA
ER — endoplasmic reticulum
ICU — intensive care unit
IF — interferon
IL — interleukin
IRF — interferon regulating factor
MI — myocardial infarction
mtDNA — mitochondrial DNA

mtDNA oxi — oxidized mitochondrial DNA
NLRP3 — Nod-like receptor of NALP family
PAMPs — pathogen-associated molecular pattern
ROS – reactive oxygen species
STING — stimulator of interferon genes
TLR9 — toll-like receptor 9
8-oxodG — 8-Hydroxydeoxyguanosine

INTRODUCTION

Despite technical progress and modernization of medical equipment, success in antibiotic therapy and the use of other modern means of treatment, mortality in critical conditions (CC) such as severe injuries, strokes, myocardial infarction, acute respiratory distress syndrome and others remains high [1-3]. The incidence of infectious complications of CC, including sepsis and pneumonia, is still high, and mortality as a result of their development reaches 30-50% [4]. Studies show that a significant proportion of patients with sepsis are characterized by various forms of neurological deficit, impaired immune system and reduced life expectancy [5, 6]. To improve the outcomes of critical conditions, a search is underway for biomarkers that would allow a patient to be in the intensive care unit (ICU) in the early stages of a patient's stay in the intensive care unit (ICU) to choose the optimal therapy methods, personalizing treatment if possible [7–11]. One of the promising biomarkers in this regard is extracellular DNA (exDNA) circulating in the blood [12-16].

The term "cfDNA" includes the entire spectrum of circulating DNA fragments. The molecules included in the exDNA pool differ in: (1) sources, (2) mechanisms of formation, (3) length of fragments, (4) forms of circulation, and (5) modifications (changes in the chemical structure of exDNA).

SOURCES OF CELL-FREE DNA

Until recently, it remained unknown which cells form the cfDNA pool. The identification of its sources became possible due to the study of the cfDNA methylation profile. Methylation is a post-replication (epigenetic) change in DNA with the aim of blocking or activating gene transcription [17]. This is accomplished by attaching a methyl group at position 5 of cytosine to the carbon atom in regions rich in cytosine-guanosine sequences (CpG sites). Methylation plays a key role in the processes of cell differentiation, determining the specificity of the latter for each type of cell and creating a specific picture of methylome (a set of cytosine-methylated DNA regions in the genome) [18]. It is the specific profile of DNA methylation that basically determines the set of transcribed, expressed genes (that is, the profile of the transcriptome) in cells of a given histological type or line of differentiation. Due to its availability, circulating DNA is an attractive target for analysis. Studies of cfDNA methylation in human blood are under way to determine the main tissue (cellular) sources of its origin [19–20]. The technique consists in sequencing cfDNA fragments to analyze the methylation profile of gene regions in comparison with tissue-specific methylome. According to a pilot study, in healthy people, the sources of cfDNA in plasma are leukocytes (only 55%, of which 32% are granulocytes, 12% lymphocytes, 11% monocytes), erythrocyte precursors (30%), endotheliocytes (9%), hepatocytes (1 %), neurons (1%) and others (4%) [20]. The authors found that in critical conditions such as sepsis, it is leukocytes (granulocytes) that in most cases also make the greatest contribution to the cfDNA pool (over 90%). In severe organ dysfunctions, the amount of cfDNA cells in these organs increases. An unexpected pattern was found: in severe liver damage, the amount of cfDNA of neuronal origin increases.

Determination of the content of cfDNA molecules in the blood plasma of patients with a methylation pattern characteristic of cells of a certain histiotype has a clear potential for characterizing the location and assessing the severity of organ damage. It is possible that such analyzes of circulating DNA methylome signatures in the near future will become the molecular basis for the development of early organ failure tests that are more informative for assessing the critical condition and predicting its course than SOFA indicators or other clinical tests based on monitoring clinical signs of organ dysfunction or systems. So far, only quantitative indicators of cfDNA are used as a promising biomarker of pathological conditions in the human body [12-16].

