NONINVASIVE DIAGNOSIS OF HEART REJECTION AS A PREDICTOR OF LONG-TERM TRANSPLANT SURVIVAL

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doi.org/10.35805/BSK2024I003
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Conflict of interest:
The authors declare no conflict of interests

Key words:
Chronic heart failure, Heart Transplantation, Endomyocardial Biopsy, donor-derived cell-free DNA.

Abstract

Background. Currently, in Kazakhstan, the issue of chronic heart failure is becoming increasingly relevant, with high mortality from the terminal stage of chronic heart failure, especially in patients with III-IV functional class. Heart transplantation represents the "gold" standard of surgical treatment for terminal chronic heart failure, but endomyocardial biopsy, used for monitoring the transplanted heart, is an invasive and inconvenient procedure.

Aim of this study is to generate data which can aid in more precise antibody-mediated rejection diagnosis, assisting in distinguishing between antibody-mediated rejectionand acute cellular rejection and helping us determine the appropriate treatment strategy

Materials and Methods. This article explores the potential of safe and accurate monitoring of acute transplant rejection using circulating donor-derived cell-free DNA(dd-cfDNA). The study included 40 patients who underwent heart transplantation.

Results. It was found that 60% of them had a repeat operation, while 40% had a primary one. Various cardiomyopathies, predominantly dilated and ischemic, were the cause of terminal chronic heart failure. The donor-derived cell-free DNA method demonstrates potential in differentiating T-cell-mediated and antibody-mediated rejection, with different patterns donor-derived cell-free DNA elevation. These differences have high clinical significance for diagnosis and treatment tactics.

Conclusion. Despite the prospects of using donor-derived cell-free DNA, further research is needed to establish threshold values and confirm its effectiveness in clinical practice.

Трансплантаттың ұзақ мерзімді өмір сүруінің предикторы ретінде жүректен бас тартудың инвазивті емес диагностикасы

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Мүдделер қақтығысы:
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Түіндеме

Өзектідігі: Бүгінгі таңда Қазақстанда созылмалы жүрек жеткіліксіздігі мәселесі барған сайын өзекті болып отыр. Созылмалы жүрек жеткіліксіздігі терминалдық сатысынан, әсіресе III-IV функционалдық класс пациенттерінде өлім-жітім жоғары.

Түйінді сөздер:
Созылмалы жүрек жеткіліксіздігі, Жүрек трансплантациясы, Эндомиокардиалдбиопсия, айналымдағы бос донорлық ДНК.

Жүрек трансплантациясы терминалды созылмалы жүрек жеткіліксіздігі үшін хирургиялық емдеудің «алтын» стандартын білдіреді, бірақ трансплантацияланған жүректі бақылау үшін қолданылатын эндомиокард биопсиясы инвазивті және ыңғайсыз процедура болып табылады.

Бұл зерттеудің мақсаты антидене арқылы қабылданбау диагностикасын дәлірек анықтауға көмектесетін, антиденелер арқылы қабылданбауды және жедел жасушалық қабылдамауды ажыратуға көмектесетін және тиісті емдеу стратегиясын анықтауға көмектесетін деректерді жасау болып табылады.

Материалдар мен әдістер: Бұл мақалада айналымдағы бос донорлық ДНҚ (dd-cfDNA) көмегімен жедел трансплантациядан бас тартуды қауіпсіз және дәл бақылау мүмкіндігі қарастырылады. Зерттеу нысандары жүрек трансплантациясынан өткен 40 пациент болды.

Нәтижелер. Олардың 60% - қайталама операция болғанын, ал 40% - бастапқы операция болғанын көрсетті. Созылмалы жүрек жеткіліксіздігі себебі әртүрлі, негізінен кеңею және ишемиялық кардиомиопатиялар болды. Айналымдағы бос донорлық DNA әдісі айналымдағы бос донорлық DNA жоғарылауының әртүрлі үлгілерімен Т-жасушалық және антидене арқылы бас тартуды саралау әлеуетін көрсетеді. Бұл айырмашылықтар диагностика мен емдеу тактикасында жоғары клиникалық маңыздылыққа ие.

