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EDITORIAL

AIMS AND SCOPE OF THE JOURNAL

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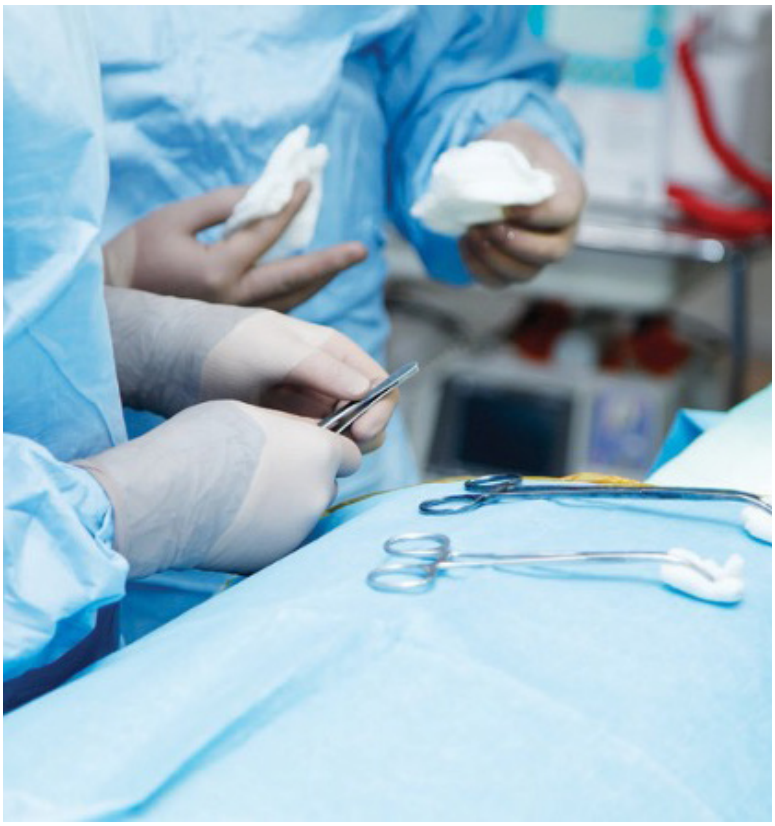

SURGERY AND TRANSPLANTATION

The key activities of these departments of NSMC are reconstructive surgery, transplantation, innovative minimally invasive approaches, microsurgical correction, cell-based therapy of various surgical diseases.
New types of minimally invasive surgical technologies, video-endoscopic and microsurgical techniques, mini-thoracoscopic operations, chemoembolization of tumors were implemented that fundamentally change the approaches to the treatment of many surgical diseases and reduce the time of hospitalization of the patients.

JOURNAL OF CLINICAL MEDICINE OF KAZAKHSTAN (E-ISSN 2313-1519)
Letter to Editor
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Twiddler's Syndrome: a Rare Complication of Pacemakers

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Abstract

Twiddler's syndrome is a rare severe entity first described in 1968 related to transvenous pacemaker placement, involving the pulse generator in a subcutaneous pocket, and the coiling, displacement, or fracture of the leads causing stimulation failure and symptoms recurrence needing immediate revision. Major risk factors for the loss of pacemaker function include bad generator fixation, manipulation, psychiatric disorder, obesity, advanced age, and an oversized pocket. The objective is to emphasize the recent case studies published in this Journal.

Keywords: deep brain stimulation, movement disorders, Twiddler's syndrome.

Dear *Journal of Clinical Medicine of Kazakhstan* Editors,

Twiddler's syndrome (TS) is a rare severe complication of transvenous pacemaker placement first described in 1968, involving the implanted pulse generator (IPG) in the subcutaneous pocket, with excessive coiling, displacement, or fracture of the leads [1-6]. The stimulation failure, and symptoms recurrence, often demand surgical revision. Risk factors for partial or total loss of pacemaker function include bad generator fixation, manipulation, psychiatric disorder, obesity, advanced age, and an oversized pocket [1-6]. This letter aims to emphasize the two recent case studies published in this Journal by Mammadinova I and colleagues describing the TS related to deep brain stimulations [3]. A 73-year-old man with Parkinson's disease and a 55-year-old woman with multifocal dystonia were longstanding well-controlled by bilateral deep brain stimulation with a left-sided dual-channel implanted pulse generator; but presented worsening motor symptoms. Their evaluations showed abnormal impedance readings, and extensive lead coiling that were surgically corrected with the lead replacement and pulse generator repositioning [3]. The authors highlighted the TS critical clinical complications of deep brain stimulation, emphasizing the early diagnosis by imaging and impedance monitoring, besides the role of prevention by adequately implanted pulse generator fixation, and the pocket's size [3].

In this setting, some short comments on new literature data seem to be opportune. A 59-year-old man had discomfort at the site of an implantable cardioverter defibrillator; the heart rhythm and pulse were normal, the ultrasound showed a reduced left ventricular ejection fraction, the atrial lead was not visualized, and the shock coil was misplaced [1]. As the X-ray confirmed lead dislodgement and significant entanglement in the pocket, the diagnosis of TS was established, and the patient underwent a surgical revision; the authors stressed the single-session extraction and re-implantation of cardiac implantable electronic device that was successfully performed with a multidisciplinary approach [1]. A 91-year-old woman with chronic renal disease, hypertension, hypercholesterolemia, and obesity grade I, had implantation of a single-chamber pacemaker through the left subclavian vein due to a complete atrioventricular block causing recurrent syncope [2]. Three weeks later, she presented with dizziness and chest discomfort, an

unpleasant rhythmic sensation in the implant site, and involuntary movements of the chest left side, the electrocardiogram showed no ventricle record, suggesting a pacemaker malfunction, while the chest X-ray revealed that the lead was fully retracted into the device pocket [2]. The authors called attention to the diagnosis of Reel syndrome, which is an uncommon variant of TS characterized by the device rotated on its transverse axis; the electrodes coil near the generator and retract away from the heart, causing pacemaker malfunction [2]. Worthy of note, they emphasized the tip of the lead fully retracted into the pouch of the device they estimated to be a phenomenon not yet described in any previous reports [2]. A 54-year-old woman with antecedents of dilated cardiomyopathy, atrial fibrillation, mechanical valve prosthesis, and single-chamber pacemaker presented with chest pain and involuntary contractions of the left pectoral and left upper limb had TS diagnosis [4]. On cardiac monitoring the pacemaker spikes were not correlated with QRS complexes; the electrocardiogram had a rhythm compatible with atrial fibrillation and pacemaker spikes without any associated QRS complexes, revealing a ventricular capture failure; the chest X-ray showed a TS, with the electrode displacement wrapped in the generator [4]. The authors stressed the suspicion of TS based on involuntary contractions of pectoral and shoulder muscles, and no ventricular capture of the pacemaker by monitor or ECG; and the role of an early diagnosis to perform the prompt and adequate management [4]. A 65-year-old woman with previous heart failure and reduced ejection fraction (HFrEF) status post the implantable cardioverter-defibrillator (Barostim) presented with decompensated heart failure 8 months later, and the chest images showed a fractured lead with the proximal extremity twisted and retracted into the pocket confirming a TS [5]. She was treated for HFrEF exacerbation and scheduled for reimplantation of Barostim; the authors commented on the benefits of the device use considering that lead design reduces the risk of carotid injury when tension is put on the lead with manipulation, and the complication may develop with a delayed presentation and less tension on artery [5]. A 65-year-old woman under cardiac resynchronization therapy-defibrillator had reduced amplitude and occasional detection failure in the atrial lead and increased pacing threshold in the right ventricular lead 13 months post-implant, and a chest X-ray showed twisted dislodged leads, and pulse generator rotation of the TS and Reel syndrome [6].

The authors highlighted the lack of guideline recommendations in the cases of risk factors, for increased follow-up, reimplantation procedures, and routine imaging studies to detect the lead dislodgements, in addition to the electrical testing procedures; moreover, the lead revision is definitively recommended in cases of their dysfunction [6].

Higher suspicion index and practical protocols for TS management can contribute to the outcome improvement among patients with cardiac implantable electronic devices.

Author Contributions: Conceptualization, V. M. S, K. M. S., R. C. N.; investigation, V. M. S, K. M. S., R. C. N.; verification, V. M. S, K. M. S., R. C. N.; writing – original draft preparation, V. M. S, K. M. S., R. C. N.; writing – review and editing, V. M. S. The authors have read and agreed to the published version of the manuscript.

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Immunogenetic Landscape of Secondary Sjögren's Syndrome in Systemic Sclerosis and Lupus Erythematosus: Insights from Kazakhstan

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Abstract

Introduction: Secondary Sjögren's syndrome (sSS) frequently develops in patients systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and systemic sclerosis (SSc). Nevertheless, its immunogenetic features remain poorly understood, particularly in underrepresented populations.

Objective: To investigate the immunological and genetic characteristics of secondary Sjögren's syndrome in Kazakh patients diagnosed with SSc and SLE.

Methods: Patients diagnosed with sSS associated with SLE and SSc were enrolled in the study. SLEDAI-2K and the modified Rodnan skin score were measured, respectively. Antinuclear factor on HEp-2 cells was analyzed by indirect immunofluorescence; autoantibody profiles were determined by immunoblotting. Interleukin (IL)-6 levels were measured by ELISA. Whole-exome sequencing was performed using a targeted panel of autoimmune-related genes. Variants were analyzed and clustered using Ion Reporter software.

Results: Antinuclear factor on Hep2 cells was positive in all SLE-sSS and 75% of SSc-sSS cases. SS-A/Ro60 and SS-A/Ro52 antibodies were frequently detected in both SLE-sSS and SSc-sSS patients, whereas SS-B antibodies were less common. Complement levels were mostly within normal ranges despite 2 SSc-sSS patients. One patient in the SSc group showed an elevated IL-6. Genetic analysis revealed likely pathogenic variants in SAMD9L, ABCC2, IL6ST, and TNFAIP3 in sSS patients.

Conclusion: This study provides insight into the immunogenetic features of secondary Sjögren's syndrome in Kazakh patients, suggesting overlapping genetic patterns in sSS among both SSc and SLE groups. Limitations include the small sample size and cross-sectional design, which limits generalizability, but provide a foundation for further larger-scale research integrating longitudinal follow-up and comprehensive genomic profiling.

Keywords: Secondary Sjögren's Syndrome, Systemic Sclerosis, Systemic Lupus Erythematosus, Antibodies, Genetic Predisposition.

Introduction

Secondary Sjogren's syndrome (sSS) is a chronic autoimmune disease occurs against the background of other autoimmune pathologies, like rheumatoid arthritis (RA), systemic sclerosis (SSc), systemic lupus erythematosus (SLE), polymyositis, and polyarteritis

nodosa [1, 2]. Sjogren's syndrome (SS) characterized by lymphocytic infiltration of the salivary and lacrimal glands, leading to xerostomia and xerophthalmia. Secondary Sjogren's syndrome has a significant impact on the daily activities of patients who often complain about dryness of oral cavity, eyes, as well as dental caries, vaginal dryness, and joint pain [3]. It is accompanied by severe fatigue, depression, anxiety, and decreased physical activity. These conditions can worsen the underlying autoimmune disorder associated with the syndrome and contribute to its more severe clinical manifestations Unfavorable prognostic indicators include delayed diagnosis, male sex, parotid gland enlargement, vasculitis, as well as immunological abnormalities such as hypocomplementemia, cryoglobulinemia, and the presence of anti-SSB antibodies [2].

The etiology of secondary Sjögren's syndrome, like many autoimmune diseases, remains unclear. It is believed that both genetic susceptibility and environmental factors contribute to its development. A variety of exogenous and endogenous elements, including stress, infections, exposure to medications, hormonal fluctuations, and the presence of silicone implants, can disturb immune regulation and initiate the pathological activation of both innate and adaptive immune responses [4]. These pathogens include human T-cell leukemia type 1 virus (HTLV-1), herpes simplex virus (HSV), hepatitis B and C viruses, and cytomegalovirus (CMV) [5]. It is assumed that these viruses are capable of provoking or enhancing autoimmune processes through molecular mimicry, activation of innate immunity, or chronic inflammation. The Epstein–Barr virus (EBV) is considered one of the key infectious triggers of Sjogren's syndrome, as it was found in the tissues of the salivary and lacrimal glands. It promotes the autoimmune process by stimulating epithelial cell apoptosis and activating the innate immune response through Toll-like receptors [6].

Genetic predisposition plays a significant role in the pathogenesis of SS, with both HLA and non-HLA genes being involved in regulating the immune response. HLA-DRB1*0301-DQB1*0201-DQA1*0501 haplotype considered one of the most significant risk factors for the development of SS. One of the most studied genes is interferon-regulating factor 5 (IRF5), which plays a role in the production of type I interferons and the activation of inflammation [7]. A polymorphism in the promoter region of IRF5, specifically the insertion/deletion of the CGGGG sequence, has been associated with increased gene expression and an increased risk of SS [8-10]. One of the first genes found to be associated with the development of SS syndrome was STAT4, this genetic variant is also linked to other autoimmune conditions, such as SLE and RA, highlighting common pathogenic pathways among these disorders [11]. Genetic variants in IL12A, BLK, PTPN22, and CXCR5 show moderate to low association with the development of SS and may be considered as potential risk factors; the same alleles have also been identified in patients with SLE [8, 12]. Disruption in the regulatory pathways involving CHEK1, ETS1, LEF1, TIMP1, and CXCL10 may lead to elevated MMP9 expression, potentially contributing to the development and progression of SS [13].