MECHANISMS OF CELL-FREE DNA FORMATION

There are several points of view regarding the origin of cfDNA. The main ones are the formation of a cfDNA pool as a result of cell death (the theory of “cell death by apoptosis and / or necrosis”), active secretion by cells (the theory of “metabolic DNA”) [21–25] and netosis (protrusion of large DNA fragments through the membrane of neutrophils with the formation of a "cloud" of DNA around the leukocyte, followed by the cleavage of DNA fragments and their release into the circulating blood as a result of exposure to nucleases) [26]. The length of the generated cfDNA fragments apparently depends on the mechanism of cfDNA formation [27]. In severe trauma accompanied by intense necrotic processes, DNA is released, which is practically not exposed to the action of nucleases, and thus is a large molecular compound [28, 29]. The appearance of such fragments over 10,000 base pairs (bp) is confirmed by electrophoresis of DNA isolated from blood [27, 28]. Simultaneously with large-molecular DNA fragments, shorter fractions characteristic of apoptotic cell death are also found. Apoptosis is accompanied by an active process of nuclease hydrolysis, which leads to the accumulation of DNA fragments about 150–180 bp in length in blood, which corresponds to internucleosomal regions [30]. However, it was shown that there are also intermediate lengths of DNA fragments from 200 to 10,000 bp, which is probably associated with the action of macrophages [27]. It has been shown that apoptotic and necrotic cells are captured by macrophages, which digest cells that have died and die as a result of necrosis and apoptosis; intracellularly, their DNA is cleaved into fragments with a lower molecular weight characteristic of apoptosis [31]. Under an excessive load on macrophages, their depletion, death and the release of fragments of both their own DNA and partially hydrolyzed DNA of phagocytosed cells into the blood can occur [32].

FORM OF CELL-FREE DNA CIRCULATION

Extracellular DNA in circulation can be free, in apoptotic bodies and in exosomes [33]. Exosomes are of particular interest in studying the role of cfDNA. They have the form of vesicles with a diameter of 0.03-0.1 μm, which are formed from the endoplasmic reticulum and intracellularly exist in the form of endosomes. Their composition may vary greatly depending on the type of cells and their condition, causing a variety of functions. The main function of endosomes is the delivery of substances from one cell to another [34–36]. For the purpose of transcellular transport, exosomes include penetration, invasion, and fusion proteins (CD81, CD63, and CD9) [37]. It is known that exosomes also carry nucleic acids, including DNA fragments, while normally circulating cfDNA is predominantly (up to 93%) in the form associated with exosomes [33].

CHANGES IN THE CHEMICAL STRUCTURE OF CELL-FREE DNA

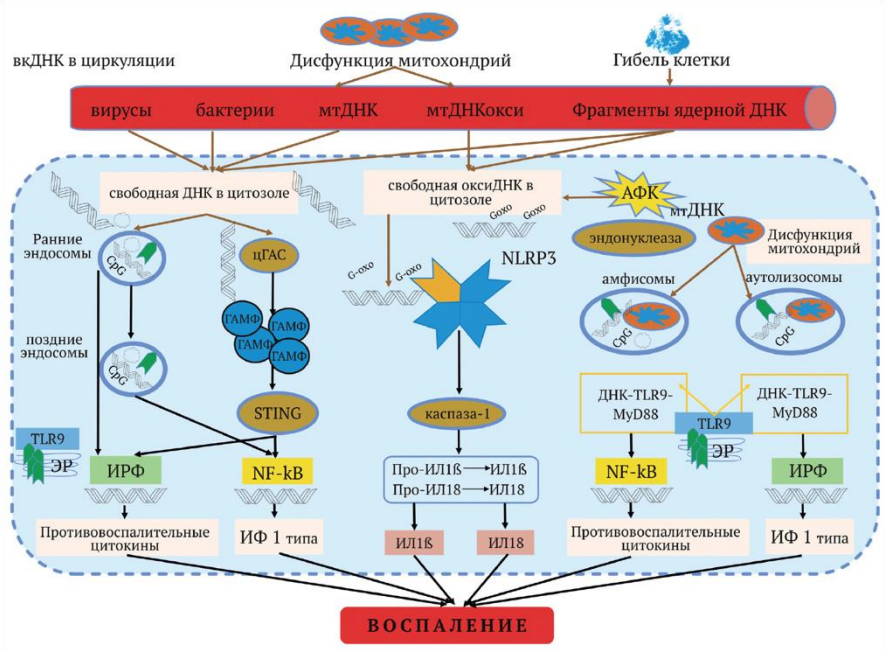
Structural changes in cfDNA can be important as a source of additional information both for prognosis and for the current state of the patient. Modifications of the DNA structure - methylation and oxidation - have been studied. It is also known that circulating cfDNA fragments can be of nuclear or mitochondrial origin. At the same time, methylation is characteristic only of nuclear DNA, and it is the latter, as noted, that has the potential to identify destructive processes in organs and assess their severity by the characteristics (signatures) of circulating DNA methylome [18–20]. Mitochondrial DNA remains predominantly unmethylated [38–40].

