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GENETIC BIOMARKERS OF ACUTE GRAFT REJECTION AFTER HEART TRANSPLANTATION

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Abstract

Heart transplantation (HT) is the standard of care for end-stage heart failure refractory to medical therapy. Patients after heart transplantation are at risk of developing various complications during their follow-up. Common complications include early allograft failure, acute graft rejection (AGR), coronary allograft vasculopathy (CAV), renal failure, infections, and cancer. Causes for secondary graft dysfunction need to be considered beyond the first week. Nowadays, histological stratification of acute rejection (AR) on endomyocardial biopsy plus histopathology (EMBx) is the standard method for diagnosing acute rejection, assessing its severity, and the response to therapy. Unfortunately, this method is invasive and has some limitations. In addition to that, acute rejection has two phenotypes, acute cellular rejection (ACR) and antibody-mediated rejection (AMR), which challenges the histopathologic diagnosis. Secondary non-genetic methods for monitoring cardiac rejection may include echocardiography, cardiac MRI, troponin, and other methods. A reliable non-invasive marker to detect acute rejection prior to the development of graft dysfunction would possibly result in better outcomes for those patients who develop allograft rejection. In the current review, we collect recent data about genetic non-invasive biomarkers including donor derived cell free DNA, DNA-methylation, RNAs Gene expression profiling (messenger RNA) and micro-RNA. cfDNA methylation analysis can help to distinguish different types of acute rejection. The fraction of cfDNA from the donor decreases rapidly post-transplantation, and increase only in case of acute rejection or myocardial injury. In summary, genetic non-invasive methods have a pivotal role in the assessing and monitoring cardiac allograft rejection. Review article established that DNA-based non-invasive tools minimize the risks of invasive procedures. It is a safe, convenient, and precise method for diagnosing heart failure after cardiac transplantation.

Keywords: *heart transplantation, acute graft rejection, genetic biomarkers, donor derived cell free DNA, cost-effectiveness*

Резюме

ГЕНЕТИЧЕСКИЕ БИОМАРКЕРЫ ОСТРОГО ОТТОРЖЕНИЯ ТРАНСПЛАНТАТА ПОСЛЕ ТРАНСПЛАНТАЦИИ СЕРДЦА

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Трансплантация сердца является стандартом лечения терминальной сердечной недостаточности, резистентной к медикаментозной терапии. Пациенты после трансплантации сердца подвергаются риску развития различных осложнений во время наблюдения. Распространенные осложнения включают раннюю недостаточность аллотрансплантата, острое отторжение трансплантата, коронарную васкулопатию аллотрансплантата, почечную недостаточность, инфекции и рак. Причины вторичной дисфункции трансплантата необходимо учитывать после первой недели трансплантации. На сегодняшний день, гистологическая стратификация острого отторжения при помощи эндомиокардиальной биопсии с гистопатологией является стандартным методом диагностики острого отторжения, оценки его тяжести и ответа на терапию. К сожалению, этот метод инвазивный и имеет некоторые ограничения. В дополнение к этому острое отторжение имеет два фенотипа: острое клеточное отторжение и антитело-опосредованное отторжение, что затрудняет постановку гистопатологического диагноза. Вторичные негенетические методы мониторинга сердечного отторжения могут включать эхокардиографию, МРТ сердца, тропонин и другие методы. Надежный неинвазивный маркер для выявления острого отторжения до развития дисфункции трансплантата, возможно, приведет к лучшим результатам для тех пациентов, у которых есть вероятность развития отторжения аллотрансплантата. В нашем обзоре мы собрали последние данные о генетических неинвазивных биомаркерах, включая внеклеточную ДНК донорского происхождения, метилирование ДНК, профилирование экспрессии генов РНК (матричная РНК) и микро-РНК. Анализ метилирования внеклеточной ДНК может помочь различить различные типы острого отторжения. Метод микро-РНК может сыграть важную роль в будущем как цель разработки иммунодепрессантов. В основе анализа внеклеточной ДНК донорского происхождения лежит обнаружение однонуклеотидных полиморфизмов, отличающих ДНК донора от ДНК реципиента. Фракция внеклеточной ДНК донора быстро снижается после трансплантации и увеличивается только в случае острого отторжения или повреждения миокарда. Таким образом, генетические неинвазивные методы играют ключевую роль в оценке и мониторинге отторжения сердечного аллотрансплантата. Анализ статей установил, что неинвазивные методы на основе ДНК минимизируют риски инвазивных процедур. Это безопасный, удобный и точный метод диагностики сердечной недостаточности после трансплантации сердца.

Ключевые слова: *трансплантация сердца, острое отторжение трансплантата, генетические биомаркеры, внеклеточная ДНК донорского происхождения, экономическая эффективность*