Autoantibodies play a crucial role in diagnosing Sjögren's syndrome, including its secondary form, which develops in the context of other autoimmune diseases such as SLE and RA. The most characteristic are anti-Ro/SSA and anti-La/SSB antibodies, associated with earlier disease onset, systemic manifestations, and exocrine gland involvement [14, 15]. However, these antibodies are not exclusive to SS and may also be found in other autoimmune conditions. Part of patients may lack these antibodies, highlighting the importance of a comprehensive diagnostic approach that includes clinical evaluation and additional serological markers. In sSS, particularly when combined with RA, rheumatoid factor (RF) and antinuclear antibodies (ANA) are frequently present, complicating differential diagnosis. Emerging markers such as anti-SP1, CA6, NA14 and PSP have been investigated, especially in early stages of the disease [16].

Pathogenesis is initiated by the activation of B- and T-lymphocytes, as well as dendritic cells, which together trigger an autoimmune cascade. T-lymphocytes, especially Th1 and Th17 populations, secrete pro-inflammatory cytokines including interferon-gamma (IFN-gamma) and interleukin-17 (IL-17). IFN-I play role in the pathogenesis of SS by enhancing the activation of immune cells, including NK cells, $CD8^+$ T lymphocytes, and macrophages. Dendritic cells - the main sources of IFN-I, founded in the tissue of the salivary glands of patients, which indicates their possible involvement in the formation of local inflammatory changes [17]. These cytokines contribute to the chronicity of inflammation and damage to exocrine glands. Activated B lymphocytes, among other things by the action of BAFF, differentiate into plasma cells that produce autoantibodies mainly against Ro/SSA and La/SBB antigens, which are found in most patients with SS [4]. Together, these mechanisms contribute significantly to the development and advancement of the disease.

The clinical manifestations of sicca are most often observed in patients with RA (in 31%), SSc (in 20.5%), and SLE (in 8.5%)[18, 19]. In patients with RA, who also suffer from sSS, arthritis is more severe and devastating than in those diagnosed with RA alone [20, 21]. Patients with RA and sSS tend to have higher disease activity by DAS28 [22].

Patients with SLE and sSS exhibit a distinct clinical and serological profile. This subgroup is characterized by a later disease onset, longer disease duration, higher prevalence among women, and a higher risk of chronic disease progression [23]. The most prevalent ocular symptom of SLE is keratoconjunctivitis sicca [24]. Clinically, sSS-SLE is more frequently associated with thyroiditis, arthritis, oral ulcers, interstitial pneumonitis, and renal tubular acidosis. Serologically, these patients demonstrate elevated levels of IgG, rheumatoid factor (RF), and anti-Ro/SSA and anti-La/SSB antibodies [23, 25].

sSS often develops in patients with SS, especially with a limited skin form of the disease. Sicca manifestations are frequently observed in patients with SSc. Among patients with SSc, those with sSS more frequently exhibit anticentromere antibodies and have a lower prevalence of pulmonary fibrosis compared to those without Sjögren's involvement [26]. While histological examination of minor salivary glands in these individuals often reveals significant fibrosis, the presence of lymphocytic infiltration typical for primary Sjögren's syndrome is noted in only a small proportion of biopsies [27].

Secondary Sjögren's syndrome is diagnosed using classification criteria originally developed for primary Sjögren's syndrome (pSS), as no specific criteria for sSS have been officially established. The 2016 ACR/EULAR classification criteria are most commonly applied; they integrate serological, histopathological, and ophthalmological findings. A diagnosis is made when a patient score ≥4 points within this framework and presents with either typical sicca symptoms or systemic activity according to the ESSDAI [28]. Although intended for pSS, these criteria have demonstrated acceptable sensitivity and specificity when applied to sSS [29]. Nonetheless, due to the variable clinical manifestations of sSS, particularly in the presence of coexisting systemic autoimmune diseases, a case-by-case clinical assessment remains essential.

Objective

To investigate the immunological and genetic characteristics of secondary Sjögren's syndrome in Kazakh patients diagnosed with SSc and SLE.

Methods

This cross-sectional study included Kazakh patients diagnosed with SSc (n=30) and SLE (n=30). Patients were recruited from National scientific medical center between July 2023 and July 2024.

Inclusion criteria:

- Confirmed diagnosis of SSc according to the 2013 ACR/EULAR classification criteria; or confirmed diagnosis of SLE according to the 2019 EULAR/ACR classification criteria;
- Diagnosis of secondary Sjögren's syndrome based on the 2016 ACR/EULAR classification criteria;
- Ethnic Kazakh origin.

Exclusion criteria:

- Primary Sjögren's syndrome;
- Other concomitant autoimmune diseases

Control group include individuals without any autoimmune pathology. All participants provided written informed consent in accordance with the Declaration of Helsinki.

Clinical and demographic data were collected from medical records and included age, sex, disease duration, organ injury, and treatment history. SLE patients were assessed for disease activity and impairment of internal organs by SLEDAI-2K score, while for SSc patients we counted modified Rodnan skin score (mRSS).

Whole blood samples were collected for further genetic and immune analysis.

Immunological analysis included ANA HEp2, anti-SSA/Ro, anti-SSB/La, as well as other antibodies common for SLE or SSc, and complement levels (C3, C4). Detection of antinuclear factor on HEp-2 cells (ANA HEp-2) was performed by indirect immunofluorescence in blood serum using an automated fluorescent system (AKLIDES). The presence of specific antinuclear antibodies (dsDNA, Sm, SS-A/60, SS-A/52, SS-B, Scl-70, CENP-B, U1-snRNP, Ribosomal P0, Jo-1, Nucleosome, Histone, RNP/Sm) was investigated using immunoblotting (qualitative result) and indirect immunofluorescence reaction (quantitative result in IU/mL) methods. Levels of C3 and C4 complement components were determined by immunoturbidimetry using a Cobas Integra 400 biochemical analyzer.

Inflammatory cytokine Interleukin-6 was determined by ELISA on an automated immunoassay analyzer Alisei.

Genetic analysis started with DNA Extraction and Library Preparation. Genomic DNA was extracted from whole blood samples using the GENEJET™ Whole Blood Genomic DNA Minikit. DNA quantity was measured with Qubit fluorometry (Qubit 1X dsDNA HS Kit), ensuring sufficient purity and concentration for downstream analysis.

Systematic review of currently available up-to-date literature sources in PubMed and Google Scholar allow to develop the Next-Generation Sequencing panel to identify gene variations relevant to connective tissue disorders, musculoskeletal conditions, and immune-related diseases autoimmune pathology in common, in SLE and SSc particularly [30]. Library preparation involved multiplex amplification using the Ion AmpliSeq Library Kit Plus. Libraries were purified with AMPure XP beads and prepared for emulsion PCR using the Ion PI™ Hi-Q™ OT2 200 system, followed by sequencing on the Ion Proton platform with Ion PI™ v3 chips.

Sequencing data was processed using Ion Reporter software. Initial quality control included filtering short or low-quality reads. High-quality sequences were aligned to a reference genome, and variants (SNPs, indels, structural changes) were identified using the SeqCut tool. Annotations were derived from ClinVar, dbSNP, and other public databases. Filters were applied to prioritize variants: minimum read depth of 30, predicted pathogenic impact (missense, frameshift), and relevance to autoimmune disease. Splice site and gene extension filters were also applied to capture regulatory variants.

Statistical analyses were carried out using Microsoft Excel and IBM SPSS Statistics version 21.0. The choice of statistical tests was guided by the distribution of the data, assessed using the Shapiro–Wilk test for normality. Continuous variables were presented as means ± standard deviation (SD). Hierarchical clustering and heatmap visualization were performed using Ion Reporter Software v5.20 (Thermo Fisher Scientific, USA). Categorical variables were analyzed using Fisher's exact test or the Chi-square (χ^2) test, as appropriate.

Results

Apart from 30 SSc patient twelve individuals were diagnosed with sSS (40%), while within SLE patients it was rare – only 13.3% suffer from sSS (4 patients out of 30 individuals). Descriptive statistics for secondary Sjogren's syndrome are presented in the table 1.

Serological markers

The most common antibodies for sSS according to clinical guidelines and up-to-date research are anti-SS-A/Ro60 and SS-A/Ro52, targeting a protein La (SSB), which plays a role in RNA processing. Due to the data obtained from the current analysis of patients with secondary Sjögren's syndrome, SS-A/Ro60 and SS-A/Ro52 antibodies were frequently detected, while SS-B was less common. Among those with SLE (n = 4), three tested positive for both SS-A/Ro60 and SS-A/Ro52, and one had SS-B antibodies. In the SSc group (n = 12), five patients were positive for SS-A/Ro60, six for SS-A/Ro52, and one for SS-B. This pattern highlights the predominance of SS-A antibodies in both disease contexts. The table 2 summarizes the presence of SS-A/Ro60, SS-A/Ro52, and SS-B autoantibodies in patients diagnosed with secondary Sjögren's syndrome within systemic lupus erythematosus and systemic sclerosis groups. No antibodies were found at the control group.

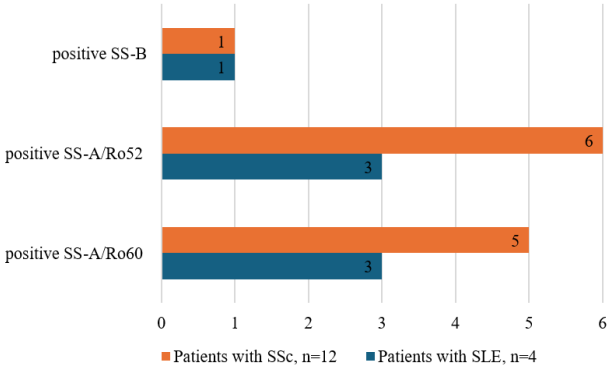

Figure 1 – Autoantibody Profile in Patients with Secondary Sjögren's Syndrome Associated within systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) groups

Table 1 Clinical Characteristics of Patients with SLE and SSc with Secondary Sjögren's Syndrome

No	Sex	Diagnosis	Disease Duration (years)	Steroids (daily dose)	Immunosuppressive Therapy (daily dose)	SLEDAI-2K/ mRSS
L02	**F**	SLE, chronic, active stage 2, with skin and appendage involvement Sjögren syndrome	11	MP 6 mg	Azathioprine 50 mg	22
L13	F	SLE, chronic, with skin and joint involvement, Sjögren syndrome	9	–	Azathioprine 100 mg	6
L14	F	SLE, chronic, with skin (erythema), blood system (anemia), stage 1–2. Sjögren syndrome	19	MP tapering to 8 mg	Hydroxychloroquine 200 mg/day, MMF 1000 mg/day	8
L22	F	SLE, chronic, with multi-organ involvement (skin, vessels, lungs, heart, blood), Sjögren's syndrome	22	MP 6 mg	MMF 1250 mg, Hydroxychloroquine 200 mg	12
S4	F	SSc, chronic, skin, GI tract (esophagitis), Sjögren's syndrome (sialoadenitis, xerostomia)	1	–	MMF 500 mg	2
S6	F	SSc, chronic, stage 2, with skin (edema, induration), joints, lungs (pneumofibrosis, PAH), secondary Sjögren's syndrome	13	MP 8 mg	Nintedanib 200 mg, Leflunomide	9
S11	F	SSc, stage 2, skin, joints, GI (esophagitis), lungs injury (pneumosclerosis), vessels, Sjögren's syndrome	10	MP 4 mg	Leflunomide 20 mg	14
S12	F	SSc, stage 2, skin, joints, vessels (Raynaud's syndrome), lungs (pneumosclerosis), Sjögren's syndrome	7	–	Methotrexate 5 mg/day	9
S15	F	SSc, chronic, with skin edema (face, perioral), Raynaud's, GI, joints, Sjögren's syndrome, Cushingoid, PAH	10	–	Leflunomide 20 mg, D-Penicillamine 500 mg	26
S16	M	Progressive SSc, subacute, stage 2, skin, lungs, vessels, GI, joints impairment, Sjögren's syndrome	3	MP 12 mg	D-Penicillamine 500 mg	15
S18	F	SSc, stage 2, skin, joints, Raynaud's syndrome	15	–	–	19
S19	F	SSc, subacute, stage 2, diffuse, with multisystem involvement, Raynaud's syndrome, Sjögren's syndrome	11	–	Methotrexate 7.5 mg, MMF 1000 mg	6
S21	F	SSc, chronic, stage 1, vascular (Raynaud's syndrome), GI, joints, skin impairment, Sjögren's syndrome	10	–	D-Penicillamine 500 mg	18
S24	F	SSc, chronic, stage 2, limited form, skin, joints, thyroid (AIT), GI injury, Raynaud's syndrome, Sjögren's syndrome	3	MP 8 mg	–	22
S27	F	SSc, generalized, stage 2, skin, joints, GI, lungs, Raynaud syndrome, Sjögren syndrome	12	–	MMF 500 mg	10
S29	F	SSc, chronic, stage 1, skin involvement with sclerodactyly, Raynaud's, GI, joints, lungs impairment	5	–	MMF 1000 mg	20

MP: Methylprednisolone; MMF: Mycophenolate mofetil; GI: Gastrointestinal tract; D-Pen: D-Penicillamine; AIT: Autoimmune thyroiditis; NFS: Functional class; PAH: Pulmonary arterial hypertension; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index; mRSS: Modified Rodnan Skin Score.