Other changes are common for both types of DNA. Both nuclear and mitochondrial DNA are susceptible to oxidative damage resulting in the formation of various oxidized nucleosides. One of the most widespread and studied structural damages of cfDNA is the formation of 8-oxo-2'-deoxyguanosine (8-oxodG), which quantitative content of circulation is detected either as individual molecules or as part of cfDNA using anti-8-oxodG antibodies or high pressure liquid chromatography associated with mass spectrometry [41–43]. It is believed that cfDNA in the oxidized form acquires special biological properties that promote the activation of inflammatory processes [42, 43]. A number of substances can lead to an increase in the production of reactive oxygen species and an increase in DNA oxidation. These include alcohol, heavy and transition metals, organic solvents, pesticides, drugs, for example, paracetamol, the inhalation anesthetic fluorotan [44, 45].

DNA changes arising under the influence of reactive oxygen species (ROS), naturally produced by mitochondria and products of their metabolism during the life of the cell and increased by oxidative stress, include oxidation of nitrogenous bases, ribose / deoxyribose, apurinization or apyrimidization, single-strand and double-strand DNA breaks [46, 47]. Some of them are being investigated as biomarkers of pathological conditions; however, information on the information content of such markers is still rare [48, 49]. It can be assumed that studying the characteristics of cfDNA modification will provide new valuable information about the patient's condition, help timely locate and assess organ damage, increase the prognostic value of cfDNA molecules, and clarify their importance in the pathogenesis of critical conditions.

Cf DNA AS ALINK IN THE CHAIN OF MOLECULAR MECHANISMS OF CRITICAL CONDITIONS DEVELOPMENT

Fragments of cfDNA, playing the role of signaling molecules, can be links in the pathogenesis of critical states, interacting with DNA sensors: TLR9 receptor and molecular complexes NLRP3 and STING [12] (scheme).



Scheme. Activation of the inflammation system by fragments of free (extra-nuclear) DNA molecules in the cytosol. Brown arrows indicate the movement of DNA fragments; black arrows show signaling paths; yellow arrows indicate TLR9 receptor movement; cfDNA, cell-free DNA; mtDNA, mitochondrial DNA; ox-mtDNA, oxidized mitochondrial DNA; IL, interleukin; IRF, interferon regulatory factor; cGAS, cyclic guanine monophosphate adenine monophosphate synthetase; GAMP, cyclic guanine monophosphate-adenine monophosphate; NLRP3, Nod-like receptor of the NALP family; ROS, reactive oxygen species; TLR9, toll-like receptor 9; IF, interferon; ER, endoplasmic reticulum; STING, interferon gene stimulator