Қорытынды. Дегенмен, айналымдағы бос донорлық DNA қолдану мүмкіндіктеріне қарамастан, шекті мәндерді белгілеу және оның клиникалық тәжірибеде тиімділігін растау үшін қосымша зерттеулер қажет.

Неинвазивная диагностика отторжения сердца, как предиктор долгосрочной выживаемости трансплантата

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Авторы заявляют об отсутствии конфликта интересов

Ключевые слова:
Хроническая сердечная недостаточность, Трансплантация сердца, Эндомиокардиальная биопсия,циркулирующие свободные ДНК донора.

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Аннотация

Фон. На сегодняшний день в Казахстане проблема хронической сердечной недостаточности становится все более актуальной, с высокой смертностью от терминальной стадии хронической сердечной недостаточности, особенно у пациентов III-IV функциональных классов. Трансплантация сердца представляет собой "золотой" стандарт хирургического лечения терминальной хронической сердечной недостаточности, но эндомиокардиальная биопсия, используемая для мониторинга трансплантированного сердца, является инвазивной и неудобной процедурой.

Целью этого исследования является получение данных, которые могут помочь в более точной диагностике антитело-опосредованного отторжения, помогая различать антитело-опосредованное отторжение и острое клеточное отторжение, а также помогая нам определить соответствующую стратегию лечения.

Материалы и методы. В данной статье исследуется потенциал безопасного и точного мониторинга острого отторжения трансплантата с использованием циркулирующей свободной ДНК донора (dd-cfDNA). Объектами исследования были 40 пациентов, перенесших трансплантацию сердца.

Результаты. Выявили, что у 60% из них операция была повторной, а у 40% - первичной. Причиной терминальной стадии хронической сердечной недостаточно-

сти были различные кардиомиопатии, преимущественно дилатационная и ишемическая. Метод определения циркулирующей свободной ДНК донора демонстрирует потенциал в дифференциации Т-клеточно-опосредованного и антитело-опосредованного отторжения, с различными паттернами повышения циркулирующихсвободных ДНК донора. Эти различия имеют высокую клиническую значимость для диагностики и тактики лечения.

Заключение. Несмотря на перспективы использования циркулирующих свободных ДНК донора, требуются дополнительные исследования для установления пороговых значений и подтверждения его эффективности в клинической практике.

Introduction

As of today, the problem of chronic heart failure is highly relevant in Kazakhstan, with annual mortality from the terminal stage of chronic heart failure significantly higher than in the general population, reaching 12% among patients with heart failure in functional classes III-IV, even under treatment in a specialized hospital. The primary method of treating the terminal stage of heart failure, when optimal medical therapy is ineffective, is surgical treatment - heart transplantation.[1,2]

According to the Republican Center for Coordination of Transplantation and High-Tech Medical Services of the Ministry of Health of Kazakhstan, more than 3 thousand Kazakhstanis are in need of organ transplantation, and 10 patients per 1 million population require heart transplantation. In recent years, not only has the number of transplantations worldwide increased significantly, but also the indicators of quality and duration of life for heart transplant recipients have improved.[1,3]

Heart transplantation is considered the "gold" standard for the surgical treatment of terminal heart failure. According to the International Society for Heart and Lung Transplantation (ISHLT) data from 2019, the overall median survival is 12.5 years, and the conditional survival is 14.8 years for those who survive the first year. Successful heart transplantation improves the quality of life and increases the survival of patients.[4,5]

One of the most serious complications, both in the early and late periods after transplantation, remains acute cellular and humoral antibody-mediated rejection (AMR). The probability of developing rejection of the heart transplant and coronary artery disease persists in patients after heart transplantation throughout their lives, necessitating continuous monitoring and correction of immunosuppressive therapy and early detection of signs of rejection.

Aim of this study is to generate data which can aid in more precise AMR diagnosis, assisting in distinguishing between AMR and acute cellular rejection (ACR) and helping us determine the appropriate treatment strategy.