Table 2 The frequency of specific antibody to Sjogren's syndrome - anti-SS-A/Ro60 and SS-A/Ro52 in the studied Kazakh cohort of patients diagnosed with secondary Sjögren's syndrome within systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) groups

Secondary Sjögren's Syndrome	positive SS-A/Ro60	positive SS-A/Ro52	positive SS-B
Secondary Sjögren's Syndrome in Patients with SLE, n=4	3	3	1
Secondary Sjögren's Syndrome in Patients with SSc, n=12	5	6	1

In patients with SLE-SS (n=4) with a history of secondary Sjögren's syndrome, other autoantibodies were also detected: dsDNA (1 out of 4), Sm (1 of 4), U1-snRNP (1 of 4), and RNP/Sm (1 of 4). In patients with SSc-SS (n=12), the following additional antibodies were found: Sm (2 of 12), U1-snRNP (4 of 12), RNP/Sm (5 of 12), Ribosomal P0 (2 of 12), and CENP-B (3 of 12). The antinuclear factor on HEp2 cells was positive (at a titer >1:160) in all 4 patients with SLE-SS and the majority of patients with SSc-SS (75%, 9 of 12, Fig. 2). The negative titer <1:80 were found in 3 patients with SSc-SS.

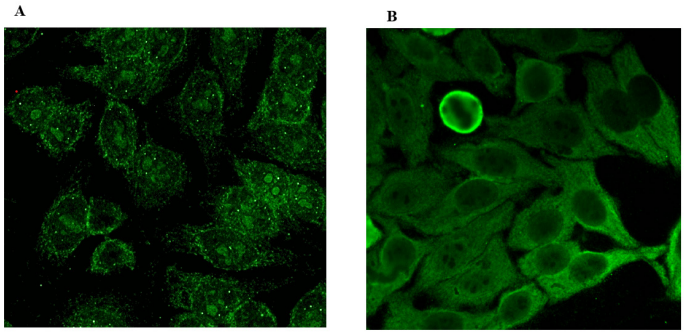

Figure 2 – Antinuclear factor (ANA HEp2). (A) Patient L13, Antinuclear factor on HEp2 cells estimated by IIFA, titre 1:320, nucleolar (AC-8/9/10) and cytoplasmic reticular (AC-21) of glow; positive antibodies to SS-A/52; (B) Patient S06, Antinuclear factor, titre 1:640, nuclear speckled (AC-4/5) and cytoplasmic dense fine speckled (AC-19/20) type of luminescence; positive antibodies to Sm, SS-A/60, U1-snRNP, RNP/Sm, ribP0. *AC – anti-cellular, anapattern.org

Complement and Cytokine Levels in Secondary Sjögren's Syndrome

As shown in Table 3, all patients with secondary SS associated with SLE (n = 4) had C3 and C4 complement levels within the normal reference range. In the SSc-associated sSS group (n = 12), C3 levels were normal in 10 patients and slightly decreased in 2, while C4 was normal in 11 and reduced in 1. Interleukin-6 (IL-6) levels remained within the normal range for all SLE patients, whereas one patient in the SSc group showed an elevated IL-6. Patient S6 (table 1) had skin impairment with edema, induration, joints injury, lungs involvement with pneumofibrosis and pulmonary arterial hypertension (PAH).

Table 3

Levels of C3 and C4 complement components, and interleukin-6 in patients with Secondary S

Indicators	Patients with Secondary SS connected to SLE, n=4			Patients with Secondary SS connected to SSc, n=12		
	normal	below reference interval	above reference interval	normal	below reference interval	above reference interval
Complement C3 (N: 0,90 - 1,80 g/l)	4	0	0	10	2	0
Complement C4 (N: 0,10 - 0,40 g/l)	4	0	0	11	1	0
Cytokine IL-6 (N: 0,0 - 10,0 pg/ml)	4	-	0	11	-	1

Genetic analysis

In patients with Sjögren's syndrome, the variants in the SAMD9L gene - chr7:92764981 T/TT (ref TC) and chr7:92761606 GT/G - were found exclusively in the systemic sclerosis patient group. Two patients, S06 and S15, carried both variants simultaneously. According to the ACMG classification, both variants are categorized as likely pathogenic. The Orphanet database associates the SAMD9L gene with a rare autoinflammatory syndrome characterized by nodular panniculitis, lipoatrophy, severe early-onset interstitial lung disease, and basal ganglia calcification. Most patients exhibit progressive isolated cytopenia affecting B-cells and natural killer (NK) cells.

Notably, two variants in the IL6ST gene were detected in one patient (L22) from the SLE cohort. The variant chr5:55265588 AT/A is classified as likely pathogenic, while chr5:55265655 G/C is of uncertain clinical significance.

Additionally, two likely pathogenic variants in the ABCC2 gene (chr10:101603641 CA/C and chr10:101559041 CA/C) were identified in the systemic sclerosis group. This gene belongs to the transporter protein family, primarily expressed in hepatocytes. While ABCC2 is linked to Dubin-Johnson syndrome (DJS)—an autosomal recessive disorder causing conjugated hyperbilirubinemia—no direct correlation with autoimmune diseases or involvement in immune regulation has been established.

In our study, variants in the IRF5 and STAT4 genes were identified among the patient cohort; however, none of the individuals exhibited clinical features characteristic of Sjögren's syndrome. For example, a variant of uncertain clinical significance in the IRF5 gene (chr7:128588751 AG/A) was detected in two patients with systemic lupus erythematosus and in three individuals diagnosed with rheumatoid arthritis. Additionally, a variant of uncertain clinical significance in the STAT4 gene (chr2:191929615 C/T) was identified in one patient from the rheumatoid arthritis group. It is worth mentioning that in our cohort of patients with systemic sclerosis and Sjögren's syndrome, a likely pathogenic variant in the TNFAIP3 gene (chr6:138199775 T/TC) was also identified.

Other genes included in the panel developed for this study, such as BLK and IKZF1, were not detected in the group of patients with Sjögren's syndrome. However, a variant of uncertain clinical significance in the BLK gene (chr8:11405631 AG/A) was identified in six individuals diagnosed with systemic lupus erythematosus. Additionally, in one SLE patient, a combination of two variants of unknown significance (chr8:11405631 AG/A and chr8:11407753 G/C) was observed.

It was not detected among patients with Sjögren's syndrome, but two variants of uncertain clinical significance in TYK2 (chr19:10476458 C/T and chr19:10472451 C/T) were identified in two individuals diagnosed with rheumatoid arthritis.

Among the participants, two individuals with systemic lupus erythematosus and two with systemic sclerosis were found to carry variants of CTLA4, including likely pathogenic changes at chr2:204732740 GT/G and chr2:204736165 G/GT, as well as a variant of uncertain clinical significance at chr2:204736181 C/T. It is important to note that the chr2:204732740 GT/G variant was also identified in the control group, indicating that no significant statistical association was established between this variant and autoimmune disorders.

The heatmap in the figure 3 provides a visual overview of genetic variants identified in patients with secondary Sjögren's syndrome. Each column represents an individual patient, while each row corresponds to a gene included in the panel. The colors indicate the predicted effect of each variant: darker shades represent more severe impacts (such as splice-site changes), while lighter tones reflect moderate or likely benign effects. Variants of uncertain significance and those with unknown clinical relevance are marked in green and blue. Most patients carried a mix of moderate and low-impact variants, with a few individuals showing high-impact changes that may affect gene function. The presence of recurrent variant patterns across multiple patients suggests potential common pathways involved in immune regulation and connective tissue pathology. This profile underscores the genetic complexity of secondary Sjögren's syndrome and highlights several candidate genes for further functional investigation.

Statistical analysis revealed no significant data in comparison with control group, potentially because of small study group.

Discussion

Our study represents the first immunogenetic investigation of secondary Sjögren's syndrome occurring alongside systemic sclerosis and systemic lupus erythematosus in a Kazakh cohort.

Clinically, the SSc cohort exhibited more extensive organ involvement — pulmonary, vascular, and glandular — while the SLE group tended toward hematologic, mucocutaneous, and glandular involvement. These differences underscore the importance of context-specific diagnosis and tailored

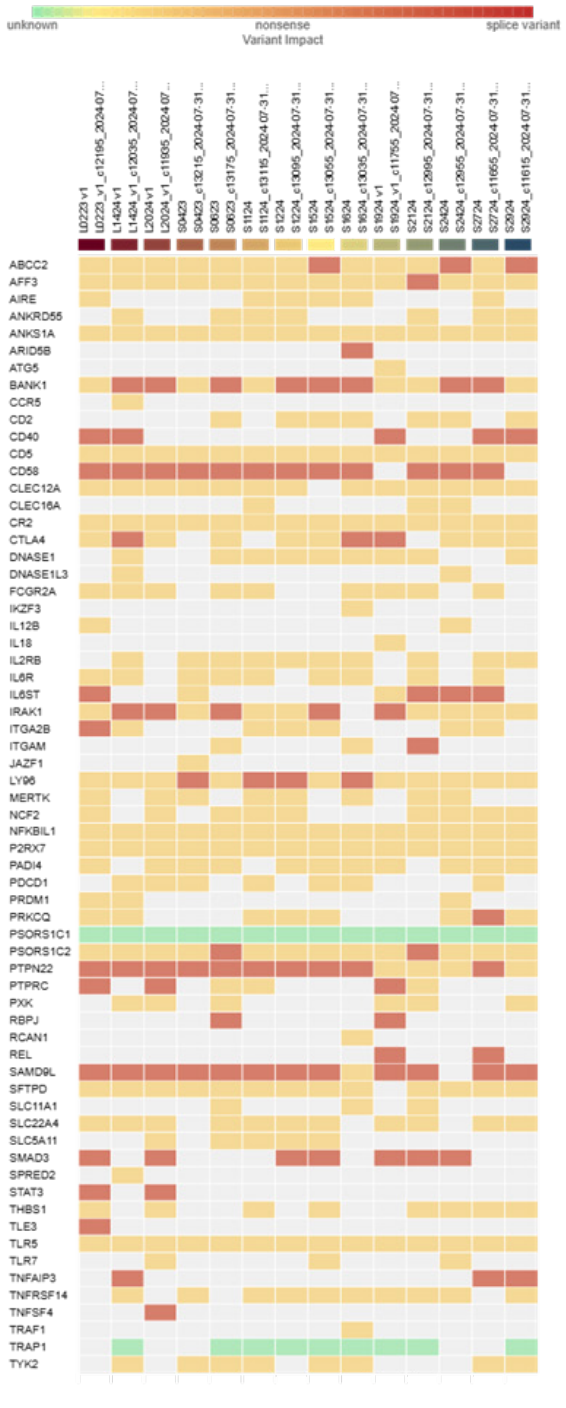

Figure 3 – Heatmap displays the distribution of germline variations across targeted genes in patient samples diagnosed with secondary Sjögren's syndrome (sSS) in the context of systemic sclerosis (SSc) and systemic lupus erythematosus (SLE)

Rows represent the targeted genes, and columns represent individual patient samples. Samples were hierarchically clustered based on the presence and predicted impact of mutations, as determined by Ion Reporter 5.20 (Thermo Fisher Scientific, Darmstadt, Germany). The color scale reflects variant classification: green indicates variants of unknown impact, orange represents missense variants, red denotes nonsense variants, maroon signifies splice variants, and white indicates no detected variant meeting the inclusion criteria. Inclusion criteria for variant reporting were set at a minimum coverage of 30 reads and a variant allele frequency (VAF) threshold of ≥20%. Hierarchical clustering and visualization of the heatmap were conducted utilizing the integrated germline variant annotation tools within Ion Reporter 5.20. Genes are organized according to alphabetical order

therapy when managing sSS depending on its autoimmune background.

We observed that anti SSA/Ro60 and anti SSA/Ro52 antibodies were frequently detected in sSS patients, with Ro52 showing slightly higher prevalence among those with SSc. This aligns with recent studies in SSc identifying anti Ro52 positivity as an independent biomarker for interstitial lung disease (ILD) and progressive lung involvement [31-33]. In fact, a longitudinal study reported that patients with anti Ro52 experienced faster declines in pulmonary function, especially those with early SSc ILD [32]. According to our data 83% of SSc patients with Sjögren's syndrome (5 of 6) had lung impairment in different forms.

Complement protein analysis yielded further insights: all SLE associated sSS patients had normal C3 and C4 levels, while a 2 of SSc associated cases exhibited lower complement values. This may indicate immune complex activation within the SSc group, reflecting differing mechanisms of inflammation in SSc versus the more immune complex–driven pathology typical of SLE.

Interleukin 6 (IL 6) emerged as another finding. The serum level of IL-6 was not elevated in Sjögren's syndrome associated with SLE, however one SSc sSS patient displayed elevated serum IL 6 levels. There was no significant difference in the serum level of IL-6 between groups of the patients with secondary Sjögren's syndrome or control group. The same was found in the literature, nevertheless, numerous recent reports link high IL 6 in SSc to disease severity, presence of digital ulcers, anti Scl 70 antibodies, and cardiopulmonary manifestations such as pulmonary hypertension [34]. Our observation, though limited to a single elevated case, echoes these trends and suggests IL 6 may be a marker of active inflammation and organ involvement in SSc related sSS.

Genetic analysis revealed variants in IL6ST, SAMD9L, CTLA4 and ABCC2 genes. Tala Shahin et al. described patients with inborn errors of immunity and pathogenic IL6ST variants presenting with hyper-IgE syndrome [35]. The strongest association signals are observed in the HLA gene region. In contrast, non-HLA genes such as IRF5 and STAT4 show consistent associations across different ethnic populations, although their effect sizes are comparatively smaller. Most genetic risk variants are located in intergenic regions, and in many cases, their functional roles remain largely unexplored [36]. In another study, in addition to the markers mentioned above, the TYK2 gene SNP rs11085725 was also highlighted [37].