TLR9 — BASIC DNA SENSOR

The cells of the innate immune system, using specific receptors, recognize pathogen-associated molecular patterns (PAMPs), which are formed during the vital activity of microorganisms or released upon their death. This leads both to the activation of cells and to the triggering of molecular mechanisms aimed at the death of the infectious agent. Similar processes are initiated in the cells of the immune system and under the influence of structurally different molecules of non-infectious type, which are formed when the integrity of eukaryotic cells or their structures is disrupted. Such molecules are called damage-associated molecular patterns (DAMPs). Mitochondria are the significant source of such structures, DAMPs , in case of organ dysfunctions. These include N-formyl peptides, TFAM, lipids, cardiolipin, succinates, ATP, and mitochondrial DNA fragments [50]. Such molecules are able, similarly to PAMPs, to bind to receptors from the toll-like receptors (TLR) family and initiate an immune response. The abundance of mitochondrial molecules that stimulate cells of the immune system and their functional similarity with PAMP molecules are possibly due to the bacterial origin of mitochondria [51].

The main function of mitochondria is the synthesis of a significant amount of energetically capacious ATP molecules to provide the metabolic processes of the cell necessary in a given time interval and in a given microenvironment. Additionally, these organelles are involved in the regulation of programmed cell death, calcium metabolism, and the formation and control of the production of reactive oxygen species [38, 52, 53]. In other words, mitochondria help maintain homeostasis throughout the cell.

Therefore, it is not surprising that mitochondria also have a signal function. They release signals for the surrounding cells about the potential danger of damage spreading into the cytosol and intercellular space in case of their own damage. Such signals are not so much informative, but rather instructive in nature, promoting the activation of mechanisms aimed at eliminating structural and functional defects of cells and restoring homeostasis [54]. How is this done?

It is known that DNA molecules are able to bind to DNA receptors, TLR9 molecules, regions containing numerous unmethylated CpG motifs [55]. Mitochondrial DNA (mtDNA), as well as bacterial and viral DNA, unlike nuclear DNA, is unmethylated or hypomethylated [39, 40, 56–58]. Thus, mtDNA should act as the main intrinsic ligand for TLR9 receptors. L. Zhang et al. (2016) decided to test whether the effects of mtDNA administration in mice differ from nuclear DNA [50]. The authors showed that nuclear DNA had practically no proinflammatory effect, while mtDNA promoted the development of inflammation and acute lung injury [50]. Differences in the proinflammatory effects of methylated and unmethylated DNA fragments could be not in the selectivity of binding to the TLR9 receptor, but in the absence of the ability of methylated fragments to interact with the receptor [55]. The authors showed that the uptake of both molecules into endosomes proceeds in the same way; however, only unmethylated cfDNA fragments are capable of efficient transfer of the TLR9 receptor from the endoplasmic reticulum to late endosomes.

NLRP3 INFLAMMASOME MASTER INFLAMMATION REGULATOR

Inflammasome is a complex of proteins involved in the process of apoptosis and inflammation [59]. It is activated in brain injury, neuroinfection, stroke, and neurodegeneration [50–54, 60–62]. In a recently published work, the authors conducted a series of experiments to study the role of the NLRP3-inflammasome under the action of cfDNA both in vitro and in vivo [43]. Mice (wild type and knockout for a number of genes) and bone marrow macrophages of mice (also wild type and with defective genes) were selected as an animal model. Based on the results, it is possible to build a signal pathway in which fragments of oxidized mitochondrial DNA play a key role (scheme).

1. Activation of receptors TLR2, TLR3 and TLR4 leads to the activation of interferon-regulating factor 1 (IRF1).

2. IGF1, being a transcription factor, activates the expression of the CMPK2 protein (cytosine monophosphate kinase 2). It is the only enzyme from the group that catalyzes phosphorylation of nucleotide mono- and diphosphates to nucleotide triphosphates, a substrate for mtDNA synthesis.