Түйіндеме

ЖҮРЕК ТРАНСПЛАНТАЦИЯСЫНАН КЕЙІН ПАЙДА БОЛАТЫН ТРАНСПЛАНТАТТЫҢ ЖЕДЕЛ ҚАБЫЛДАНБАУЫНЫҢ ГЕНЕТИКАЛЫҚ БИОМАРКЕРЛЕРІ

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Жүрек трансплантациясы медициналық терапияға жауап бермейтін жүрек жеткіліксіздігінің соңғы сатысына арналған стандартты көмек болып табылады. Жүрек трансплантациясынан кейінгі науқастар бақылау кезінде әртүрлі асқынулардың даму қаупіне ұшырайды. Жиі кездесетін асқынуларға ерте аллотрансплантаттың жетіспеуі, трансплантаттың жедел қабылданбауы, коронарлық аллотрансплантат васкулопатиясы, бүйрек жеткіліксіздігі, инфекциялар және қатерлі ісік жатады. Трансплантацияның бірінші аптасынан кейін қайтадан трансплантат дисфункциясының себептерін қарастыру қажет. Қазіргі таңда, эндомиокард биопсиясы және гистопатология арқылы жасалған гистологиялық стратификация жедел қабылданбаудың диагностикасының, оның ауырлығын және терапияға жауапты бағалаудың стандартты әдісі болып табылады. Өкінішке орай, бұл әдіс инвазивті және кейбір шектеулері бар. Бұған қоса, жедел қабылдамаудың екі фенотипі бар, жедел жасушалық қабылдамау және антидене арқылы бас тарту, бұл гистологиялық диагнозды қиындатады. Жүректің қабылданбауын бақылайтын екінші реттегі генетикалық емес әдістеріне эхокардиография, жүрек магнитті-резонанс томография, тропонин және басқа әдістер кіруі мүмкін. Сондықтан трансплантат дисфункциясы дамуынан бұрын жедел қабылдамауды анықтауға арналған сенімді инвазивті емес маркер аллотрансплантат қабылданбауы дамуы мүмкін науқастар үшін жақсы нәтижелерге әкелуі мүмкін. Біздің шолуда біз генетикалық инвазивті емес биомаркерлер туралы соңғы

деректерді жинадық, соның ішінде донордан алынған жасушасыз ДНҚ, ДНҚ-метилдену, РНҚ гендік экспрессия профилін жасау (ақпараттық РНҚ) және микро-РНҚ. Жасушасыз ДНҚ метилдену талдауы жедел бас тартудың әртүрлі түрлерін ажыратуға көмектеседі. Микро-РНҚ әдісі болашақта иммуносупрессантты препараттарды әзірлеу мақсаты ретінде маңызды рөл атқаруы әбден мүмкін. Донордан алынған жасушасыз ДНҚ талдауының мағынасы донорды реципиент ДНҚ-сынан ажыратын жалғыз нуклеотидті полиморфизмдерді анықтауға негізделген. Ал донордан алынған жасушасыз ДНҚ фракциясы трансплантациядан кейін тез төмендейді және олар жедел қабылданбау немесе миокард зақымдануы жағдайында ғана жоғарылайды. Қорытындылай келе, генетикалық инвазивті емес әдістер жүрек аллотрансплантатының қабылданбауын бағалауда және бақылауда шешуші рөл атқарады. Шолу мақаласы ДНҚ негізіндегі инвазивті емес құралдар инвазивті процедуралардың тәуекелдерін азайтатынын көрсетті. Бұл жүрек трансплантациясынан кейін жүрек жеткіліксіздігін диагностикалаудың қауіпсіз, ыңғайлы және дәл әдісі.

Түйінді сөздер: *жүрек трансплантациясы, трансплантаттың жедел қабылданбауы, генетикалық биомаркерлер, донордан алынған жасушасыз ДНҚ, экономикалық тиімділік*

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Introduction

Heart transplantation has profoundly impacted the management of end-stage heart disease, offering renewed life to patients with otherwise limited treatment options. The journey began with the world's first successful human heart transplant by Dr. Christiaan Barnard in 1967, a monumental event that paved the way for the procedure's future developments [4,8,34]. In Kazakhstan, for the first time, a heart transplant was performed on August 8, 2012 [26]. Heart transplantation (HT) has become an established therapy for patients with heart failure (HF), representing the gold-standard treatment for end-stage HF [30]. Thus, in Kazakhstan, the prevalence of HF dramatically increased from 4393 to 22,088 people per million population during the 2014-2021 years [65]. It is worth noting that age-standardized CVD mortality rates among countries in Central Asia ranged from 331.8 to 542.3 per 100,000 in 2022. Of the 21 regions, Central Asia ranked 4th in 1990 and 2nd in 2022 for age-standardized CVD mortality, and 1st in CVD age-standardized prevalence in 2022. Moreover, epidemiologically, it addresses the high burden of cardiomyopathies and ischemic heart disease prevalent in the region [40,64].

Due to limited donor supply, a decline in the number of HTs was observed between the 1990s and early 2000s; however, in recent years, the number of HT patients has increased, and about 5500 HTs have been performed annually worldwide in recent years [39]. Since then, advancements in surgical techniques and immunosuppressive therapy have significantly improved patient outcomes. The introduction of drugs like cyclosporine has been pivotal in reducing graft rejection and enhancing survival rates. The median lifespan following adult heart transplants conducted from 2002 to 2009 is approximately 12.5 years, with an extension to about 14.8 years among those who survive the first year after transplantation [30]. Advances in immunosuppression and patient management have led to significant improvements in survival, with one-year post-transplant survival rates now exceeding 80%, and substantial increases in longer-term survival [37,51]. Survival rates within the first year after a heart transplant and overall longevity differ based on the initial diagnosis. For example, individuals receiving transplants due to nonischemic and ischemic cardiomyopathies exhibit the highest survival rates after one year, whereas those undergoing retransplantation show the lowest. As expected, older recipients tend to have shorter long-term survival rates, and older donor ages correlate with increased mortality rates shortly after transplantation. Moreover, female recipients have consistently demonstrated longer median survival compared to male recipients, with women averaging 12.2 years and men 11.4 years [30]. Currently, the one-year survival rate approximates 90%, the five-year survival reaches around 70%, yet the 20-year survival drops to about 20% [2].