The involvement of additional gene polymorphisms in disease pathogenesis has also been described, with several identified as risk factors. These include polymorphisms in the CD28 cluster of differentiation (CD28 haplotype GC, showing an odds ratio of 2.5 and $P < 0.001$), cytotoxic T-lymphocyte–associated antigen 4 (CTLA4), protein tyrosine phosphatase non-receptor type 22 (PTPN22W), tumor necrosis factor alpha (TNF-α, allele 308 A), interleukin-10 (IL-10, allele 1082 G), and C-X-C chemokine receptor type 5 (CXCR5). Each of these molecules contributes to lymphocyte function at different stages, such as tissue migration (CXCR5), activation and proliferation (CD28, CTLA4), receptor-mediated signaling (PTPN22W), and immunoglobulin secretion (IL-10). For instance, CTLA4 serves as an inhibitory molecule that restrains cell cycle progression and downregulates transcription factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thereby preventing abnormal T-cell proliferation [38].

Subsequent studies should expand the scope of immunogenetics by utilizing large-scale genome-wide association studies (GWAS) focused on the Kazakh population. Due to the unique ethnicity and genetics of this population, population-based comparative studies would deepen our understanding of the complex genetic factors influencing the risk and disease course of secondary Sjögren's syndrome in the context of SSc and SLE. Clarification of gene-environment relationships, especially with common viral stimuli like Epstein–Barr virus (EBV) and cytomegalovirus (CMV), could aid in formulating tailored precision interventions rooted in genetic data when advanced bioinformatics frameworks are harnessed.

Longitudinal cohort studies are imperative for the characterization of predictive biomarkers for preemptively identifying individuals at increased risk of developing secondary Sjögren's syndrome. The integration of longitudinal clinical phenotyping together with molecular and serological data, including cytokine expression, microRNA profiles, and developing autoantibodies, stands to greatly enhance the prognostic models and early intervention frameworks. In particular, the application of machine learning approaches to diverse datasets has the potential to identify previously uncharacterized clusters of biomarkers.

Prospective, long-term cohort studies are recommended, encompassing detailed clinical evaluations alongside periodic molecular assessments. Incorporating serial measurements of circulating cytokine levels (such as IL-6, IFN-γ, and IL-17), evolving microRNA expression profiles, and autoantibody dynamics (including anti-Ro/SSA and anti-La/SSB antibodies) can significantly enhance predictive accuracy. Implementing advanced bioinformatics approaches, including machine learning algorithms, could facilitate real-time risk stratification, personalized patient management, and target therapeutic interventions aimed at delaying or halting disease progression.

To deepen our understanding of the genetic architecture underlying secondary Sjögren's syndrome, future studies should conduct expansive genome-wide association studies (GWAS) and comprehensive whole-genome sequencing analyses. Emphasis should be placed on recruiting large, ethnically diverse populations, especially considering the distinctive genetic backgrounds present within the Kazakh cohort. Such investigations will likely identify novel susceptibility loci and elucidate the roles of rare variants that may contribute disproportionately to disease pathogenesis. Incorporating population genetics methods, comparative analyses with international genetic databases, and rigorous statistical validation will be instrumental in uncovering critical genetic determinants and previously unexplored pathogenic pathways.

Comprehensive functional analyses are required to substantiate the pathogenic relevance of genetic variants identified in secondary Sjögren's syndrome. In-depth cellular experiments using primary patient-derived immune cells, complemented by CRISPR/Cas9 genome editing technology, will help elucidate variant-specific impacts on cellular signaling pathways, immune cell activation, and cytokine production. Additionally, the generation and analysis of genetically modified animal models will offer indispensable insights into disease mechanisms, organ-specific immune responses, and the efficacy of novel therapeutic interventions. These functional studies hold great promise in advancing personalized treatment strategies and uncovering therapeutic targets tailored specifically to the unique genetic profiles observed in secondary Sjögren's syndrome.

Several notable methodological constraints warrant explicit acknowledgment in the context of interpreting our study findings. Primarily, our investigation included a relatively modest sample size, inherently constraining statistical power and potentially impacting the generalizability and extrapolation of our results to broader patient populations. This limitation increases the possibility of Type II statistical errors, potentially masking genuine associations or exaggerating observed findings. Moreover, the sampling methodology employed may have introduced inadvertent selection biases, particularly given the specific clinical setting and inclusion-exclusion criteria that guided patient recruitment. Such biases could limit the representation of diverse clinical phenotypes and stages of disease progression, thereby narrowing the applicability of findings across different patient subgroups. Additionally, due to practical constraints, the genetic findings in our study were not extensively validated through complementary experimental methodologies, thereby potentially affecting the robustness, reliability, and clinical interpretability of identified genetic variants.

Conclusion

This study offers a first glimpse into the immunogenetic features of secondary Sjögren's syndrome in patients with systemic sclerosis and systemic lupus erythematosus within the Kazakh population. Our findings reveal distinct patterns of autoantibody profiles, complement component levels, cytokine expression, and genetic variants, suggesting both shared and disease-specific immunopathogenic mechanisms. The observed genetic heterogeneity, particularly in immune-related genes, supports the notion of sSS as a complex, polygenic condition influenced by underlying autoimmune disease context. These insights contribute to a more personalized understanding of sSS and may guide future efforts toward targeted diagnostics and therapeutic approaches in ethnically diverse populations.

Author Contributions: L.Z. conceptualized the study, supervised the research process, and contributed to manuscript preparation. A.B. conceived of the presented idea. L.Z. and N.K. were responsible for patient recruitment, D.M. – for clinical data collection, A.B. and Zh.Zh. for sample processing and immune analysis. M.S. and Zh.Zh. performed the genetic analyses and bioinformatic interpretation. L.Z. and L.K. drafted the manuscript, and all authors reviewed and approved the final version.

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Data availability statement: The corresponding author can provide the data supporting the study's conclusions upon request. Due to ethical and privacy constraints, the data are not publicly accessible.

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Comparison of Pericapsular Nerve Group Block and Femoral Nerve Block for Ease of Positioning During Spinal Anaesthesia in Hip Fracture Surgeries: A Prospective Randomized Controlled Trial

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Abstract

Background: Hip fractures often require spinal anaesthesia for surgery, but positioning for spinal anaesthesia is painful, necessitating preoperative regional nerve blocks. This study compares ultrasound-guided pericapsular nerve group (PENG) and femoral nerve block (FNB) for positioning efficacy in hip fracture patients.

Methods: In current prospective, randomized controlled trial, 70 patients with unilateral hip fractures were allocated to either PENG (n=35) or FNB (n=35) group. Pain was assessed via Numerical Rating Scale (NRS), Positioning quality was categorized by hip flexion angle and rescue analgesia requirement was recorded.

Results: NRS scores were lower in the PENG group at 20 minutes (4.74 vs. 6.20, p=0.001), 30 minutes (2.80 vs. 4.83, p=0.001), and during positioning of spinal anaesthesia (1.74 vs. 2.97, p=0.001). The PENG group had better positioning quality, with Category A achieved by 20 (57.14%) patients versus 12 (34.29%) in the FNB group (p=0.036). Rescue analgesia was needed by 1 (2.86%) patient in PENG roup versus 7 (20%) patients in FNB group (p=0.027).

Conclusions: The PENG block offers superior pain relief and positioning quality for spinal anaesthesia in hip fracture patients, with less rescue analgesia required compared to FNB.

Keywords: Pain Management, Patient Positioning, Bupivacaine, Ultrasonography, Regional Anaesthesia

Introduction

The hip joint, commonly known as a "ball and socket" joint, is an important joint where the acetabulum (the socket) cradles the femoral head (the ball). The femoral neck connects the head to the shaft and intertrochanteric region, forming an essential link in the hip's anatomy[1,2]. Hip fractures can occur in any of these areas and are particularly common among older adults with existing health conditions [3]. It poses high morbidity and mortality, with an derived estimate of 200,000 hip fractures annually in India over 50 years [4]. There are several treatments available [5], but ultimately they often require specialised surgical interventions [6]. Hip surgery is generally performed under spinal anaesthesia due to its simplicity, effectiveness and low perioperative complications [7]. However, positioning these patients for spinal anaesthesia can be difficult and extremely painful requiring regional nerve blocks to enhance patient comfort and procedural ease [7,8]. Preoperative regional nerve blocks such as the femoral

nerve block (FNB), fascia iliaca block , and pericapsular nerve group (PENG) block are employed to enhance spinal anaesthesia [9,10,11]. These blocks improve patient positioning, provide immediate pain relief, reduce postoperative pain, enabling earlier mobilization and shorter hospital stays [8,12].

As a relatively new technique, the PENG block has limited research comparing it to the well-established FNB. To bridge this gap, this study was aimed to evaluate the analgesic efficacy of ultrasound-guided PENG and FNB in facilitating spinal anaesthesia positioning for hip fracture patients. The primary objective was to compare the analgesic effects, while the secondary objectives were to assess quality of position during spinal anaesthesia and postoperative pain relief among the two blocks.

Methods

In this prospective, randomized study conducted at a tertiary care centre in northern India, researchers compared two regional anaesthesia techniques for positioning during spinal anaesthesia in hip fracture patients from January to December 2024. The study received approval from the Biomedical Research Ethics Committee (BREC/23/TH-Anaesthesia/36) and was registered with the Clinical Trial Registry of India (CTRI/2024/01/061612).

The study included ASA Physical Status I–III adult patients aged 18–85 years, of any gender, with a unilateral hip fracture scheduled for surgery under spinal anaesthesia. However, individuals with bleeding tendencies, multiple fractures, anticoagulant therapy, peripheral neuropathy, fractures older than a week, allergies to amide local anaesthetics, mental health conditions, local infections at the injection site, inability to report pain scores, or those who declined participation were excluded.

Sample size was determined based on a study by Lin Dy et al.[13], using an α of 1.96, β of 0.84, 80% power, a 7% effect size (▲), and a standard deviation (σ) of 0.1.

Each patient underwent a thorough clinical history review and examination. Subjects were explained about the study's purpose, protocol, Numerical Rating Scale (NRS) [14,15] for pain (0–10) and a written informed consent was obtained. Using computer-generated randomization, patients were assigned to one of two groups:

Group PENG (n=35): Received a Pericapsular Nerve Group (PENG) block before spinal anaesthesia and

Group FNB (n=35): Received a Femoral Nerve Block (FNB) before spinal anaesthesia.

Following ASA fasting guidelines, patients were brought to the premedication room 30 minutes before surgery. There, standard monitoring was done with ECG, non-invasive blood pressure (NIBP), and oxygen saturation (Spo2), and an IV access was secured and ringer lactate infusion was started.

Both PENG and FNB blocks were performed under ultrasound-guidance as it improves the efficacy and safety of the regional block, reducing the risk of adverse events while providing effective analgesia [16]. Sonosite Edge-II ultrasound machine with a curvilinear (3.5–5 MHz) transducer was used. A consultant anaesthesiologist with five years of ultrasonography experience administered the blocks, injecting 20 ml of 0.25% bupivacaine in 5-ml increments, ensuring proper spread and performing negative aspiration at the start and after each 5-ml dose.

A different consultant anaesthesiologist, unaware of the study group recorded the study observations.

Pain was assessed using Numerical Rating Scale (NRS) at different time points : before the block (T0), 20 min after the block (T1), 30 minutes after the block (T2). Patients were then shifted to the operating theatre. Spinal anaesthesia was performed using a 23G Quincke's needle in the sitting position via a midline approach. Pain score was assessed during positioning for spinal anaesthesia (T3). If a patient reported an NRS score ≥4, they received 1g of paracetamol, and positioning was reattempted. A block was deemed optimal if the NRS remained <4 during positioning. Postoperative NRS scores were also noted at 4 hours (T4) and 24 hours (T5), with additional analgesia provided as and when needed by the surgical team.

The quality of patient positioning was measured using a goniometer to assess hip flexion angle, categorized as shown in Figure 1: Category A: Good flexion (>90 degrees), Category B: Average flexion (<90 degrees without twisting or using hand support), Category C: Poor flexion (and/or twisting or using hand support) [12].

Number of spinal anaesthesia attempts, the time taken, and the need for rescue analgesics were also recorded.

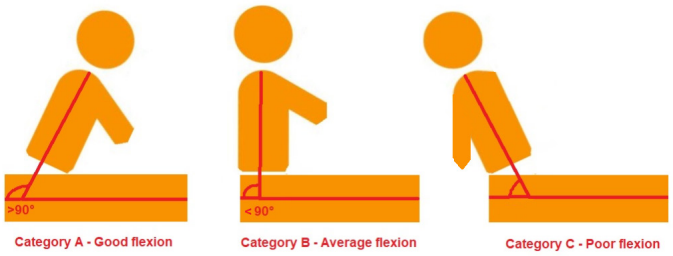

Figure 1 – Quality of positioning Categorization
Image Credit: Tarun Yadav.

Results

This study compared the efficacy of Pericapsular Nerve Group Block (PENG) with Femoral Nerve Block (FNB) in facilitating spinal anaesthesia focusing on comparing pain relief, quality of positioning, rescue analgesic requirements. Study assessed a total of 78 patients, out of which 8 patients refused to participate so finally 70 patients were enrolled and were subsequently randomized into either PENG group or FNB group (Figure 2).

Demographics between PENG and FNB groups were comparable, with no significant differences in age, sex, weight, height, BMI, or ASA grades. Both groups had similar mean ages, sex distributions, and physical characteristics and ASA grades (Table 1).

Comparison of quality of positioning during spinal anaesthesia demonstrated that PENG group had a better quality of position compared to the FNB group with Category A having 20 (57.14 %) of PENG group patients versus 12(34.29%) of FNB group patients, Category B having 13 (37.14%) of PENG group patients versus 14(40.00%) of FNB group and Category C having only 2 (5.71%) of PENG group patients versus 9 (25.71%) of FNB group with a significant statistical difference (p-value 0.036) (Figure 3).