3. With the participation of the TFAM protein, the synthesis of mtDNA by gamma polymerase occurs. Further, with the participation of the TFAM protein, mitochondrial ROS production (mtROS) increases, leading to the oxidation of mtDNA (mtDNAoxy). Further, under the action of endonuclease, mtDNAoxy is fragmented and leaves the mitochondria into the cytosol.

4. mtDNAoxy binds to NLRP3 inflammasomes.

5. The complex of mtDNAoxy and NLRP3 inflammasome activates caspase 1.

6. Caspase 1 hydrolyzes pro-IL1β to IL1β, triggering an inflammatory response.

It has been shown that oxidized fragments of nuclear or artificially synthesized DNA are also capable of activating the NLRP3 protein of the inflammasome.

SIGNAL PATH OF *CGAS-STING*

Intrinsic or outside (bacterial or viral) free DNA fragments in the cytosol are able to bind to another DNA sensor — cGAS (cyclic adenosine monophosphate - cyclic guanosine monophosphate (cAMP-cGMP) synthetase) [63]. After interaction, the enzyme changes its conformation and is activated, which leads to the synthesis of cyclic cGAS, the ligand of the endoplasmic reticulum protein STING (a protein that stimulates the expression of interferon genes) through interferon regulatory factor 3 (IRF3) and NF-kB. IRF3 and NF-kB, by binding to certain regions of nuclear DNA, stimulate the transcription of interferon genes of the 1st type of proinflammatory interleukins, respectively (see diagram).

CELL-FREE DNA IN CRITICAL AND EMERGENCY CONDITIONS

Extracellular DNA is a biomarker of cellular damage in various conditions, in which a multiple increase in its concentration in plasma can occur relative to healthy donors. Such an increase is observed in critical conditions such as severe trauma, sepsis, vascular accidents, as well as physiological and borderline conditions, including physical exertion and acute and chronic psychoemotional stress [49, 64–70].

CARDIOVASCULAR DISEASE (CVD)

According to the WHO, cardiovascular diseases rank first in terms of mortality in the world. They claim 17.7 million lives annually, accounting for 31% of all deaths. The most common causes of death in patients with CVD are coronary artery disease (7.4 million) and ischemic stroke (6.7 million).

ISCHEMIC STROKE

Ischemic stroke is an acute cessation or significant decrease in blood circulation to the brain, accompanied by a sudden dysfunction of the brain [69]. The reasons may be the formation of a blood clot in the artery, embolism and severe hemodynamic disturbances [72-73]. The affected area develops gradually. The nucleus of the lesion (necrosis) focus is quickly formed, which corresponds to the blood supply zone of the damaged artery. Gradually, the affected area expands with the formation of "penumbra". Cell damage products are released into the intercellular space. Further, in order to enter the bloodstream, substances must pass through the difficult-to-penetrate blood-brain barrier (BBB). The BBB consists of endothelial cells, pericytes, astrocytes, neurons, and extracellular matrix. Endothelial cells adhere tightly to each other, having a minimal capacity for pinocytosis, and promote the selective transport of substances into the microenvironment of brain cells [74–75]. During ischemia, the BBB cells are exposed to hypoxia, lactic acidosis, hypoglycemia, which triggers a cascade of processes leading to the destruction of connections between the components of the neurovascular unit due to endothelial cell dysfunction and increased BBB permeability [75–78]. Violation of the integrity of the BBB leads to an increased transport of substances and cells both to the substance of the brain and from it into the blood. Substances entering the circulation are studied as biomarkers of the severity and prognosis of the outcomes of ischemic stroke, but their sensitivity and specificity remain insufficient [7]. Taking into account the ongoing cell death in the ischemic zone and their release of molecules and DNA fragments into the interstitium, cfDNA can be used as a biomarker of stroke. It has been shown that the cfDNA concentration in ischemic stroke increases statistically significantly on the day of the vascular accident and remains elevated in relatively healthy donors up to 30 days ($p < 0.001$) [64, 65, 67]. Changes in the concentration of circulating DNA fragments make it possible to differentiate with statistical significance ischemic stroke with stroke-like conditions (convulsions, complicated migraines and other conditions causing pathological neurological symptoms) (AUC = 0.857, $p < 0.001$) [66]. The authors point to a statistically significant relationship between the amount of circulating DNA and the amount of brain damage, established in experimental models ($R = 0.78$, $p < 0.0001$) [80].