HT has significantly advanced, yet it is associated with various complications that can affect the outcomes and quality of life post-surgery. HT patients are at risk of developing various complications during their follow-up. Common complications include early allograft failure, acute graft rejection (AGR), CAV, renal failure, infections, and cancer [2]. The most immediate surgical issues post-transplant can include primary graft failure and complications from the surgical procedure itself. Acute kidney injury is a notable complication, particularly affecting those undergoing more extensive surgeries such as valve replacements or aortic surgeries. This can significantly impact in-hospital mortality and long-term outcomes [21]. Graft rejection remains a primary concern, with both acute and chronic forms. Acute cellular rejection, which is more

common in the first six months post-transplant, involves T cell-mediated attacks against the donor heart. Chronic rejection, often manifested as cardiac allograft vasculopathy, involves the slow narrowing of the heart's arteries, which can lead to heart failure or arrhythmias [9]. CAV is a leading cause of long-term graft dysfunction and graft loss after heart transplantation. While CAV pathogenesis is complex, and involves both alloimmune and nonimmune processes, it is apparent that both donor and recipient risk factors predispose to CAV development [31].

Recent advancements in the field of HT include the development of new immunosuppressive agents that show promise in reducing the incidence of acute rejection and improving overall graft survival. Additionally, techniques in donor heart preservation and novel monitoring methods for rejection [9]. Therefore, it is crucial to establish an effective follow-up protocol for HT patients' right from the early post-transplant stages [45].

Aim of the review: To study international experience in conducting new genetic non-invasive diagnostic markers of acute graft rejection after heart transplantation and determine the most effective ones**.**

Search Strategy.

To ensure a comprehensive review of the literature on non-invasive genetic diagnostic markers of acute graft rejection after heart transplantation, we conducted systematic searches in the following databases: PubMed, Google Scholar, Web of Science, and Scopus. The search was performed from the inception of each database up to June 2024. This extensive search ensured the inclusion of the most recent studies relevant to our topic. We utilized a combination of keywords and Medical Subject Headings (MeSH) terms to maximize the search sensitivity. The primary search terms included: "Heart transplantation", "Acute graft rejection", "Non-invasive diagnostics", "Genetic markers", "Cell-free DNA", "microRNA", "Donor-derived cell-free DNA", "Biomarkers". These terms were used in various combinations to capture a wide range of relevant studies.

Inclusion Criteria

Studies were selected based on the following criteria:

1.Population: Patients who underwent heart transplantation.

2.Intervention: Use of non-invasive genetic markers for diagnosing acute graft rejection.

3.Outcome: Sensitivity, specificity, and overall diagnostic accuracy of the genetic markers.

4.Study Design: Randomized controlled trials, cohort studies, case-control studies, and cross-sectional studies.

Exclusion Criteria

The exclusion criteria were as follows:

1.Non-English Articles: Studies not published in English were excluded.

2.Non-Original Research: Reviews, meta-analyses, editorials, and case reports were excluded.

3.Non-Heart Transplantation Studies: Studies focusing on transplantation of organs other than the heart.

4.Non-Genetic Markers: Studies that did not investigate genetic markers for diagnosing acute graft rejection.

Selection Algorithm

An initial search using the specified terms was conducted in each database. Titles and abstracts were screened for relevance based on the inclusion and exclusion criteria. Full texts of potentially relevant articles were retrieved and reviewed in detail. Data from the selected studies were extracted and summarized, focusing on the study design, population, intervention, outcomes, and key findings. The quality of the included studies was assessed using standardized tools appropriate for the study design.

Diagnosis of Graft Rejection in Heart Transplantation

Early and accurate diagnosis of graft rejection following HT is crucial to extend the survival and improve the outcomes of transplant recipients. Current strategies emphasize both invasive and noninvasive methods to detect and manage rejection episodes promptly. The goal is to preserve graft function, extend patient survival, and avoid cardiac allograft vasculopathy (CAV), a leading cause of long-term allograft failure and mortality [1]. Causes for secondary graft dysfunction need to be considered beyond the first week [24].

The cornerstone of rejection diagnosis often involves endomyocardial biopsy (EMB), which remains the gold standard for detecting acute cellular rejection (ACR) and antibody-mediated rejection (AMR). This method allows for direct tissue assessment, providing crucial information before clinical symptoms manifest, thereby enabling early intervention. Histological grading of AR on endomyocardial biopsy plus histopathology (EMBx) is the standard method for diagnosing AR, assessing its severity, and the response to therapy [1]. Most heart transplant programs implement routine surveillance through endomyocardial biopsies beginning the first week after surgery. The typical schedule involves weekly biopsies for the initial six weeks, biweekly up to three months, monthly until six months, and then every two to three months for the first year. These biopsies are crucial for detecting any significant acute cellular rejection (ACR) or antibody-mediated rejection (AMR) and aid in the careful tapering of the intense immunosuppressive treatment initiated at transplantation. Additionally, functional cardiac assessment typically starts with echocardiography during the first week, followed by assessments at one, three, and six months, and then annually. Annual checks for vasculopathy are commonly conducted with angiography starting one year post-transplant and every five years thereafter, often using computed tomography coronary angiography. In the years between, functional testing may involve stress echocardiography or nuclear medicine scintigraphy to reduce the risk of nephrotoxicity from contrast agents used in imaging [24].