In the PENG group, 32 (91.43%) patients achieved spinal anaesthesia in Ist attempt, 3 (8.57%) patients in IInd attempt,

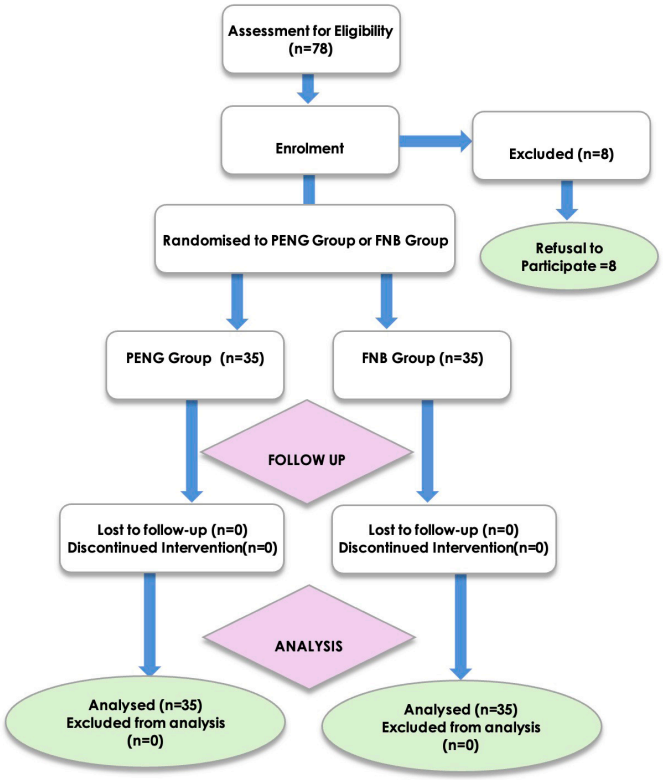

Figure 2 – CONSORT flow chart

Table 1 Demographic data among PENG and FNB group

Parameter	PENG Group	FNB Group	p-value
Mean Age (years)	61.89 ± 15.26	61.71 ± 15.93	0.754#
Male	23 (65%)	17 (48%)	0.160*
Female	12(35%)	18 (52%)	
Weight (kg)	64.34 ± 10.80	66.69 ± 6.69	0.280#
Height (cm)	166.17 ± 6.03	167.17 ± 7.80	0.950#
BMI (kg/m^2)	24.02 ± 2.41	23.60 ± 2.58	0.484#
ASA Grade I	3 (8.57%)	3(8.57%)	0.727*
ASA Grade II	23 (65.71%)	20(57.14%)	
ASA Grade III	9 (25.71%)	12(34.29%)	

*# Unpaired t test, * chi-square test*

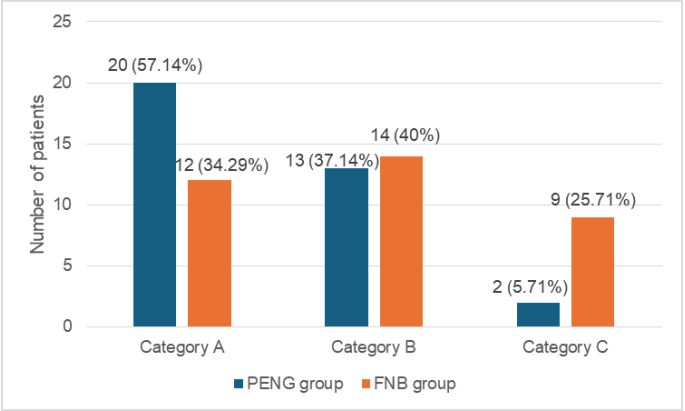

Figure 3 – Comparison of quality of positioning during spinal anaesthesia among PENG and FNB groups

X axis: Hip flexion angles categories; Y axis: Number of patients; PENG: Pericapsular nerve group block ; FNB: Femoral nerve block; Category A: Good flexion; Category B: Average flexion; Category C: Poor flexion.

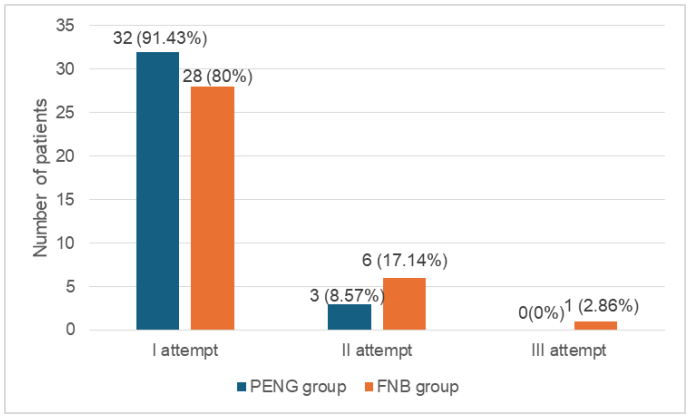

Figure 4 – Number of spinal anaesthesia attempts among PENG and FNB groups

X axis: Number of attempts to achieve spinal anaesthesia;
Y axis: Number of patients;
PENG: Pericapsular nerve group block ;
FNB: Femoral nerve block.

and none of the 0 (0%) patients required IIIrd attempt. While in the Femoral group, 28 (80%) patients achieved spinal anaesthesia in Ist attempt, 6 (17.14%) patients in IInd attempt and 1 (2.86%) patient in IIIrd attempt. Comparison of number of attempts between two groups during spinal anaesthesia showed statistically non-significant difference (p-value 0.32) (Figure 4).

The mean time taken for spinal anaesthesia was 46.14±8.70 sec in PENG group and 46.29±10.31 sec in FNB group which were comparable with non-significant difference (p=0.48).

In the PENG group, only 1 (2.86%) patient needed rescue analgesia while positioning during spinal anaesthesia whereas, in the FNB group, 7 (20 %) participants needed rescue analgesic. Rescue analgesia requirement in PENG group was significantly lower when compared to FNB group (p-value 0.027) (Figure 5).

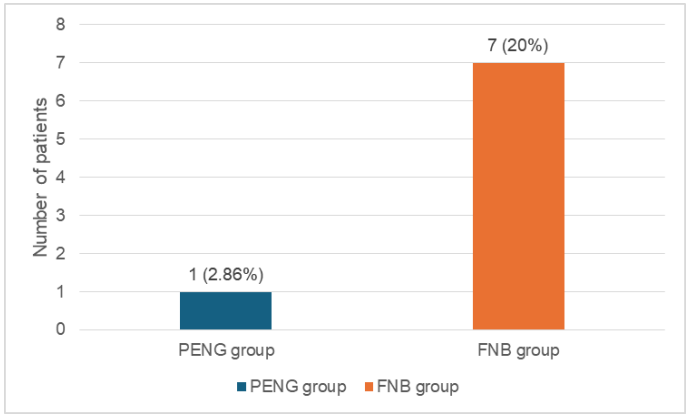

Figure 5 – Rescue Analgesic requirement among PENG and FNB groups

X axis: Rescue Analgesic requirement;
Y axis: Number of patients;
PENG: Pericapsular nerve group block;
FNB: Femoral nerve block.

When NRS pain score was compared among PENG and FNB groups, PENG group had significantly lower pain scores at 20 min after block (T1) (4.74±0.74 vs. 6.20±0.87; p=0.001), 30 min after block (T2) (2.80±1.05 vs. 4.83±0.93; p=0.001), and during positioning of spinal anaesthesia (T3) (1.74±0.74 vs. 2.97±0.99; p=0.001). However, difference was not statistically

Table 2 Comparison of NRS pain score at different time intervals among PENG and FNB groups

Conditions	PENG group	FNB group	p-value
Baseline (T0)	8.91 ± 1.04	8.91± 1.01	1.000
20 mins after regional group (T1)	4.74 ± 0.74	6.20 ± 0.87	0.001(S)
30 min after regional group (T2)	2.80 ± 1.05	4.83 ± 0.93	0.001(S)
During spinal anaesthesia positioning (T3)	1.74 ± 0.74	2.97 ± 0.99	0.001(S)
4hr Postoperatively (T4)	2.97 ± 0.75	2.89 ± 1.18	0.718
24hr Postoperatively (T5)	5.17 ± 0.66	5.43 ± 0.78	0.141

significant at baseline (T0) (p=1.0), 4 hrs postoperatively (T4) (p=0.718) and 24 hrs postoperatively (T5)(p=0.141) (Table 2).

Discussion

The present study aimed to compare pericapsular nerve group block (PENG) and femoral nerve block (FNB) for ease of positioning during spinal anaesthesia in hip fracture surgeries.

The PENG block demonstrated superior pain relief, with significantly lower Numeric Rating Scale (NRS) scores at 20 minutes after block (T1) (4.74 vs. 6.20, p=0.001), 30 minutes after block (T2) (2.80 vs. 4.83, p=0.001), and during spinal anaesthesia (T3) (1.74 vs. 2.97, p=0.001) compared to the FNB. This is likely due to the PENG block's targeting of the femoral, accessory obturator, and obturator nerve articular branches, which innervate the anterior hip capsule's nociceptive mechanoreceptors [9]. In contrast, the FNB may not adequately block these branches, resulting in suboptimal pain relief. Similar findings were reported by Jeevendiran et al. [17] and Chaudhary et al. [18], who noted significant reductions in pain scores with the PENG block.

In current study positioning quality, assessed by hip flexion angle, was significantly better in the PENG group, with 57% of patients achieving Category A (Good flexion) compared to 34% in the FNB group (p=0.036). This aligns with Jeevendiran et al. [17], who reported better sitting angles in the PENG group (63% vs. 26%, p=0.001), and Alrefaey et al. [12], who observed 87% of PENG patients achieving flexion >90°. Improved positioning likely results from enhanced analgesia, facilitating patient cooperation. However, Chaudhary et al. [18] found no significant difference, possibly due to differing measurement scales, suggesting a need for standardized assessment tools.

No significant difference was observed in spinal anaesthesia attempts, with 91.43% of PENG group and 80% of FNB group patients receiving spinal anaesthesia on the first attempt (p=0.32). This is similar with Erten et al. [19] and may reflect the expertise of the consultant anaesthesiologists, minimizing procedural variability.

The PENG group required significantly less rescue analgesia during spinal anaesthesia (3% vs. 20%, p=0.027) over FNB group, supporting its superior analgesic profile. Jeevendiran et al. [17] reported similar results (5.7% vs. 25.7%, p=0.045), highlighting the PENG block's effectiveness in reducing supplemental analgesia needs. Sahoo et al. [20] in a case series also found a significant reduction in VAS scores at rest (baseline: 6.77 ± 1.20; 30 minutes post block: 0.44 ± 0.52).

Postoperative NRS scores at 4 hours (2.97 vs. 2.89, p=0.718) and 24 hours (5.17 vs. 5.43, p=0.141) showed no significant differences, likely due to the waning effect of single-shot blocks and postoperative use of other analgesics (e.g., tramadol, NSAIDs). Allard et al. [21] reported similar findings, while Chaudhary et al. [18] noted significant pain relief in the PENG group at 6 and 8 hours, possibly due to protocol differences. Continuous blocks may improve postoperative outcomes.

No complications (e.g., bleeding, nerve injury, infection) were reported in either group, likely due to ultrasound guidance and experienced consultants.

Limitations

The study's single-centre design and small sample size (n=70) limit generalizability. Postoperative mobility, muscle function, and hospital stay were not assessed, and single-shot blocks may have restricted prolonged postoperative analgesia. Future studies should explore continuous blocks, larger multicentre cohorts, and functional outcomes.

Conclusion

In conclusion, the PENG block demonstrated significantly superior positioning quality for spinal anaesthesia compared to the femoral nerve block. Patients in the PENG group reported significantly lower NRS pain scores and required less rescue analgesia during positioning. These findings suggest that the PENG block offers clear advantages in terms of patient comfort and procedural facilitation. Further multi-centre, large-scale studies incorporating continuous postoperative nerve blocks are warranted to confirm these results and further explore the clinical benefits of the PENG block.

Author Contributions: Conceptualization, T. Y., M. G., H. C. ; methodology, T. Y., M. G., H. C. ; validation, T. Y., M. G., H. C.; formal analysis, T. Y., M. G., H. C.; investigation, T. Y., M. G., H. C.; data curation, T. Y., M. G., H. C., D. W.; writing – original draft preparation, T. Y., M. G., H. C., D. W., J. L.; writing – review and editing, T. Y., M. G., H. C., D. W., J. L., S. T. All authors have read and agreed to the published version of the manuscript.

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Health and Treatment-Related Anxiety among Kidney Transplant Recipients in Kazakhstan

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Abstract

Background: Kidney transplantation (KTx) is a life-saving intervention, but post-transplant psychological well-being and treatment adherence remain critical challenges. This study aimed to (1) develop and validate a Health and Treatment-Related Anxiety Scale in Kazakh and Russian languages, (2) identify predictors of such anxiety, and (3) examine its impact on adherence to treatment and well-being among kidney transplant recipients in Kazakhstan.

Methods: A cross-sectional study was conducted among 223 adult kidney transplant recipients. The newly developed anxiety scale was tested for psychometric validity using exploratory and confirmatory factor analyses. Internal consistency and criterion-related validity were assessed through item-rest correlations and correlation with related outcomes. Multiple linear regression and mediation models were used to identify predictors of health and treatment-related anxiety and psychological mechanisms affecting treatment adherence and well-being.

Results: The scale demonstrated strong psychometric properties with a one-factor structure and high internal consistency (Cronbach's α=0.893). Anxiety negatively correlated with both treatment adherence ($p<0.001$). Regression analysis identified employment under disability status and perceived availability of post-KTx monitoring as protective factors. Emotional support and anxiety levels significantly predicted treatment adherence. Mediation analyses revealed that anxiety partially mediated the relationship between perceived post-KTx monitoring and both adherence and well-being ($p<0.05$).