To improve the prognosis of patients with ischemic stroke, the patency of the occluded artery is restored in order to reduce the zone of necrosis and penumbra [81]. At the same time, long-term (more than 3 months) neurological outcomes are ambiguous and correlate with cfDNA concentration after recanalization [82]. The resumption of blood flow can lead to reperfusion syndrome, which contributes to additional damage to the brain tissue, as well as to the BBB structures [76–79], and this, in turn, can potentiate an increase in the cfDNA content in plasma. Additionally, it was found that reperfusion is associated with a number of contraindications, such as late arrival at the hospital (patients outside the therapeutic window), convulsions at the onset of ischemic stroke, hypertensive crisis, low scores on the NIHSS scale, recent surgical interventions and advanced age [8, 81, 83]. The presence of such criteria (the main of which is the late start of therapy) is associated with the low share of reperfusion in developed countries which is less than 15% [83]. In the absence of restoration of blood flow in the ischemic area, the classic picture of stroke, described earlier, develops. Cells in the zone of the nucleus and penumbra die, releasing DNA fragments into the environment. It is believed that the mechanisms of cell death in stroke are different. Thus, the first zone is characterized by necrosis, while the “shadow” zone is characterized by apoptosis [74]. During necrosis, high molecular weight DNA fragments are formed containing more than 10,000 bp, while during apoptosis much smaller fragments ((150 bp) are formed [28–30]. The accumulation of high molecular weight DNA molecules in the blood can affect the rheological properties of blood and disrupt the processes of thrombolysis [8, 65].

MYOCARDIAL INFARCTION

Myocardial infarction (MI) is a critical condition resulting from acute circulatory disorders in the heart muscle as a result of critical narrowing of the vessel lumen in the coronary artery basin [84]. Currently, the determination of the blood concentration of cardiospecific troponin is used as the gold standard for the diagnosis of MI [85]. However, in some patients, including the intensive care cases, the sensitivity of the troponin test may be reduced. A false positive test is possible during exercise, sepsis, renal failure (due to impaired clearance) and taking cardiotoxic drugs [9, 85–88]. Another disadvantage of the method is the short period of troponin informativeness after a vascular catastrophe, in connection with which it is necessary to search for additional biomarkers of MI [85]. Jin Xie et al. (2018) traced the dynamics of cfDNA concentration in patients with MI over two periods: the first 5 days and then up to 5 months [89]. Healthy donors were selected as the control group, and patients with chronic cardiovascular diseases were the comparison groups. According to the presence of complications, all patients were divided into 2 groups: with complications - angina pectoris, arrhythmia, repeated myocardial infarction (group 1) and without complications (group 2). In the first 5 days, all patients with MI had a 5-10-fold increase in the cfDNA concentration relative to that in healthy donors (comparison group), while in the first group the cfDNA content was statistically significantly higher by 1.8 times ($p < 0.001$).The results of determining the cfDNA concentration over the next 5 months were compared by the authors in groups 1 and 2 with each other and with the values obtained in the control group. In the latter, only insignificant fluctuations in the level of cfDNA were observed over 5 months ($p > 0.05$). In patients after AMI, the dynamics of cfDNA was different. Thus, in group 1, the cfDNA concentration remained elevated relative to its value in the comparison group and higher than in group 2 ($p = 0.001$), in which it decreased in 3