Advances in noninvasive techniques have added significant value to the monitoring protocols. Imaging techniques like echocardiograms and electrocardiograms are routinely used to assess heart function and detect early signs of graft dysfunction. More sophisticated methods such as coronary angiography, intravascular ultrasound, and cardiac stress testing are employed to investigate chronic rejection scenarios.

Recent developments have introduced molecular approaches such as gene expression profiling (GEP) and the measurement of donor-derived cell-free DNA (dd-cfDNA) levels, which provide a more dynamic and less

invasive way to monitor transplant recipients. These methods can detect rejection episodes earlier than traditional methods and are particularly useful in continuous monitoring setups

Non-genetic methods for monitoring and assessing cardiac allograft rejection

Echocardiography

Echocardiography has emerged as a crucial non-invasive modality for monitoring and assessing cardiac allograft rejection in heart transplant recipients. Echocardiography's versatility allows for regular assessments of graft function. It is especially effective in detecting changes associated with both acute and chronic allograft rejection. Speckle-tracking echocardiography (STE), for instance, uses myocardial strain measurements to detect subtle changes in myocardial function that might indicate acute cellular rejection, even in patients with preserved left ventricular ejection fraction. This method has shown promise in predicting severe rejection, helping guide timely therapeutic interventions [13, 60]. Despite its advantages, the diagnostic accuracy of echocardiography in detecting acute cardiac allograft rejection when compared to the gold standard of endomyocardial biopsy (EMB) shows some limitations due to variability in sensitivity and specificity. A meta-analysis indicates that while echocardiography is a useful tool, there is heterogeneity in its clinical application, emphasizing the need for combining it with other diagnostic methods or using it in specific clinical contexts [36]. According to The International Society for Heart and Lung Transplantation (ISHLT) Guidelines for Heart Transplant Recipients echocardiography is not recommended as a primary method for rejection monitoring due to certain limitations specific to HT patients [62].

Endomyocardial biopsy

Endomyocardial biopsy (EMB) is considered the gold standard for monitoring cardiac allograft rejection following heart transplantation. EMBs were introduced in the cardiac transplant field about 40 years ago in many centers, first in the US and then worldwide. Its primary role is to detect cellular or antibody-mediated rejection and guide the management of immunosuppressive therapy [19]. Monitoring EMBs for heart transplants is particularly important for post-transplanted patients, who are subjected to about 14 EMBs during the first year post-transplant. Despite its widespread use and critical role, EMB has notable limitations and risks associated with its invasive nature:

1) this is invasive procedure associated with some minor unavoidable clinical complications;

2) the close correlation between the clinical and histological resolution of rejection is debarred by interobserver variability and sampling errors;

3) EMBs, systemically used for surveillance during the first year after heart transplantation, represent an expensive medical procedure [20,47]. However, EMB is highly effective in diagnosing acute and chronic rejection. Its effectiveness is underpinned by the ability to provide histopathological diagnosis, which remains unmatched by non-invasive methods. However, the procedure's diagnostic yield is highly dependent on the sampling technique and the experience of the clinical team [19].

Cardiac MRI

Cardiac MRI (CMR) is increasingly recognized as a valuable non-invasive tool for monitoring and assessing cardiac allograft rejection in heart transplant recipients. Its usage leverages advanced imaging techniques to evaluate myocardial tissue characteristics without the need for invasive biopsy procedures. CMR is considered as the gold-standard imaging modality for assessing cardiac morphology, ventricular volumes, systolic function, and myocardial mass in HT patients. CMR is particularly useful for its ability to perform detailed tissue characterization using T1 and T2 mapping, LGE and parametric mapping, as well as the measurement of extracellular volume fraction (ECV). These quantitative markers are effective in detecting changes in myocardial tissue that are indicative of allograft rejection. Studies have demonstrated that T2 mapping has high diagnostic accuracy, showing elevated T2 values in patients with acute cardiac allograft rejection. Similarly, ECV measurements are also elevated in rejection cases, providing critical diagnostic information. These techniques can help detect inflammation, myocardial edema, fibrosis, and irreversible injury, providing valuable insights into graft health and the presence of complications. Therefore, a multisequential CMR examination could operate as a non-invasive tool for excluding subclinical ACR in heart transplant patients [14, 20, 23, 45, 63]. While CMR offers significant advantages, it also has limitations. The technique's sensitivity and specificity can vary, and it may not detect all cases of rejection, particularly those that are less severe or localized. Additionally, the availability of high-quality CMR can be limited by the need for specialized equipment and expertise. Interpretation of CMR results requires experienced radiologists or cardiologists trained in advanced cardiac imaging techniques. There is also the challenge of integrating CMR findings with clinical management, as CMR is a complement to, but not a replacement for, traditional methods like endomyocardial biopsy in many clinical settings [23].

Troponin

Cardiac troponins T and I are exclusively present in cardiomyocytes and are highly sensitive and specific non-invasive markers of myocardial injury [47]. Cardiac troponin has been studied for its potential to detect acute cellular rejection in heart transplant recipients. While it's a non-invasive option, its diagnostic accuracy varies. A systematic review revealed a pooled sensitivity of around 48% and specificity of approximately 70% for detecting acute cellular rejection, suggesting moderate effectiveness. However, significant heterogeneity exists across studies, which could be due to variations in troponin assay sensitivity, rejection criteria, and study designs. Elevated troponin levels do not consistently correlate with rejection, limiting its standalone diagnostic use in this context [35]. It is not used extensively in transplant rejection assessment due to low sensitivity in the setting of anything less than severe rejection. While the positive predictive value of high-sensitivity cardiac troponin (hs-cTn) is low, the negative predictive value is acceptable, suggesting there may be a role as a “rule out” test for severe rejection. The low sensitivity of convention troponin makes its use questionable. The value of a negative hs-cTn in avoiding endomyocardial biopsy remains to be proven in a prospective trial [24].