Conclusion: The validated anxiety scale is a reliable tool for assessing psychological vulnerability in kidney transplant recipients. Addressing treatment-related anxiety may enhance post-transplant care by improving both adherence and emotional well-being.

Keywords: kidney transplantation, anxiety, validation, adherence, well-being.

Introduction

Kidney transplantation is the preferred treatment option for patients with end-stage renal disease, offering significantly improved survival rates and quality of life. However, despite these clinical benefits, the psychological impact of transplantation remains an important and often underexplored issue. In Kazakhstan, much of the existing literature has focused on the clinical and immunological outcomes of kidney transplantation such as graft rejection, biomarkers, and pathophysiology [1–4]. In contrast, fewer studies have addressed the psychological consequences that follow transplantation, even though mental health plays a crucial role in patient recovery and long-term outcomes.

Anxiety and depression are common psychological disorders among transplant recipients [5]. Studies show that depression affects 13% to 40% of transplant patients and doubles the risk of mortality [6]. Anxiety, reported in up to 35% of

kidney transplant recipients [7], is similarly linked to poorer outcomes, including lower quality of life (QOL), higher rates of hospitalization, and increased complications such as rejection and graft dysfunction [8]. What is more, depression has been linked to significantly lower adherence to post-transplant medical regimens, with affected patients being up to three times more likely to be non-adherent [9].

Understanding the factors contributing to psychological distress, particularly anxiety, among kidney transplant recipients is therefore essential. Research indicates that several things, such as stress, depression, long-term medication treatment, dread of the unknown, and uncertainty about the future, may cause such anxiety in transplant recipients [10]. A recent study conducted in Kazakhstan further highlights the importance of contextual and demographic factors in shaping psychological outcomes. It identified key determinants of psychological well-being among kidney transplant recipients, including educational level, access to healthcare services, and anxiety related to post-transplant treatment [11]. Notably, patients residing in rural areas faced greater barriers to care and reported higher levels of psychological distress, with anxiety emerging as a strong negative predictor of overall well-being.

In this regard, it is essential to assess anxiety in kidney transplant recipients using validated psychological tools to address psychological challenges. These tools usually provide a standardized and reliable method for identifying patients at risk of developing anxiety-related complications. For instance, the Hospital Anxiety and Depression Scale is an effective screening tool for anxiety and depression among kidney transplant recipients, with high sensitivity and specificity [8]. Similarly, the Spielberger State-Trait Anxiety Inventory (STAI) has been widely used to assess both state and trait anxiety in transplant populations [12]. Implementing such assessments can guide the development of targeted psychological interventions and inform healthcare policies aimed at improving both mental health and clinical outcomes.

Despite growing awareness, there is limited understanding of the specific mechanisms through which anxiety influences treatment adherence and psychological well-being among kidney transplant recipients in Kazakhstan. Thus, this study aims to (1) develop and validate a Health and Treatment-Related Anxiety Scale (HTRAS) in Kazakh and Russian languages, (2) identify predictors of such anxiety, and (3) examine its impact on adherence to treatment and well-being among kidney transplant recipients in Kazakhstan.

Methods

Study Design and Participants

A cross-sectional study was conducted among adult kidney transplant (KTx) recipients in Kazakhstan between February and March 2025. Participants were recruited through outpatient nephrology and transplant clinics affiliated with major urban hospitals, as well as via patient WhatsApp chat groups moderated by transplant coordinators or peer volunteers. Eligible individuals were aged ≥18 years, had received a kidney transplant at least three months prior, and were fluent in either Kazakh or Russian.

Participants were recruited through outpatient nephrology and transplant clinics affiliated with major urban hospitals, as well as through patient WhatsApp support group chats moderated by transplant coordinators or peer volunteers. The online survey link (Google Forms) was distributed via these support chats, while additional participants were contacted and invited by phone. All participants received comprehensive information about the study's objectives, procedures, and their rights as participants. Informed consent was obtained electronically or verbally for phone interviews. Anonymity and confidentiality were rigorously upheld: no personal identifiers were collected, and all responses were securely stored and accessed only by the research team. Participation was entirely voluntary and involved no physical or psychological risk. Participants could withdraw at any stage without consequence.

A total of 223 recipients participated, with 96 completing the Kazakh-language version and 127 completing the Russian-language version of the survey. The final sample was linguistically and socio-demographically diverse, including rural and urban residents and individuals with varying education and occupational statuses.

Instrument Development and Translation

Patients' concerns regarding post-transplant (post-KTx) health and treatment were assessed using a newly developed 7-item scale (HTRAS), with each item rated on a 5-point Likert scale (1 = never to 5 = always), adapted from the framework of Rhu et al. (2019) [13]. The items addressed specific anxiety-provoking domains, including: (1) risk of infection due to immunosuppressive therapy, (2) concerns about maintaining kidney function, (3) anxiety over unfavorable laboratory results, (4) long-term health stability, (5) financial burden of treatment, (6) emotional distress such as depression or low mood, and (7) side effects of immunosuppressive medications. Higher cumulative scores indicated greater health and treatment-related anxiety [9].

The item pool and wording were refined through expert consultation with professionals fluent in English, Kazakh, and Russian. The expert panel included clinical nephrologists, health psychologists, and researchers with experience in psychometric validation. Their input ensured that the scale was both culturally appropriate and clinically relevant for the multiethnic transplant recipient population in Kazakhstan.

Measures

In addition to the HTRAS, the questionnaire included several validated and self-developed measures:

- Subjective well-being was assessed using the WHO-5 Well-Being Index, a widely used and validated tool for measuring psychological well-being [14]. The scale consists of five positively worded items, each rated on a 6-point scale from 0 ("At no time") to 5 ("All of the time"), with total raw scores ranging from 0 to 25. To standardize results, the raw score was multiplied by four to yield a percentage score ranging from 0 to 100, where higher scores indicate greater well-being.

- Self-reported adherence to the post-transplant treatment regimen was assessed with a single item: "How do you assess your ability to follow your post-transplant treatment regimen (including taking immunosuppressive medication)?" Participants responded on a 5-point scale ranging from "Very easy to follow" to "I do not follow."

- Perceived emotional care after kidney transplantation was evaluated using a single item asking whether participants

received emotional support from family, friends, or healthcare providers. Response options were categorized as "All of the time," "Sometimes," or "Never."

- Perceived availability of follow-up care was assessed using a 5-point Likert scale ranging from 1 ("Very unavailable") to 5 ("Very available").

- Sociodemographic and clinical data were also collected, including age, gender, marital status, number of children, place of residence (urban vs. rural), education level, occupation, and receipt of disability benefits. Clinical variables included the presence of comorbid conditions.

Psychometric Validation

Internal consistency was assessed using Cronbach's alpha and item-rest correlations (IRC). Criterion validity was tested by examining Spearman correlations between HTRAS scores and well-being, adherence, and perceived monitoring availability.

Exploratory Factor Analysis (EFA) was conducted separately for each language version and the full sample to assess the dimensionality of the HTRAS. Suitability for factor analysis was evaluated using the Kaiser-Meyer-Olkin (KMO) measure and Bartlett's test of sphericity. Confirmatory Factor Analysis (CFA) was then employed to test the model fit of the one-factor solution. Model fit was evaluated using the Comparative Fit Index (CFI), Tucker–Lewis Index (TLI), and Root Mean Square Error of Approximation (RMSEA).

Statistical Analysis

Descriptive statistics were used to characterize the sample. Group comparisons were performed using Chi-square and independent-sample t-tests. To identify predictors of anxiety and adherence, multiple linear regression models were fitted, controlling for sociodemographic, clinical, and psychosocial variables. Two mediation analyses were conducted to examine whether HTRAS scores mediated the relationship between perceived monitoring availability and: (1) treatment adherence, and (2) psychological well-being. All statistical analyses were performed using Jamovi software (version 2.6.17), and results were considered statistically significant at $p < 0.05$.

Results

A total of 223 kidney transplant recipients participated in the study, with 96 individuals in the Kazakh-language group and 127 in the Russian-language group. The mean age was 37.7±10.2 years in the Kazakh-language group and 39.5±10.5 years in the Russian-language group. The median duration since kidney transplantation was 47.5 months (IQR: 72.5) in the Kazakh-language group and 36 months (IQR: 79.0) in the Russian-language group, with no statistically significant difference between the groups.

The gender distribution was similar between groups, with 44.8% males in the Kazakh-language group and 43.3% in the Russian-language group (p=0.825, Table 1). Participants aged 18–39 years constituted 57.3% of the Kazakh group and 49.6% of the Russian group (p=0.255).

Educational attainment differed marginally between groups (p=0.052), with a higher proportion of college-educated individuals in the Kazakh group (34.4%) compared to the Russian group (19.7%). Notably, a significant difference was found in place of residence, with 22.9% of Kazakh-speaking participants residing in rural areas compared to only 8.7% in the Russian-speaking group (p = 0.003).

Table 1 Study participants (N=223)

Variable	Kazakh-language group (n=96)		Russian-language group (n=127)		χ^2, p
	n	%	n	%	
Gender					
Male	43	44.8	55	43.3	0.05, p=0.825
Female	53	55.2	72	56.7	
Age group					
18-39 years	55	57.3	63	49.6	1.30, p=0.255
40-64 years	41	42.7	64	50.4	
Education					
Middle-school	6	6.3	5	3.9	9.37, p=0.052
High-school	11	11.5	11	8.7	
College	33	34.4	25	19.7	
Undergraduate	45	46.9	85	66.9	
Post-graduate	1	1.0	1	0.8	
Residence					
Rural	22	22.9	11	8.7	8.81, p=0.003
Urban	74	77.1	116	91.3	
Occupation					
Student	6	6.3	13	10.2	6.13, p=0.294
Employed	39	40.6	43	33.9	
Self-employed	11	11.5	15	11.8	
Unemployed	11	11.5	7	5.5	
On disability benefits	27	28.1	42	33.1	
Pensioner	2	2.1	7	5.5	
Marital status					
Single	24	25.0	42	33.1	3.12, p=0.374
Married	63	65.6	72	56.7	
Divorced	8	8.3	9	7.1	
Widow	1	1.0	4	3.1	
Children					
No	32	33.3	47	37.0	0.323, p=0.570
Yes	64	66.7	80	63.0	
Comorbidities					
No	34	35.4	49	38.6	0.235, p=0.628
Yes	62	64.6	78	61.4	

Occupational status, marital status, parenthood, and presence of comorbidities did not differ significantly between groups. However, a relatively high proportion of participants in both groups reported being on disability benefits (28.1% in the Kazakh group vs. 33.1% in the Russian group).

Validity of the Health and Treatment-Related Anxiety Scale

Descriptive statistics and IRC for the newly developed Health and Treatment-Related Anxiety Scale (HTRAS) are presented in Table 2. Across both language versions, the most frequently endorsed item was concern about the financial costs associated with prescribed post-transplant treatment, with a mean score of 4.18 (SD=1.21) in the Kazakh-language group and 3.65 (SD=1.28) in the Russian-language group. The least endorsed item was the experience of depression or low mood after transplantation, with mean scores of 3.22 (SD=1.52) and 2.77 (SD=1.28) in the Kazakh- and Russian-language groups, respectively. Most items showed strong item-rest correlations, indicating good internal consistency. In the Kazakh-language

Table 2 Post-KTx health and treatment-related anxiety scale items descriptive statistics and item-rest correlations

Item	Kazakh-language			Russian-language		
	M	SD	IRC	M	SD	IRC
Concern about the risk of infection due to taking immunosuppressants after transplantation	3.97	1.33	0.712	3.50	1.20	0.770
Concern about how well the function of the transplanted kidney will be maintained	4.11	1.19	0.633	3.67	1.24	0.790
Concern about poor laboratory test results during clinic visits	3.81	1.24	0.708	3.83	1.13	0.774
Concern about how long health will remain stable after transplantation	4.04	1.26	0.705	3.73	1.24	0.833
Concern about financial costs associated with prescribed post-transplant treatment	4.18	1.21	0.394	3.65	1.28	0.601
Experience of depression or low mood after transplantation	3.22	1.52	0.538	2.77	1.28	0.671
Concern about potential problems with the transplanted kidney and overall health due to taking immunosuppressive medications	3.57	1.43	0.643	3.43	1.31	0.808

version, IRC values ranged from 0.394 to 0.712, while in the Russian-language version they ranged from 0.601 to 0.833. These results support the individual item contributions to the overall construct of post-transplant anxiety.

Internal consistency reliability was also strong. Cronbach's alpha values were 0.853 for the Kazakh-language version, 0.918 for the Russian-language version, and 0.893 in the total sample, confirming the scale's high internal reliability (Table 3).

Table 3 Post-KTx health and treatment-related anxiety scale validity

Language	Cron-bach's α	Bartlett's test of sphericity	KMO MSA	CFI	TLI	RMSEA
Kazakh	0.853	p<0.001	0.859	0.968	0.953	0.078
Russian	0.918	p<0.001	0.903	0.954	0.931	0.126
Total	0.893	p<0.001	0.901	0.967	0.951	0.093

To examine the scale's construct validity, EFA was first performed. The analysis supported a one-factor solution for both language versions. Sampling adequacy was confirmed with a KMO measure of 0.859 for the Kazakh-language group, 0.903 for the Russian-language group, and 0.901 for the total sample. Bartlett's test of sphericity was significant ($p < 0.001$ for all), indicating that the data were appropriate for factor analysis.

Confirmatory factor analysis (CFA) was subsequently conducted to validate the unidimensional structure identified by EFA. Model fit indices supported good fit in both language versions and in the overall sample. For the Kazakh-language scale, the model yielded a CFI of 0.968, TLI of 0.953, and RMSEA of 0.078. The Russian-language version showed similarly adequate fit, with CFI = 0.954, TLI = 0.931, and RMSEA = 0.126. In the combined sample, CFI and TLI remained high (0.967 and 0.951, respectively), with an RMSEA of 0.093. These results are detailed in Table 3.