Material and methods

This is a cross-sectional analysis of a single-center, retrospectiveand prospective, observational clinical study-from 2023 to 2025. The study included 40 patients who had previously undergone orthotopic heart transplantation at the National Scientific Cardiac Surgery Center in the conditions of the Republic of Kazakhstan. The sample size was calculated from the 58 surviving patients after the orthotopic heart transplantation. Accordingly, a sample size of 40 adult patients (confidence level: 95%, margin of error: 5%) was determined. Clinical material samples (venous blood) were collected from participants aged 18 and older who had undergone heart transplantation. Patients who refused to undergo diagnostic procedures specified in the study protocol were excluded from the research. Participants provided questionnaire data, and informed consents for study participation were obtained. Inclusion criteria for study participants were adults after heart or kidney transplantation:

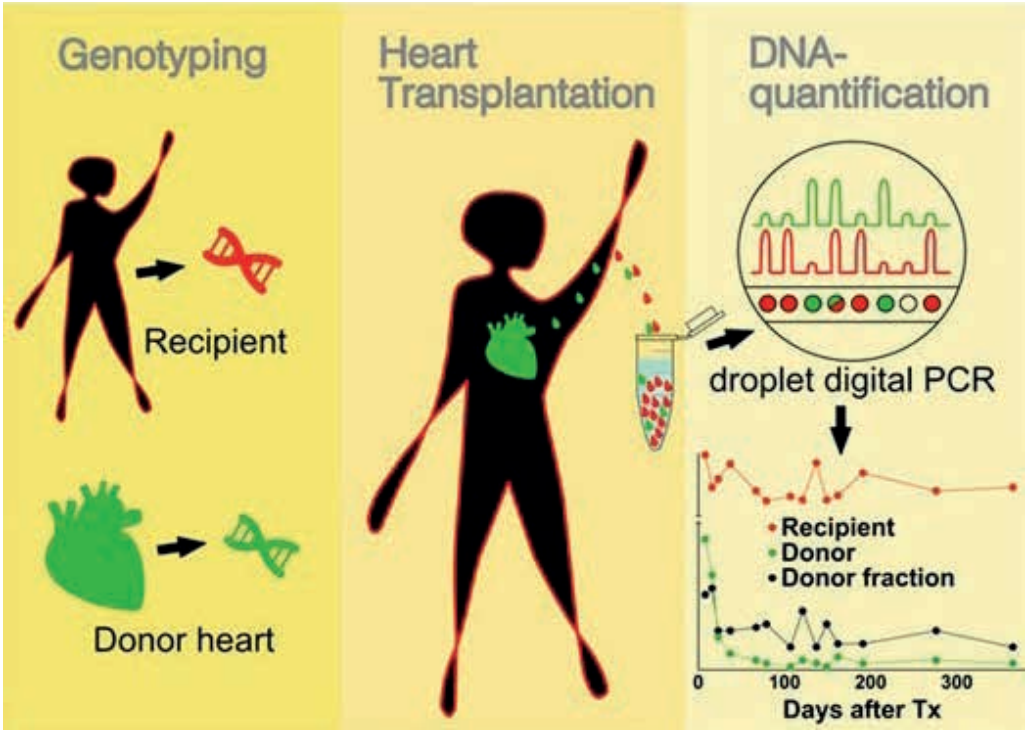
- Age over 18 years
- Both male and female gender
- Presence of transplanted heart, kidney, or liver
- Patients who signed informed consent to participate in the study.
- Exclusion criteria for study participants were:

- Refusal to undergo diagnostic procedures as specified in the study protocol
- Participation inanother study

Presence of anatomical or concomitant diseases, or other medical, social, or psychological conditions that, in the researcher's opinion, could limit the subject's ability to participate in the clinical study or meet the requirements of subsequent observation or affect the scientific validity of the results of the clinical study.