months to the level detected in patients with chronic heart diseases. Thus, the data of this work indicate the possibility of using cfDNA to predict the outcomes of MI with a wider time interval. Hai Zemmour et al. (2018) confirm that AMI is accompanied by an increase in the concentration of total cfDNA in the blood, but the sensitivity of this test compared to troponin was lower [85]. To increase the specificity of cfDNA determination in MI, the authors, using the technology developed by them earlier, estimated the contribution of DNA fragments from dead cardiomyocytes to the total cfDNA pool in the first 56 hours after the onset of AMI [85]. Determination of the cfDNA concentration of cardiomyocytes in AMI had high sensitivity and specificity (AUC = 0.94, $p < 0.0001$) and was similar to the troponin test data ($r = 0.79$, $p < 0.0001$) (statistically significant in both cases). It is likely that the determination of the concentration of not only the total cfDNA in patients after MI, but also the specific DNA of cardiomyocytes in the postinfarction period will provide additional information on the cellular processes occurring in the heart muscle and the body as a whole.

SEPSIS

Sepsis is a life-threatening condition resulting from organ dysfunction due to maladjustment of the body's response to the infectious process (Sepsis-3). With the development of circulatory disorders, cellular or metabolic dysfunction, septic shock occurs, which further increases the risk of death - up to 60% or more [5, 90, 91]. Studies show that a significant proportion of discharged patients with sepsis have a decrease in the quality of life, they suffer from neurological disorders and have an immunosuppressive status [6]. Fragments of cfDNA are currently considered from two sides: (1) as a biomarker and (2) as an element of the pathogenesis of sepsis.

Rannikko et al. (2018) determined the prognostic value of DNA circulating in plasma in sepsis [69]. The study included 469 patients with sepsis. Thus, the level of cfDNA in patients who died by day 7 was statistically significantly higher during this time ($p < 0.001$, $p = 0.017$). The authors revealed a statistically significant association between the cfDNA concentration and mortality by the 7^{th} day (OR = 7.7 (95% CI 3.9-15.3), AUC = 0.73 (95% CI 0.65-0.82, $p < 0.001$) and day 28 (OR = 6.8 (95% CI 3.9-11.8), AUC = 0.721 (0.65-0.79) $p < 0.001$). The results were confirmed in other studies [92–93] The mechanisms of such a correlation have not yet been precisely established, but it is assumed that they represent a reflection of the pathogenetic patterns of the development of sepsis as an infectious life-threatening systemic state.

The reason for the development of sepsis is the entry of microorganisms and their toxins into the bloodstream. Under the influence of foreign substances, an uncontrolled process of inflammation and coagulopathy is triggered. Disorders of blood clotting leads to vascular thrombosis of the microvasculature, multiple organ failure, which is associated with high mortality [94–95]. Another pathogenetic link in sepsis is associated with the action of leukocytes activated by bacterial products, their adhesion to the vascular endothelium, their production of ROS, damaging the endothelial structure and causing microcirculation disorders with the development of perfusion disorders and secondary oxidative stress in cells suffering from oxygen deficiency. To find the place of cfDNA in the pathways of sepsis pathogenesis, Schneck et al. (2017) examined its effect on the hemostatic system [70]. It turned out that with an increase in cfDNA concentration, the clotting time is reduced and the process of fibrinolysis is disrupted. There are data that clarify the molecular mechanisms of cfDNA that lead to coagulopathy in sepsis [96]. Under the action of DNase 1, neutrophilic extracellular traps formed due to DNA protrusion through the membrane of leukocytes are destroyed (netosis), and fragments of cfDNA and histones are released, entering the circulating blood. The latter, in turn, activate thrombin synthesis by platelets. In a subsequent work, the same team of authors investigated the effect of cfDNA on the anticoagulant link in hemostasis [60]. It was found that a high level of cfDNA leads to a decrease in the activity of fibrinolysis. Thus, in an in vitro experiment in the presence of a high concentration of cfDNA (30–40 μg / ml), the duration of thrombus lysis was significantly increased by a factor of 5 ($p < 0.001$). At the same time, according to electron microscopy data, the thrombus, pierced with cfDNA threads, becomes denser. Considering that the size of cfDNA can vary greatly from 150 bp to (with apoptosis) up to more than 10,000 bp. (with netosis and necrosis), the effect of the fragment length on the duration of thrombus lysis was investigated. We found that short fragments (less than 150 bp) do not affect thrombolysis. Only long (more than 10,000 bp) fragments of cfDNA were able to bind to plasmin and fibrin, preventing thrombolysis. Thus, the duration of thrombus lysis mediated by tissue plasminogen activator increased by 2 times ($p = 0.002$), by plasmin - by 1.7 times ($p < 0.001$), and the destruction of the fibrin alpha chain slowed down 3 times ($p < 0.001$). When treating a blood clot with DNase in vitro, the rate of clot destruction was restored. In another study of the therapeutic use of DNase 1 in an in vivo model of abdominal sepsis, it was expected that enzymatic cleavage of excess cfDNA would have a beneficial effect. This turned out to be true with a delayed (by 4–6 hours) introduction of the enzyme [61]. Increasing this interval to 24 hours, however, did not have a positive effect on survival [62]. DNase therapy in the first 2 hours led to an increase in the level of proinflammatory interleukins (IL6 and IL10) in the blood and a greater death of animals. Thus, an increase in the concentration of cfDNA in sepsis, including long fragments and histones released during netosis, has a procoagulant effect, which may contribute to an unfavorable outcome of sepsis. The hydrolysis of cfDNA in such patients most likely has a therapeutic potential, which possibilities yet to be verified in clinical trials.