Table 4 Correlation analysis

Variable	M±SD	(1)	(2)	(3)	(4)	(5)
Months after KTx (1)	57.0± 47.0	-				
WHO-5 well-being index (2)	66.1± 24.6	0.012 (p= 0.862)	-			
Health and treatment-related anxiety (3)	3.65± 1.00	-0.136 (p= 0.043)	-0.253 (p< 0.001)	-		
Availability of post-KTx monitoring (4)	3.22± 0.97	0.025 (p= 0.711)	0.171 (p= 0.011)	-0.192 (p= 0.004)	-	
Ability to follow treatment regime (5)	4.16 ± 0.90	-0.039 (p= 0.561)	0.413 (p< 0.001)	-0.249 (p< 0.001)	0.173 (0.009)	-

To further evaluate the criterion-related validity of the Health and Treatment-Related Anxiety Scale, Spearman correlation analysis was conducted among key psychological and treatment-related variables (Table 4).

Health and treatment-related anxiety was negatively correlated with the WHO-5 well-being index (rho=-0.253, $p < 0.001$), indicating that higher anxiety levels were associated with lower subjective well-being. Additionally, anxiety showed a modest but significant negative correlation with perceived availability of post-transplant monitoring services (rho=-0.192, p=0.004), and with the self-reported ability to follow the treatment regimen (rho=-0.249, p<0.001). These findings suggest that greater anxiety may hinder both psychological well-being and treatment adherence behaviors, thereby supporting the scale's criterion-related validity.

In contrast, the WHO-5 well-being index positively correlated with both perceived monitoring availability (rho=0.171, p=0.011) and ability to follow treatment (r=0.413, p<0.001), further reinforcing the interconnection between psychological health and treatment-related outcomes in kidney transplant recipients.

Predictors of health and treatment-related anxiety among kidney transplant recipients

Perceived emotional care following kidney transplantation, defined as emotional support received from relatives, friends, or healthcare workers, was reported as consistently available ("all of the time") by 72.2% of participants, occasionally ("sometimes") by 24.7%, and absent ("never") by 3.1%. Although a one-way ANOVA indicated a statistically significant difference in health and treatment-related anxiety levels across these groups ($p = 0.03$), subsequent post-hoc comparisons did not reveal significant pairwise differences. Participants who reported never receiving emotional support had the highest mean anxiety score (4.35± 0.67), followed by those who sometimes received support (3.78

Table 5 Predictors of health and treatment-related anxiety among kidney transplant recipients in Kazakhstan (F=2.22, p=0.004, R^2=0.164)

Variable	β	β 95%CI	p
Gender			
Female – Male	0.035	-0.238 – 0.308	0.801
Age group			
40–64 – 18–39 years	0.178	-0.149 – 0.506	0.284
Months after KTx	-0.100	-0.235 – 0.035	0.146
Education	0.023	-0.123 – 0.169	0.756
Residence			
Urban – Rural	-0.276	-0.670 – 0.116	0.166
Occupation			
Student – on disability benefits	-0.538	-1.084 – 0.008	0.053
Employed – on disability benefits	-0.606	-0.939 – -0.273	<0.001
Self-employed – on disability benefits	-0.411	-0.862 – 0.039	0.073
Unemployed – on disability benefits	-0.100	-0.619 – 0.418	0.703
Pensioner – on disability benefits	-0.138	-0.839 – 0.563	0.699
Marital status			
Married – Single	0.084	-0.463 – 0.630	0.763
Divorced – Single	-0.161	-0.769 – 0.447	0.603
Widow – Single	-0.543	-1.590 – 0.503	0.307
Children			
Yes – No	-0.187	-0.720 – 0.347	0.491
Comorbidities			
Yes – No	0.043	-0.236 – 0.321	0.763
Perceived emotional care after KTx			
All of the time – Never	-0.520	-1.288 – 0.249	0.184
Sometime – Never	-0.450	-1.239 – 0.339	0.262
Availability of post-KTx monitoring	-0.147	-0.288 – -0.006	0.042

Table 6 Predictors of ability to follow treatment regime among kidney transplant recipients in Kazakhstan (F=6.27, p<0.001, R^2=0.370)

Variable	β	β 95%CI	p
Gender			
Female – Male	0.015	-0.222 – 0.253	0.898
Age group			
40–64 – 18–39 years	0.066	-0.220 – 0.352	0.649
Months after KTx	-0.088	-0.206 – 0.030	0.142
Education	0.105	-0.022 – 0.232	0.106
Residence			
Urban – Rural	0.307	-0.037 – 0.650	0.080
Occupation			
Student – on disability benefits	-0.076	-0.555 – 0.403	0.756
Employed – on disability benefits	-0.113	-0.412 – 0.186	0.458
Self-employed – on disability benefits	-0.118	-0.513 – 0.277	0.557
Unemployed – on disability benefits	-0.129	-0.581 – 0.323	0.574
Pensioner – on disability benefits	0.048	-0.658 – 0.563	0.878
Marital status			
Married – Single	-0.104	-0.579 – 0.372	0.668
Divorced – Single	-0.877	1.407 – -0.347	0.001
Widow – Single	-0.362	-1.275 – 0.551	0.435
Children			
Yes – No	0.026	-0.439 – 0.490	0.912
Comorbidities			
Yes – No	-0.158	-0.400 – 0.085	0.201
Perceived emotional care after KTx			
All of the time – Never	1.509	0.837 – 2.180	<0.001
Sometime – Never	0.591	-0.098 – 1.279	0.093
Availability of post-KTx monitoring	-0.032	-0.156 – 0.092	0.612
Health and treatment-related anxiety	-0.199	-0.319 – -0.078	0.001

±1.03), and those who reported consistent emotional support (3.58±1.00).

To identify predictors of health and treatment-related anxiety among kidney transplant recipients in Kazakhstan, a multiple linear regression analysis was conducted (F=2.22, p=0.004, R^2=0.164). The model included sociodemographic, clinical, and psychosocial variables (Table 5). Among the predictors, being employed while on disability benefits was significantly associated with lower anxiety levels (β=-0.606, p < 0.001).

Availability of post-transplant monitoring was another significant predictor (β=-0.147, p=0.042), suggesting that patients who perceived greater monitoring support experienced lower anxiety. In contrast, gender, age group, months after transplantation, education level, marital status, presence of children or comorbidities, and emotional care were not statistically significant predictors in the adjusted model. Emotional support showed a protective trend but did not reach significance. These findings suggest that both occupational context and perceived healthcare support may play important roles in shaping transplant-related anxiety.

Predictors of Ability to Follow Treatment Regimen

To examine the factors associated with adherence to the post-transplant treatment regimen (Table 6), a multiple linear regression model was fitted, including sociodemographic, psychosocial, and clinical predictors, as well as health and treatment-related anxiety (F=6.27, p<0.001, R^2=0.370).

Among all variables, two emerged as statistically significant predictors. Perceived emotional care following transplantation was strongly associated with better treatment adherence. Specifically, participants who reported receiving emotional support "all of the time" demonstrated significantly higher adherence compared to those who reported never receiving such support (β=1.509, p < 0.001). In contrast, health and treatment-related anxiety was negatively associated with treatment adherence (β=-0.199, p=0.001), indicating that higher anxiety levels were associated with lower self-reported ability to follow medical recommendations.

Marital status also had an effect, with divorced participants reporting significantly lower adherence compared to those who were single (β=-0.877, p=0.001). Other variables, including gender, age, months post-transplant, education level, residence, occupation, comorbidities, and perceived availability of monitoring services, were not significantly associated with adherence in the adjusted model.

To further investigate the mechanisms underlying the impact of perceived post-transplant care on treatment outcomes,

two mediation models were tested with health and treatment-related anxiety as the proposed mediator.

In the first model, we examined whether health and treatment-related anxiety mediated the relationship between the perceived availability of post-transplant monitoring and the ability to follow the treatment regimen (Table 7). The indirect effect was statistically significant (β =0.056, p=0.017), indicating that perceived monitoring availability reduced anxiety, which in turn was associated with better adherence. Component paths confirmed that availability of monitoring was associated with reduced anxiety, and that lower anxiety predicted higher adherence (p<0.001). While the direct effect of monitoring availability on adherence did not reach significance (p=0.06), the total effect was significant (p=0.009), supporting a partial mediation model.

The second model tested whether health and treatment-related anxiety mediated the effect of perceived monitoring availability on psychological well-being, as measured by the WHO-5 index (Table 8). Again, the indirect effect was significant (β=1.038, p=0.032), indicating that perceived availability of post-KTx monitoring improved well-being indirectly through its anxiety-reducing effect. Specifically, better perceived monitoring was associated with reduced anxiety (p<0.001), and lower anxiety was significantly related to higher well-being (p=0.005). The direct effect of perceived monitoring on well-being remained significant (p=0.005), and the total effect was also significant (β=4.596, p=0.006), again supporting a partial mediation model.

Together, these findings highlight that health and treatment-related anxiety is a key psychological mechanism linking perceived healthcare support with both behavioral (treatment adherence) and emotional (well-being) outcomes in kidney transplant recipients.

Table 7 Indirect Effect of Health and Treatment-Related Anxiety on the Relationship Between Availability of Post-Transplant Monitoring and Ability to Follow Treatment Regimen Among Kidney Transplant Recipients

Type	Effect	beta	SE	p
Indirect	[Availability of post-KTx monitoring] → [Health and treatment-related anxiety] → [Ability to follow treatment regime]	0.056	0.019	0.017
Component	[Availability of post-KTx monitoring] → [Health and treatment-related anxiety]	-0.229	0.068	<0.001
	[Health and treatment-related anxiety] → [Ability to follow treatment regime]	-0.199	0.059	<0.001
Direct	[Availability of post-KTx monitoring] → [Ability to follow treatment regime]	0.116	0.061	0.060
Total	[Availability of post-KTx monitoring] → [Ability to follow treatment regime]	0.161	0.061	0.009

Table 8 Indirect Effect of Health and Treatment-Related Anxiety on the Relationship Between Availability of Post-Transplant Monitoring and Psychological Well-Being Among Kidney Transplant Recipients

Type	Effect	beta	SE	p
Indirect	[Availability of post-KTx monitoring] → [Health and treatment-related anxiety] → [Well-being]	1.038	0.483	0.032
Component	[Availability of post-KTx monitoring] → [Health and treatment-related anxiety]	-0.229	0.068	<0.001
	[Health and treatment-related anxiety] → [Well-being]	-4.530	1.628	0.005
Direct	[Availability of post-KTx monitoring] → [Well-being]	3.558	1.688	0.005
Total	[Availability of post-KTx monitoring] → [Well-being]	4.596	1.679	0.006

Discussion

This study aimed to develop and validate a Health and Treatment-Related Anxiety Scale (HTRAS) in Kazakh and Russian languages, identify predictors of such anxiety, and examine its impact on adherence to treatment and well-being among kidney transplant recipients in Kazakhstan. The HTRAS demonstrated strong psychometric properties and proved to be a reliable, culturally appropriate tool for measuring transplant-specific anxiety. Overall, our results indicated that elevated anxiety was significantly associated with reduced treatment adherence and lower psychological well-being. Conversely, perceived emotional support, post-transplant medical monitoring, and being employed under disability benefits were associated with lower anxiety. These findings emphasize the importance of addressing psychological and structural factors in post-transplant care.

Validity of the HTRAS

The HTRAS demonstrated robust psychometric validity, with a unidimensional structure and high internal consistency (Cronbach's α = 0.893), confirmed through CFA. Unlike generic anxiety tools, the HTRAS in this study directly targets post-transplant stressors specific to adult kidney transplant recipients in Kazakhstan, highlighting dominant anxieties such as concern about financial costs associated with prescribed post-transplant treatment (M=4.18) in Kazakh and (M=3.65) in Russian, concern about how well the function of the transplanted kidney will be maintained (M=4.11). Financial strain emerged as the most severe anxiety driver, mirroring findings in heart transplant populations where long-term financial burdens were the leading stressor [15]. This aligns with finding that cost-related anxiety predicts non-adherence, as patients balancing medication expenses against perceived benefits often experience heightened emotional distress [16]. By focusing on context-specific stressors, the HTRAS addresses critical gaps in anxiety assessment.

The HTRAS's cultural adaptation process including dual-language validation and patient-centric item refinement ensures relevance to Kazakhstan's multiethnic population, adhering to transcultural guidelines. Its unidimensional design prioritizes systemic challenges over broad psychosocial domains, resonating with calls for nuanced tools tailored to underserved populations [17]. This contrasts with multidimensional scales like the Transplant Effects Questionnaire (TxEQ) [18] or donor-

specific tools such as the Fear of Kidney Failure questionnaire [19], which emphasize broader psychosocial or pediatric/donor-centric anxieties.

Clinically, the HTRAS enables early identification of high-risk patients, such as those reporting severe financial strain (M=4.18), allowing for targeted interventions like subsidized immunosuppressants or adherence counseling. This is critical in mitigating non-adherence risks, which are strongly linked to graft rejection and mortality [1, 16]. Furthermore, mediation analyses revealed that anxiety partially explains the relationship between perceived care monitoring and adherence (indirect effect: β=1.038, p = 0.032), underscoring the dual necessity of emotional support and medical oversight in post-transplant care. Such insights align with Bosworth et al.'s (2010) model, where clinician-patient relationship fosters trust, reducing anxiety and enhancing compliance [20].