The assessment of the donor's donor-derived cell-free DNA (ddcfDNA) fraction relative to the total cell-free DNA in the recipients' blood is a non-invasive diagnostic method for acute rejection in patients after heart transplantation. Below are the interim results of the study, and all calculations were performed using Excel, presenting average statistical values.

Figure 1.
The principle of the diagnostic method.[6]



All patients are examined according to the schedule Table 1

Table 1.
Schedule of diagnostic procedures and studies

Criteria	0 day	6 month	12 month	24 month	30 month
Signing of informed consent	+				
Blood for sensitization	+			+	
CBC with differential	+	+	+		
Glycated hemoglobin, glucose	+	+	+		
AST, ALT	+	+	+		
Urea, Creatinine, Total Bilirubin, Direct Bilirubin, Total Protein, High-Sensitivity C-Reactive Protein (hs-CRP), Ferritin, Uric Acid, Total Cholesterol.	+	+	+		
Fibrinogen, D-dimer	+	+	+		
Expanded lipid profile (TC, LDL-C, HDL-C, TG, apoB)	+	+	+		
ApoA, Lipoprotein(a)	+				

Markers for hepatitis	+				
NTproBNP	+	+	+		
coronary angiography	+				
echocardiogram with myocardial deformation assessment	+	+	+		
24-hour Holter monitoring of ECG, blood pressure	+	+	+		
Duplex ultrasound of brachiocephalic vessels.	+	+	+		
12-lead electrocardiogram (ECG)	+	+	+		
ultrasound of the liver	+		+		
EMG	+		+		
bioinformatic analysis				+	+

Ethical approval

This study was conducted in strict accordance with the principles outlined in the Helsinki Declaration. Before commencing the research, approval was obtained from the Local Bioethics Committee of the Corporate Fund "University Medical Center."

Statistical analysis

Data were analyzed using IBM SPSS Statistics software (IBM SPSS Inc.). Numerical variables were expressed as mean ± SD and categorical variables as numbers and percentages. Nonparametric statistics were performed for dataset analysis. Between-group comparisons were assessed for numerical variables, and the Chi-square test was used for categorical variables. P value ⩽0.05 was considered statistically significant.

Results

A representative sample was collected based on the National Scientific Cardiac Surgery Center in Astana. The sample included 40 patients who had previously undergone orthotopic heart transplantation. The total sample size was 40 patients, of themsignificant more males 32 (80%) than 8 (20%) females, Chi-squared 10.32, 95%CI [21.6:78.2], p= 0.0013. The age range was 17 to 59 years. Of the patients, 24 (60%) had undergone previous surgeries, and 16 (40%) had heart transplantation as their primary surgical intervention, p= 0.221.

The terminal stage of heart failure was primarily caused by dilated cardiomyopathy in 19 cases (47.5%), ischemic cardiomyopathy in 7 cases (17.5%), hypertrophic cardiomyopathy in 4 cases (10%), non-compaction myocardium in 2 cases (5%), familial forms of cardiomyopathies in 4 cases (10%), and valvular cardiomyopathy in 4 cases (10%).

The echocardiography data were characterized by a pronounced decrease in left ventricular myocardial contractility - left ventricular ejection fraction (hereinafter - LVEF) of 17.6 ± 4.9% (8–27%), cardiomegaly (left ventricular end-systolic dimension 71.3 ± 9.8 mm (35–95 mm), left ventricular end-diastolic volume 273.25 ± 84.2 ml (52–524 ml), high pulmonary hypertension (mean pulmonary artery pressure 55.6 ± 13.27 mmHg (range 25–82 mmHg), laboratory data CRP 1.05 ± 0.95 mg/dl (normal 0.5 mg/dl), NT-ProBNP (B-type natriuretic peptide) 6000.8 ± 1699.2 pg/dl (normal range 125-700 pg/dl depending on age).