Genetic biomarkers of cardiac allograft rejection

During the last two decades, important resources have been allocated to the search for an accurate non-invasive biomarker of allograft rejection. These biomarkers can be

classified into two categories: those reflecting allograft injury and those reflecting the inflammatory and allo-immune processes underlying allograft rejection [11]. Genetic biomarkers have shown great potential in monitoring and assessing cardiac allograft rejection, offering a less invasive alternative to traditional endomyocardial biopsies. Here are several types of genetic biomarkers that are under investigation:

- Gene Expression Profiling (GEP): This technique assesses the expression levels of multiple genes simultaneously to predict and identify acute cellular rejection (ACR). GEP can provide valuable insights into the immune processes involved in rejection and has been shown to predict rejection events before they occur [6].
- MicroRNAs (miRNAs): These small, non-coding RNA molecules regulate gene expression and have been identified as potential biomarkers for allograft rejection. MiRNAs are stable in the bloodstream, making them excellent candidates for non-invasive monitoring of transplant rejection [20].
- Donor-Derived Cell-Free DNA (dd-cfDNA): The levels of dd-cfDNA in the blood of transplant recipients can indicate allograft injury. This biomarker is gaining attention because it can reflect the overall amount of injury to the donor organ and is useful for both diagnosing active rejection and potentially guiding immunosuppression therapy [38].

Since the introduction of gene-expression profiling (GEP) as a rule out test for ACR, alternative methods are being developed with potentially better diagnostic, monitoring, and prognostic performance in heart transplantation. These methods include donor-derived cell-free DNA (dd-cfDNA), cfDNA methylation, microRNAs (miRs), protein biomarkers, extracellular vesicles (EVs) and donor specific antibodies (DSA) [47]. We focus our review on DNA-based non-invasive biomarkers such as donor derived cell free DNA, DNA-methylation, RNAs Gene expression profiling (messenger RNA) and micro-RNA.

Donor derived cell free DNA

Donor-derived cell-free DNA (dd-cfDNA) is a rapidly emerging biomarker for monitoring and assessing cardiac allograft rejection in transplant medicine. This method offers a non-invasive alternative to traditional endomyocardial biopsies, potentially reducing the need for these invasive procedures while providing timely insights into graft health. Dd-cfDNA are small fragments of DNA released into the bloodstream by the death of donor cells in a transplanted organ. When the transplanted heart is under stress or undergoing rejection, injured or dying cells release more DNA fragments into the circulation. By measuring the levels of dd-cfDNA, clinicians can gauge the extent of graft injury, providing a functional insight into the state of the transplant. The detection of dd-cfDNA involves collecting a blood sample from the transplant recipient and analyzing it using highly sensitive molecular techniques such as PCR or next-generation sequencing (NGS). These techniques quantify the amount of dd-cfDNA that is specific to the donor, distinguished by single nucleotide polymorphisms (SNPs) that differ from the recipient's DNA [27,47,50].

Dd-cfDNA can be found in both the urine and serum of transplant recipients. Post-transplant, the proportion of dd-cfDNA typically diminishes rapidly but will increase during episodes of acute rejection or cardiac injury. To avoid early post-operative changes that could affect the results, studies often do not analyze serum from the first month after the transplant. Additionally, a noticeable decrease in dd-cfDNA levels has been linked to the effective treatment of acute cellular rejection, as confirmed by EMB [24,56]. Böhmer et al. demonstrated that the proportion of donor-derived DNA increased significantly during episodes of rejection [7].

Furthermore, nongraft-related effects on total cf-DNA levels due to other biological variables (e.g., inflammation, infection and exercise) should be considered when interpreting results. Combining dd-cfDNA with other informative biomarkers and infectious disease detection will improve diagnostic potential [16]. Noninvasive approach was associated with the ability to lower immunosuppression, increase satisfaction, and reduce anxiety in HT recipients [3]. Comparative data on genetic biomarkers of cardiac allograft rejection presented in Table 1. This table showcases that dd-cfDNA is emerging as a reliable non-invasive biomarker for detecting heart transplant rejection. The highlighted studies utilized different genetic methods like SNP-based differentiation and quantitative PCR, which provide high sensitivity and specificity in detecting rejection episodes. These findings suggest a strong potential for dd-cfDNA to reduce the frequency of invasive biopsies and improve post-transplant care through early rejection detection.

Table 1.

Genetic biomarkers of cardiac allograft rejection.

№	References	Study design	Method	Main results
1	2	3	4	5
Donor-derived cell-free DNA (dd-cfDNA)				
1	Snyder T.M. et al., 2011 [54]	Cohort of 112 consecutive patients	Microfluidic digital PCR and NGS	At a threshold of 1.70% donor DNA captured an 83% true positive rate with a 16% false positive rate. Cell-free DNA can reveal a unique signature specific to an organ that is associated with rejection, and this detection is feasible with any combination of donor and recipient DNA.
2	Hidestrand M. et al., 2014 [25]	Not specified	Quantitative PCR based analysis of 94 SNPs	of dd-cfDNA levels less than 1% were shown to be negative for rejection. Targeted quantitative genotyping of dd-cfDNA offers a sensitive, quick, and cost-efficient non-invasive method that could serve as an alternative to EMB for monitoring rejection

Continuation of Table 1.