PROBLEMS OF CF-DNA USE IN CLINICAL PRACTICE

Despite the presence of associations between the concentration of total cfDNA and the outcome of pathological conditions, quantitative determination of cfDNA as a full diagnostic and prognostic biomarker of such conditions has not yet been used in clinical medicine. The available research results suggest that cfDNA is still a candidate biomarker ("candidate biomarker"). This status is largely facilitated by variable data on the "normal" cfDNA concentration. The authors of various publications provide figures characterizing the content of cfDNA in relation to the "norm", which range from several to ~ 1200 ng / ml [13, 14, 49, 64–71, 89, 97]. There are several sources of such variability. (1) The content of cfDNA can increase several times in a healthy person depending on physical or emotional stress, and after a short time (about 60 minutes) return to the initial value [68]. In such cases, the authors methodically approach the recruitment of the control group, minimizing the stressful effect on donors before blood sampling (using a pre-installed venous catheter, physical and emotional rest for 30 minutes, etc.). (2) Currently, there is a variety

(in the absence of standard methods) approaches to the quantitative determination and isolation of cfDNA: quantitative polymerase chain reaction, spectrophotometric or fluometric determination of its concentration, extraction of cfDNA from plasma by the column method or organic solvents [97]. (3) Samples from which DNA is isolated — plasma or serum, the specificity of collection, duration and storage conditions — all contribute to the variability of cfDNA concentration [97]. Therefore, when analyzing the results, the authors use the data of their own control group, which are correlated with the subject. The feasibility of developing and introducing a reasoned standard (s) for cfDNA analysis is currently undeniable.

CONCLUSION

Due to its ability to accumulate in the bloodstream in the event of damage to body cells and to initiate receptor-mediated signaling processes, cfDNA can act as a pathogenetically significant biomarker of critical and emergency conditions in medicine and be a key link in the development of both protective and damaging inflammatory responses in resuscitation patients. Investigation of the characteristics of cfDNA, such as the primary structure (nucleotide sequence), specific methylation profile, dynamics of accumulation in circulation, and the presence of oxidative modifications can make it possible to assess the severity of damage to the organ of interest and to predict the disease. Obtaining new data on the signaling functions of cfDNA molecules, including its structurally modified variants, and the effect of cfDNA on the hemostatic system has significant potential in terms of elucidating the key mechanisms of the development of emergency conditions and developing new approaches to personalized treatment of patients. Verification of the prognostic value of cfDNA in various critical conditions, including life-threatening infectious complications, will make it possible to solve this problem in a timely manner.

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