In the interim result of this clinical study, a positive cross-match reaction between the donor and the recipient was recorded in 5%. In 40 patients, the post-heart transplant survival duration at the time of the study ranged from 6 months to 11 years (average 6.9±4.07 years). All patients reported an improvement in their quality of life. Echocardiographic data showed a left ventricular ejection fraction (LVEF) of 56.6 ± 6.2% (range 49–61%), left ventricular end-systolic volume of 39.6 ± 17.9 ml (23–58.8 mm), left ventricular end-diastolic volume of 92 ± 53.3 ml (60–129 ml), and laboratory data CRP 0.63 ± 0.56 mg/dl (normal 0.5 mg/dl), NT-ProBNP (B-type natriuretic peptide) 1001.8 ± 866.25 pg/dl (normal range 125-700 pg/dl depending on

age).The average age of patients was 38.85±13.23 years, and dilated cardiomyopathy (47.5%) was the predominant cause of the terminal stage of heart failure in most cases.

Discussion

Endomyocardial biopsy (EMB) is the standard for monitoring and assessing a transplanted heart. Despite its increasing prevalence and widespread recognition, EMB is an invasive procedure prone to errors and may be associated with both procedural complications and long-term consequences.[7,8]

Furthermore, EMB, routinely used for monitoring during the first year after heart transplantation, is a costly medical procedure that is inconvenient for patients. Additionally, about 25% of biopsy samples are deemed unsuitable for use. In light of these limitations, extensive efforts have been made to develop non-invasive monitoring methods that could reduce the need for subsequent EMB. This emphasis is on monitoring the recipient's immune response to detect the onset of rejection. Currently, there is ongoing development of an analysis that directly assesses the health of the transplanted heart.[9,10]

Considerable efforts have been made to develop non-invasive diagnostic biomarkers that could replace or reduce the need for endomyocardial biopsy.

Episodes of acute rejection are most common in the first weeks after transplantation and can be categorized into T-cell-mediated and AMR. During acute cellular rejection, lymphocytes infiltrate and proliferate in the interstitial space. The adaptive immune system plays a central role in ACR. Direct allorecognition involves the interaction between the T-cell receptor (TCR) on recipient T cells and mismatched human leukocyte antigens (HLA) on donor antigen-presenting cells.[7,8,9,10] Indirect allorecognition also plays a role. The interaction of HLA/peptide-TCR and co-stimulatory signals promotes the proliferation and differentiation of T cells. CD8+ T cells release perforin and granzyme B, inducing apoptosis of target cells. Monocytes and myeloid dendritic cells (DCs) also infiltrate the graft and contribute to acute rejection.[8]

AMR can occur within the first year after transplantation. AMR is mediated by donor-specific antibodies targeting HLA or non-HLA antigens on the donor's endothelium. The antigen-antibody interaction leads to antibody-dependent cellular cytotoxicity and complement activation, causing lysis of target cells. Damage to endothelial cells results in platelet aggregation and recruitment of leukocytes through cytokines, chemokines, and chemo attractants, ultimately leading to acute rejection.[4]

Biomarkers are categorized into two groups: those reflecting allograft injury and those reflecting inflammatory and alloimmune processes underlying allograft rejection. Given the potential consequences of not diagnosing and treating acute rejection of a cardiac allograft, these biomarkers must be highly sensitive to rejection, even at the expense of low specificity.[3,11]

Non-invasive methods include AlloMap, detection of dd-cfDNA, microRNAs, extracellular vesicles, and donor-specific antibodies. Despite dozens of promising studies and potential biomarkers, only two have been approved by the Food and Drug Administration (FDA) and are used in everyday clinical practice: AlloMap and dd-cfDNA.[6,7,12,13]

Cell-free DNA of donor origin (dd-cf DNA) is present ubiquitously in biological fluids and various environments, including soil and water biotopes. Recently, studies have shown that certain types of extracellular DNA can play a significant role in living organisms and indicate pathological conditions. Cell-free DNA refers to all non-encapsulated DNA in the bloodstream and was first detected in the blood plasma of healthy individuals in 1948. Cell-free DNA consists of approximately 150 base pairs of double-stranded DNA released from nucleosomes during apoptosis and necrosis. cf-DNA molecules exist as monomers, dimers, and trimers. Most cf-DNA circulates as nucleosomes or chromatosomes, as free DNA is vulnerable to rapid degradation by nucleases. An important characteristic of cf-DNA is its half-life in the bloodstream (30 minutes to 2 hours), indicating continuous release from apoptotic or necrotic cells.[14,15,11]