1	2	3	4	5
3	Beck J. et al., 2015 [5]	Mixed method study, 34 patients	Droplet digital PCR (ddPCR), SNP-based differentiation	Rejection episodes are marked by a substantial rise in dd-cfDNA (>5-fold) compared to patients without any complications. This elevation in DNA levels typically precedes any clinical symptoms or biochemical indicators of rejection.
4	Ragalie W.S. et al., 2018 [48]	Prospective study, 88 patients	Genotyping of 94 SNPs, qPCR	Targeted assay for the quantification of donor fraction had exquisite sensitivity in ruling out the presence of acute cellular rejection.
5	Richmond M.E. et al., 2020 [49]	Prospective multicenter study, 241 heart transplant recipients	qPCR-based commercially available cfDNA assay (myTAI$_{HEART}$, TAI Diagnostics Inc.).	ROC analysis using a cutoff value of 0.3, revealed an AUC of 0.814 with a sensitivity of 0.65, specificity of 0.93, and an NPV of 81.8% for the absence of any allograft rejection.
6	North P.E. et al., 2020 [41]	Longitudinal study, 76 patients	qPCR-based commercially available cfDNA assay (myTAI$_{HEART}$, TAI Diagnostics Inc.).	Donor fraction cutoff (0.32%) produced 100% NPV for ≥2R ACR. myTAI$_{HEART}$ is clinically validated for heart transplant recipients ≥2 months old and ≥8 days post-transplant
7	Agbor-Enoh S. et al., 2021 [1]	Multicenter, prospective cohort study, 171 patients	Shotgun sequencing	%ddcfDNA ≥ 0.25% detected AR with an AUC of 0.92, a sensitivity of 81%, a specificity of 85%, a PPV of 19.6%, and a NPV of 99.2%.
8	Knuttgen F. et al., 2021 [33]	Cohort Study, 87 patients	40 SNPs-based ddPCR	Using a cutoff of 0.35%, sensitivity and specificity of dd-cfDNA for cardiac rejection were 0.76 and 0.83 (AUC: 0.81)
9	Sorbini M. et al., 2021 [55]	Pilot, 19 patients	Targeting polymorphic HLA-DRB1	%ddcfDNA ≥ 0.11% detected AR with an AUC of 0.72.
10	Gondi K.T. et al., 2021 [22]	Single-center experience of combined GEP and dd-cfDNA, 153 patients	Not specified	Routine dd-cfDNA testing alongside GEP testing yielded a significant reduction in EMB volume by re-classifying GEP (+) patients into a lower risk group, without reduction in AR detection. The addition of dd-cfDNA identified patients at higher risk for AR.
12	Kim P.J. et al., 2022 [32]	Observational study, 223 patients	SNP-based massively multiplexed-PCR	dd-cfDNA fraction ≥0.15% as AR yielded 78.5% sensitivity and 76.9% specificity.
13	Feingold B. et al., 2022 [18]	Prospective study, 64 patients	qPCR-based commercially available cfDNA assay (myTAI$_{HEART}$, TAI Diagnostics Inc.), AlloSure (CareDx)	dd-cfDNA assessment and per-protocol EMB decreased surveillance EMB by 81% in cohort with no short-term adverse outcomes.
14	Böhmer J. et al., 2023 [7]	Prospective study, 52 patients	dPCR	Using a cut-off of 7.5 copies/mL, the sensitivity/specificity were 92%/43% for donor fraction (AUC ROC-curve: 0.75).
DNA-Methylation				
15	Sun K. et al., 2015 [58]	Not specified	NGS-based	The graft-derived contributions to the plasma in the transplant recipients correlated with those determined using donor-specific genetic markers.
MicroRNAs				
16	Duong Van Huyen J.P. et al., 2014 [15]	Cohort, 113 patients	qPCR	miRNAs strongly discriminated patients with allograft rejection from patients without rejection: miR-10a (AUC = 0.975), miR-31 (AUC = 0.932), miR-92a (AUC = 0.989), and miR-155 (AUC = 0.998)
18	Constanso-Conde I. et al., 2020 [10]	Cohort, 121 patients	RT-PCR, miR-181a-5p	AUC=0.80, NPV=98%
19	Kennel P.J. et al., 2021 [29]	Case-control, 43 patients	miRNA sequencing	Authors identified miRNAs that may serve as potential predictors for the subsequent development of ACR: hsa-miR-29c-3p (ACR) and hsa-miR-486-5p (AMR). AUC=0.63-0.96

Continuation of Table 1.

1	2	3	4	5
RNAs Gene expression profiling				
20	Bodez D. et al., 2016 [6]	Case-control study nested within a retrospective heart transplant patients cohort included 126 patients	Microarray	Myocardial GEP is a helpful method to accurately diagnose ACR, and predicts rejection one month before its histological occurrence.
21	Shannon C.P. et al., 2019 [53]	Prospective observational study, 160 patients	Transcriptomic profiling, HEARTBiT	HEARTBiT achieved 47% specificity given ≥ 90% sensitivity, with an AUC 0.69.
22	Tarazon E. et al., 2021 [59]	Cohort of 40 patients	RNA-sequencing	MCU (AUC = 0.944, p < .0001), MCU/MCUR1 ratio (AUC = 0.972, p < .0001), MCU/MCUB ratio (AUC = 0.970, p < .0001), and MCU/MICU1 ratio (AUC = 0.970, p < .0001)

DNA-Methylation

Advancements in cfDNA methylation analysis now permit simultaneous tracking of cfDNA from various tissue sources, offering potential methods to distinguish between antibody-mediated rejection (AMR) and acute cellular rejection (ACR) [47]. The determining of methylation signatures may provide further insights into the origin of dd-cfDNA elaboration and help differentiate unique phenotypes of rejection [50]. The latest study organized by Cox et al. pointed out major practical advantages of combination of two analysis – dd-cfDNA methylation and dd-cfDNA monitoring [12].