There are various types of cf-DNA, with the most important being cell-free mitochondrial DNA, tumor DNA, and fe-

tal DNA, all possessing similar properties. Concentrations of cfDNA vary under both normal physiological conditions (7-18 ng/ml in healthy individuals) and diagnosed diseases (800 ng/ml in patients with esophageal cancer). The initial discovery of cell-free DNA in 1948 *by Mandel P. and Metais P.* led to numerous studies assessing the role of cfDNA in various diseases. Initially used to study oncological markers in cancer patients, the most successful application of cf-DNA as a clinical biomarker is non-invasive prenatal testing (NIPT) for detecting fetal pathologies, showing higher accuracy compared to biochemical screening. Recently, interest in cell-free DNA has increased in the field of transplantation. Determining the quantity of donor-derived cell-free DNA in a patient's blood plasma can aid in early detection of organ rejection after transplantation.[6,7,12,13]

Non-cellular DNA serves as a marker for transplant viability. During graft rejection, caused by the breakdown of its cells, dd-cfDNA is released into the bloodstream, leading to increased levels in the recipient's body. Early elevation of dd-cfDNA levels is observed in patients during acute graft dysfunction, suggesting potential use of quantitative dd-cf DNA levels as an alternative rejection marker. Some studies report temporary elevation of dd-cfDNA levels in the early post-transplant period; however, in stable patients receiving immunosuppressive therapy, this indicator decreases to baseline levels around 7-10 days post-transplantation. Overall, research indicates that donor non-cellular DNA levels demonstrate high accuracy and can predict acute rejection of the transplanted organ, with consistent predictive ability across all organ types. Highest cf-DNA levels are observed during acute antibody-mediated graft rejection. Several studies have compared dd-cf DNA with other markers of graft injury.[10,14] Methods for quantifying recipient dd-cfDNA in plasma include real-time PCR, droplet digital PCR (ddPCR), and massively parallel sequencing, also known as next-generation sequencing (NGS). ddPCR and NGS require donor genotyping, evaluating the presence of a single nucleotide polymorphism where the recipient is homozygous for a specific allele and the donor is not.[11]

As a non-invasive quantitative marker of allograft injury, dd-cfDNA promises to become a safe, accurate, and feasible method for monitoring acute rejection in heart transplant recipients. Although further research is necessary to confirm specific threshold values for routine clinical use, dd-cfDNA currently demonstrates the greatest potential as a monitoring tool, screening patients who would benefit most from preemptive biopsy. Advancements in rejection monitoring using dd-cfDNA further our efforts towards developing precise medicine methods for heart recipients. Patterns of dd-cfDNA elevation also vary between AMR and ACR, which can facilitate diagnosis and have different fragment lengths of cfDNA with shorter fragments. These unique data can aid in more precise AMR diagnosis, assisting in distinguishing between AMR and ACR and helping us determine the appropriate treatment strategy.[10,11]

The diagnosis of acute and chronic rejection of cardiac allograft remains a complex task, as rejection often occurs asymptomatically, impacting short-term and long-term transplant outcomes. Significant progress has been made in molecular diagnostics for non-invasive monitoring of acute rejection after heart transplantation over the last decade. Alternative non-invasive biomarkers may replace or reduce the need for endomyocardial biopsy. The effectiveness of this rejection diagnosis method has been actively studied over the last 10 years.

Cell-free DNA is widely used as a prognostic and predictive biomarker, entering the bloodstream due to cell death and being present in much higher concentrations in diseased individuals compared to healthy ones. Each fragment of cfDNA carries molecular characteristics of the cell it originated from, such as DNA methylation status.[16] Donor-derived cfDNA detected in transplant recipients' blood has been proposed as a potential biomarker for organ rejection or cell transplant damage.[17]

The first method of detecting cfDNA involved genetic differences, such as donor-recipient sex mismatch, where the Y-chromosome was detected in a female recipient.[18] cfDNA data were evaluated

based on HLA donor-recipient mismatch in the HLA-DRB1 locus using optimized droplet digital PCR. Another method of cfDNA detection involves a quantitative approach using PCR and genomic sequencing.[19,20]

According to *De Vlaminck et al.,* by comparing endomyocardial biopsy results and cfDNA fraction, the latter was significantly elevated by 5 months post-transplantation, whereas biopsy results were negative. Their results indicate that determining the amount of cfDNA may replace endomyocardial biopsy and that these measurements can be used for other aspects of patient management, such as rejection event prediction and immunosuppressant dosage management. As explained by Zangwill, a higher overall level of cfDNA in the early stages post-transplant predicted death.[21]

This method will allow differentiation between acute cellular and antibody-mediated rejection, which have different therapeutic approaches, and has high clinical significance. At the same time, this method requires further study: first, new clinical trials are needed to obtain compelling evidence for this method; second, to determine its clinical effectiveness compared to invasive approaches (transplant biopsy); third, by studying the sensitivity and specificity of the method, to determine threshold values at which clinicians could diagnose acute rejection. Moreover, current approaches to ddCF-DNA determination have several limitations, including the labor intensity and high cost of these methods.

Limitation

During the course of this study, several limitations were encountered. Firstly, patient refusal posed a challenge as some individuals declined to participate, potentially introducing selection bias and impacting the generalizability of the findings. Secondly, the scope of the research was confined to primary investigation regarding cell-free DNA as a biomarker for detecting acute rejection in heart transplant recipients. Further exploration involving larger cohorts and diverse clinical settings is imperative to validate and extend the results. Lastly, the external validity of the findings may be constrained by the specific population and context in which the research was conducted, limiting their applicability to broader patient populations and clinical settings.

Conclusion

The study highlights the potential of cell-free DNA as a non-invasive biomarker for detecting acute rejection in heart transplant recipients. The findings underscore the importance of early rejection detection and differentiation between acute cellular and antibody-mediated rejection for tailored therapeutic interventions. However, further research is needed to address methodological limitations, validate the clinical utility of cfDNA testing, and establish threshold values for accurate rejection diagnosis. Despite these challenges, cfDNA testing holds promise for revolutionizing rejection monitoring post-heart transplantation, potentially enhancing patient outcomes and quality of life.

What is already known on this topic: Values of non-invasive diagnostic biomarkers of organ transplant rejection. AlloMap method and determination of donor-derived cell-free DNA. The quantitative marker of allograft damage donor-derived cell-free DNA is a safe, accurate method for monitoring acute rejection in heart transplant recipients.

What this study adds: For the first time in Kazakhstan, donor-derived cell-free DNA was assessed as an early predictor of heart transplant rejection.

Acknowledgment: The authors would like to express their sincere appreciation to the Corporate Fund "University Medical Center" for their generous support which made this research possible. Also, authors would like to express their gratitude to the surgery team for their valuable contributions to this research. Additionally, heartfelt thanks are extended to the personnel of the center for their unwavering support and assistance throughout the research process. Special appreciation is reserved for the patients whose participation and cooperation made this study possible.

Authors' Contributions: P.Y: Study conception and design, surgeries, revising discussion section of the manuscript. M.G: Study design, data analysis, and interpretation, revising discussion section of the manuscript. N.S: Data acquisition, analysis, and interpretation; surgeries, revising results section of the

manuscript. Y.Y: Data collection, drafting, revising results section. B.M: Data collection, medical diagnoses, surgical pathologic evaluations. A.S: Study conception and design, overall responsibility of the study, data analysis and interpretation. All authors have approved the final version of the article.

Conflict of Interest: There are no conflicts of interest.

Funding: This research is funded by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant title: Non-invasive methods for diagnosis of transplant rejection as a predictor of long-term graft survival, Grant No. BR21882206).

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