RNAs Gene expression profiling (messenger RNA)

RNA gene expression profiling (GEP) is becoming an increasingly valuable tool for monitoring and assessing cardiac allograft rejection, providing a non-invasive alternative to traditional endomyocardial biopsies (EMBs), with AlloMap being one of the most clinically integrated tests. AlloMap, developed by CareDx, analyzes the expression of 20 genes (11 informative and 9 housekeeping) from peripheral blood mononuclear cells, boasting a high negative predictive value (NPV) of 99% for acute cellular rejection (ACR). However, its positive predictive value (PPV) is relatively low at about 10%, and it does not detect antibody-mediated rejection (AMR) [44,47].

In recent advancements, the HEARTBiT study by Shannon et al. evaluated a 9-gene panel for early detection of ACR post-transplant. The study involved 160 patients and over 1600 samples, utilizing the NanoString nCounter technology, and found an AUC of 0.69, indicating moderate accuracy in detecting ACR at a median of 42 days post-transplant [53].

Additionally, research by *Tarazon E. et al.* explored the potential of mitochondrial gene expression as biomarkers for ACR in a cohort of 40 heart transplant patients. This study involved RNA sequencing of 112 mitochondrial genes and found several that were differentially expressed during episodes of ACR. These genes not only served as markers but also appeared to play a role in stimulating the immune response, suggesting their dual functionality as mediators of rejection. This approach showed a promising AUC of 0.90 for detecting ACR [59].

MicroRNAs

MicroRNAs (miRNAs) have emerged as promising non-invasive biomarkers for monitoring and assessing cardiac allograft rejection. These small, non-coding RNA molecules can regulate gene expression and have significant potential in the context of heart transplantation. Several studies have demonstrated the utility of miRNAs as biomarkers for detecting ACR. For example, a study identified miR-142-3p, miR-92a-3p, miR-339-3p, and miR-21-5p as significantly enriched in exosomes from serum samples of patients undergoing acute cardiac allograft rejection. These miRNAs have shown potential in reflecting the immunological status of the allograft, providing a basis for non-invasive monitoring of rejection [11, 57]. The use of miRNAs in clinical settings has been tested in various studies, where specific miRNAs profiles from blood samples correlated well with biopsy-proven allograft rejection. This correlation helps in reducing the dependency on invasive biopsy procedures, which are the current gold standard but come with risks and limitations such as sample bias and inter-observer variability [15, 61]. Further validation of miRNA profiles is ongoing, with studies such as those involving large cohorts of heart transplant recipients, where specific miRNAs have been linked to different stages and types of cardiac allograft rejection. These studies enhance the understanding of the role miRNAs can play in the early detection of rejection and the potential adjustment of immunosuppressive therapy [11, 57]. The integration of miRNA profiling into clinical practice for heart transplantation could significantly advance the management of transplant recipients, providing a more detailed, real-time assessment of graft health and reducing the need for invasive procedures [52]. This research area continues to evolve, with ongoing studies aimed at confirming these findings and developing standardized protocols for wider clinical application.

Comparative data summarizing various methods used for monitoring and assessing cardiac allograft rejection, each with their own advantages and disadvantages, presented in Table 2.

Cost-effectiveness of genetic biomarkers

The evidence currently available confirms that dd-cfDNA is a safe, convenient, and reliable method for transplant monitoring [28]. Acute rejection is associated with a significant increase in treatment costs, and ACR and AMR have contributed to graft loss in more than 60% of graft failures. Compared to these costs, dd-cfDNA testing with ddPCR is reasonable: on average US$401 per test, based on the German Health Charges Code (GOÄ). Potential cost savings would result from fewer biopsies as a result of the tests' high negative predictive value, fewer re-transplantations, and less organ failure [42].

Table 2.

Methods for monitoring and assessing cardiac allograft rejection.

Method	Advantages	Disadvantages
Endomyocardial Biopsy (EMB)	Gold standard, provides direct tissue assessment	Invasive, associated risks, potential sampling errors, inter-observer variability
Echocardiography	Non-invasive, widely available, provides real-time functional information	Indirect measure of rejection, may miss subclinical changes
Cardiac MRI	Non-invasive, detailed tissue characterization, can detect inflammation and fibrosis	Expensive, limited availability, may require gadolinium contrast which has associated risks
Troponin	Non-invasive, widely available, indicator of myocardial injury	Non-specific, elevated levels can result from other cardiac or systemic issues
Gene Expression Profiling	Non-invasive, can detect molecular changes specific to rejection types	Requires sophisticated laboratory setup and data interpretation, may not reflect local changes
Donor-Derived Cell-Free DNA	Non-invasive, can indicate overall graft injury, promising for detecting acute rejection	Expensive, requires baseline levels for comparison, still being validated
MicroRNAs	Non-invasive, potential for specific and sensitive detection of rejection types	Still under research for standardization and validation in clinical settings
DNA-Methylation	Non-invasive, can provide insights into cellular changes specific to rejection	Early in research, requires further validation and standardization

Hospital management, insurance companies/public payers, and policy makers benefit from cost savings due to a decreased burden for care-givers [43]. According to the HT, dd-cfDNA-led surveillance showed less invasive and cost saving alternative to endomyocardial biopsy-led surveillance among pediatric and young adult heart transplantation recipients. Thus, over 20 years from HT, dd-cfDNA-led surveillance is projected to cost $8545 less than endomyocardial biopsy-led surveillance [17]. Among patients who had a heart transplant more than 6 months ago and who had a low risk of rejection, a rejection monitoring strategy involving gene expression profiling (AlloMap test, CareDx, Brisbane, CA), compared with conventional biopsies, was not associated with an increased risk of serious adverse outcomes and resulted in significantly fewer biopsies [46].

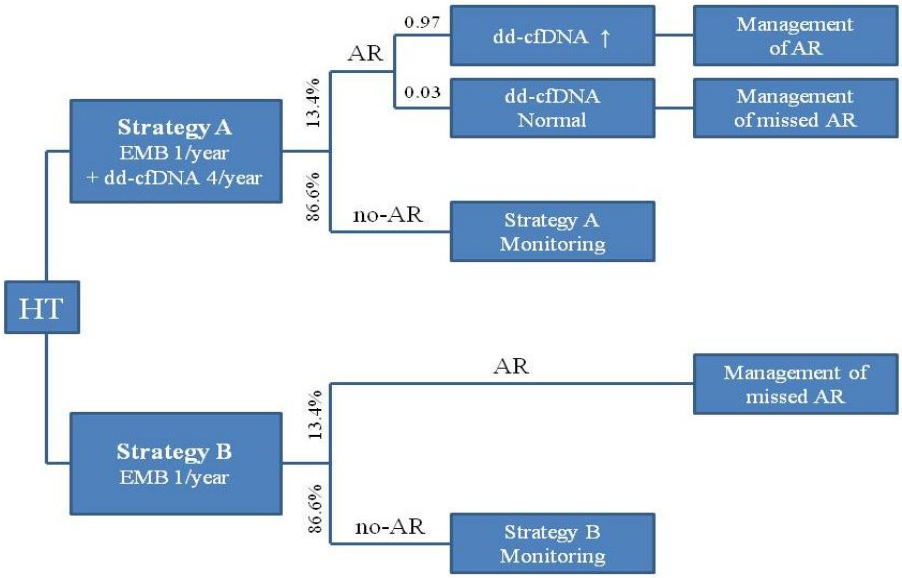

Figure 1. Diagram of the "decision tree" method for monitoring after the HT.

To assess the economic effectiveness of the use of dd-cfDNA-based genetic monitoring to assess the risk of acute rejection (AR) after HT in Kazakhstan, a diagram using the "decision tree" method was constructed, shown in Figure 1. The main clinical and economic parameters for calculating economic efficiency are presented in Table 3.

Strategy A provided for endomyocardial biopsy (EMB) once a year and assessment of the level of dd-cfDNA 4 times a year. Whereas Strategy B only provided for EMB once a year.

Table 3.

Model parameters and data sources.

Variable	Data	Reference
Costs		
Endomyocardial biopsy (EMB)	482 294.17 KZT	estimated
dd-cfDNA test	240148 KZT	
Treatment of acute rejection (AR)	3 000 000 KZT	
Treatment of fatal/non-fatal “missed ” acute rejection (AR)	3 242 232,15 KZT	
Probabilities		
Probabilities of AR	1st year after HT – 13.4% 2nd year after HT – 4% 3rd year and beyond after HT 1.7%	[46]
NPV of dd-cfDNA test	97%	

Based on these calculations, Strategy A was more expensive in comparison with strategy B, namely: in the 1st year after HT - by an average of 230 749.4 KZT per on patient, in the 2nd year - 236 153.6 KZT, and in the third year and more after HT - by 236 153.6 KZT per patient. It is worth noting that in comparison with early studies [17,46], where the cost of treating a patient with "missed" AR exceeded twice the amount for treatment of “non-missed” AR, in Kazakhstan the “missed” case exceeds the “non-missed” case by only 8%. Moreover, this calculation did not take into account such parameters as: the risk and rate of terminal condition of the transplanted heart after a “missed” case of acute rejection, quality of life and survival rate after "missed" and "timely detected" AR. However, further replacement of invasive EMB with a non-invasive dd-cfDNA test will save monitoring costs by an average of 246,140 KZT per patient-year.

Thus, further clinical and economic studies are needed to evaluate the effectiveness of non-invasive monitoring methods for assessing the risk of acute rejection after heart transplantation, including in Kazakhstan.

Conclusion

Complications after heart transplantation have serious consequences, such as a sharp deterioration in quality of life, shortened survival after transplantation, and many others. To date, many methods have been developed for diagnosing early transplant rejection. Invasive diagnostic methods, clinical laboratory data and imaging methods, which were aimed at further monitoring of the patient after transplantation, cannot timely detect acute cellular damage and/or antibody-mediated rejection. Hence, before the development of secondary graft dysfunctions, it is necessary to monitor using non-invasive genetic diagnostic methods. The latest studies established that DNA-based non-invasive tools minimize the risks of invasive procedures. It is safe, convenient and accurate method for diagnosing heart failure after cardiac transplantation.

Declarations

Conflict of interest. *The authors declare no conflict of interest.*

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