Predictors of Health- and Treatment-Related Anxiety

Interestingly, consistent with the second aim, regression analyses identified two protective factors against health- and treatment-related anxiety, addressing the study's second aim: employment under disability benefits ($p<0.001$) and perceived availability of post-transplant monitoring ($p<0.05$). These predictors highlight the interplay of socioeconomic stability and healthcare accessibility in mitigating anxiety.

Disability employment in Kazakhstan provides a guaranteed income, reducing stress over medication costs and follow-up care expenses. This directly alleviates a primary anxiety driver. Cukor et al. (2007) reported that income instability and occupational stress exacerbate psychological distress in patients with chronic kidney disease (CKD), aligning with broader evidence that socioeconomic pressures often amplify mental health burdens in chronically ill populations [21]. Also, in some studies, being employed after transplantation showed a better quality of life than unemployed patients [22, 23]. This distinction underscores the importance of contextualizing employment's impact: while being unemployed may heighten distress, regulated employment under disability benefits can alleviate anxiety by addressing systemic vulnerabilities (e.g., financial strain, fragmented care, access to medications).

Another significant predictor was the availability of post-transplant monitoring, which suggests that patients who perceived monitoring experienced lower anxiety. The other study supported that monitoring of kidney function at least every 2–3 months after the first-year post-transplantation is important [24]. However, our findings extend beyond frequency to emphasize patient perceptions of care accessibility. In Kazakhstan's context, where rural-urban disparities often limit healthcare access [11], perceived monitoring availability likely reduces anxiety by fostering trust in clinical oversight and reducing fears about graft rejection. Moreover, this discrepancy may reflect the specific characteristics of kidney transplant recipients, whose anxiety levels are more strongly influenced by clinical and treatment-related factors than by general sociodemographic variables [25].

Predictors of ability to follow treatment regimen

Results from this study identified perceived emotional care after kidney transplantation and marital status as predictors for treatment adherence among kidney transplant recipients in Kazakhstan. Perceived emotional care or consistent psychosocial support from healthcare workers, family, or peers was strongly positively related to adherence ($p<0.001$). These findings are in line with existing literature where emotional interventions were said to lower distress and facilitate self-management in chronic disease groups [26, 27]. Furthermore, the mediation analyses specify the protective function of emotional care, in that anxiety mediates at least in part the link between perceived post-transplant monitoring and adherence (indirect effect: $p<0.05$). This dual mechanism implies that care models should include both medical monitoring and emotional support because the last one lowers anxiety, which mediates the effect of monitoring on adherence.

In addition, marital status constituted another significant predictor: divorced participants were less adherent than were those who declared themselves as single ($p<0.01$). This confirms findings of Reber et al. (2016), whereby social isolation and lack of spousal support somehow fostered non-adherence in transplant populations [28]. The absence of a stable support network may exacerbate practical barriers (e.g., medication management) and amplify emotional burdens, increasing risks of missed doses or clinic visits. Non-adherence, whether due to inadequate emotional care or social instability, carries severe consequences, including graft rejection, graft loss, and mortality. These risks underscore the importance of addressing psychosocial determinants of adherence in post-transplant care.

The mediation models helped explain the interactions between perceived monitoring availability, anxiety, and outcomes. Perceived availability of monitoring reduced anxiety ($p<0.001$), while lower anxiety predicted better adherence ($p<0.01$). The post-transplant monitoring's indirect effect on well-being ($p<0.05$) further confirms the mediation role of anxiety between health-care support and psychological outcomes. The findings from previous study indicates that monitoring of emotional state of patients after transplantation is necessary to address anxiety [29]. Such results recommend interventions that target anxiety such as structured counseling, peer-support programs and others to maximize the outcome of clinical monitoring.

Implications

Our findings suggest several actionable recommendations. First, the HTRAS could be used for routine screening in post-transplant care programs to identify patients experiencing high anxiety. Early detection may allow for timely psychological intervention. Second, integrating emotional support services such as counselling into post-transplant care could enhance adherence and emotional resilience. Third, policy-level interventions to support low-income and rural patients, including financial subsidies, transfer to healthcare settings for rural patients and other initiatives may help reduce structural barriers to recovery.

Strengths and limitations

This study offers several strengths, including the development of a validated instrument for measuring transplant-specific anxiety and the inclusion of a linguistically and geographically diverse sample. Mediation analysis added depth to the interpretation of psychological mechanisms linking system-level variables to behavioral outcomes. However, the cross-sectional design limits causal inference. Self-reported data may be subject to bias, and the absence of clinical outcomes (e.g., graft function, hospitalization) restricts broader clinical interpretation. Additionally, the use of disability benefits as a proxy for social support should be interpreted cautiously. Because receiving economic support does not necessarily mean social

support. Someone might receive benefits but still be isolated, unsupported. Future longitudinal studies should incorporate objective clinical indicators and explore the effectiveness of targeted interventions for anxiety reduction.

Future studies should examine longitudinal changes in anxiety and adherence across the transplant timeline, ideally integrating clinical metrics like graft survival or clinical complications. Validating the HTRAS in other solid organ transplant populations, as well as in other regions, could expand its use. Finally, a deeper examination of structural inequities, including regional disparities and systemic barriers, will be essential for improving equity in transplant care.

Conclusion

This study is the first in Kazakhstan to develop and validate a culturally and linguistically appropriate instrument – the Health and Treatment-Related Anxiety Scale (HTRAS) – to assess anxiety specific to kidney transplant recipients. The HTRAS demonstrated robust psychometric properties and effectively captured core concerns related to post-transplant health, including medication side effects, financial strain, and uncertainty about graft function. Importantly, higher anxiety levels were associated with reduced treatment adherence and lower psychological well-being, underscoring the psychological burden faced by this patient population.

Key protective factors against anxiety included employment under disability benefits and perceived availability of post-transplant monitoring, both of which reflect the importance of structural and contextual supports. Emotional care from family, healthcare providers, and peers emerged as a significant predictor of adherence, highlighting the need for integrated psychosocial support in post-transplant care. Mediation analyses confirmed that anxiety plays a critical role in linking healthcare system factors, such as perceived care availability, to patient outcomes, including both adherence and well-being. These findings emphasize that managing anxiety is not only a matter of mental health but also a determinant of medical success in transplantation.

Routine use of the HTRAS may allow clinicians to identify at-risk individuals early and implement timely interventions. Future research should focus on longitudinal validation of the HTRAS and evaluate the impact of targeted psychosocial interventions. Addressing the psychological dimensions of transplantation care will be essential to improving long-term outcomes, particularly in regions with socioeconomic and healthcare access disparities such as Kazakhstan.

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Data availability statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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A Study To Compare Ultrasound Guided Modified 4 In 1 Block With Combined Adductor Canal And Injection Between Popliteal Artery And Capsule Of The Knee (IPACK) For Postoperative Analgesia In Total Knee Arthroplasty – A Prospective Randomized Controlled Trial

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Abstract

Introduction: TKA has ensured a better quality of life for geriatric population apropos of eliminating pain, early mobilization and discharge with a better rehabilitation. It is a painful surgery and scarce management of post-operative pain may prolong the hospital stay with increased risk of adverse effects. We compared a modified 4 in 1 block with combined ACB + IPACK for postoperative analgesia after TKA.

Methods: The present randomized trial was conducted on patients between the ages 45 and 75 of either sex belonging to ASA I, II, and III who were posted for TKA under SAB.

Results: Non-Parametric tests were used as data were not normally distributed. Wilcoxon-Mann-Whitney Test was used to compare the two groups at each of the timepoints. The Friedman test was used to examine the changes in parameters over time within each group. The Generalized Estimating Equations method was employed to assess the differences in parameter changes between the two groups over time. The two groups showed significant differences in terms of VAS. at: 8, 16, 24, 36 hours after surgery. Hence Group A patients had better pain relief when compared with Group B (p=<0.001). There was a significant difference in the QMST at 36 Hours (W = 700.000, p = 0.036), with patients in Group A having better range of motion. Majority (80%) of the patients had no pain. Ten patients required single dose of Inj. PCM 500mg IV, out of which six belong to Group B.

Conclusion: ACB + IPACK and modified 4 in 1 block are effective in pain management of TKA patients. The combination of ACB + IPACK provides an excellent analgesia, prompt rehabilitation, and early ambulation of the patients. The modified 4 in 1 still needs to prove its efficacy in varied surgeries and in large population sample.

Keywords: adductor canal, modified 4 in 1, arthroplasty.

Introduction

Osteoarthritis of the knee is a pathological condition characterized by degenerative changes of the cartilage leading to joint destruction. About 40% of the population in the age group > 65 years is suffering the implications of osteoarthritis, and approximately 15% of the patients continue to have knee pain after 3-4 years of undergoing total knee arthroplasty, which is the most promising treatment [1]. The number of joint replacement surgeries in India is steadily increasing, with estimates suggesting that approximately 200,000 knee arthroplasties were performed in 2020[2]. TKA has improved the quality of life for our elderly population. Of eliminating pain, minimal morbidity, mortality, as well as early mobilization and discharge, restoring mobility and function of the knee joint [3].

It is a painful surgery, and scarce management of post-operative pain may prolong the hospital stay with increased risk of adverse effects like pulmonary dysfunction, myocardial ischemia and infarct, urinary retention, paralytic ileus, along with thromboembolism, DVT, as well as mental depression due to chronic pain [4]. The knee joint has a complex nerve supply. The protocol of postoperative pain management following TKA may vary with individual institutes, and myriad options are available. Still, optimal coverage of the complete anatomical supply of the knee joint is an art, and exuberant debates continue to date.

Several methods have pros and cons. Central neuraxial blocks, like epidurals, have debilitating complications such as autonomic disturbances leading to urinary retention and motor blockade. Opioids can cause postoperative nausea and vomiting (PONV), respiratory depression, and pruritus, along with addiction [5]. The precise deposition of drugs near the nerve or plexus under real-time ultrasound guidance has revolutionized Regional anaesthesia, establishing a niche in pain management practice [6].

PNB for TKA includes non-motor-sparing blocks like the femoral nerve block (FNB) and popliteal sciatic nerve block (PSNB), and motor-sparing blocks such as the adductor canal block (ACB), infiltration between the popliteal artery and knee joint capsule (IPACK), 4-in-1 block, and modified 4-in-1 block [4, 7]. FNB is not standard due to quadriceps weakness, causing falls and injuries postoperatively. The ACB's rationale is that the saphenous nerve, a sensory nerve, and part of the obturator nerve traverse the adductor canal [7, 8].

Since 2014, IPACK has been clinically employed. It blocks the superomedial and lateral genicular nerves, branches of the sciatic nerve, and articular branches of the obturator nerve in the popliteal region. This approach provides analgesia to the knee joint's posterior capsule while preserving limb motor function [9].

The newly developed regional techniques, like the USG-guided 4-in-1 block and Modified 4-in-1 blocks, provide effective post-operative analgesia for TKA [10, 11]. In the later, the Nerve to vastus medialis (NVM) is targeted separately through a peripheral nerve stimulator (PNS) guided injection of LA [4, 11].

When carefully planned, a procedure-specific RA approach can provide effective pain relief without compromising motor function, facilitating early mobilization, faster recovery, and reduced opioid consumption, thereby minimizing associated side effects [6].

IPACK and ACB have proven superior in studies for postoperative analgesia in TKA. The modified 4-in-1 block is a newer option for lower limb surgery. This study compares the modified 4-in-1 block with ACB + IPACK for postoperative analgesia after total knee arthroplasty (TKA), hypothesizing that the modified 4-in-1 block may provide better pain management and reduce analgesic needs without excessive motor weakness or side effects.

The primary objective was to compare the differences in VAS scores between the two groups for 72 hours after surgery. The Secondary objectives were to study the duration of postoperative analgesia using VAS up to 72 hours of surgery, the site of pain, whether it is medial, lateral or upper side of knee, the amount and type of rescue analgesia required, the extent of ambulation and quadriceps strength after 24 hours of surgery, patients overall experience score and the complications if any.

Methods

The present prospective, randomized, single-blind study was conducted in the Department of Anaesthesiology and Critical Care at Pt. B. D. Sharma PGIMS, Rohtak, from July 2023 to September 2024, after institutional biomedical research ethical committee approval (BREC/Th/20/Anesth12). The trial was registered (CTRI/2022/07/043759). Patients aged 45 to 75 years of either sex, classified as ASA I, II, or III, who were scheduled for TKA under subarachnoid block (SAB), were included in the study. Patients with known hypersensitivity or allergy to the study drugs, bleeding disorders, infections at the site of the block, uncontrolled diabetes mellitus, hypertension, a history of cardiac, liver, or kidney disease, and those who declined to participate in the study were excluded.

Randomization and group allocation

Our estimated sample size is determined using the VAS score across groups. For this calculation, we defined a mean difference of 0.6 and a standard deviation of 0.75. We calculated the sample size using a 95% confidence interval, 80% power, and an alpha level of 0.05. The resulting sample size is 35.

The patients were divided into two groups of 35 each using a randomization table. Random numbers were printed and placed into labeled, opaque, sealed envelopes, opened only during the procedure, assigning patients to a group on surgery day. Group-A: Patients received USG-guided combined adductor canal and IPACK block with 12 ml and 10 ml of 0.25% bupivacaine, respectively, with 8mg dexamethasone as an adjuvant. Group B: Patients received USG-guided modified 4 in 1 block with 20 ml of 0.25% bupivacaine and 2 ml of dexamethasone (total 22ml).

Preparation of patients

All patients underwent a clinical history assessment and physical exam one day before surgery. Any history of hypertension, diabetes, cardiovascular issues, renal problems, or liver disease was noted. Medications like NSAIDs, aspirin, and opioids were recorded. Routine blood tests were done as needed. All patients provided informed written consent after the procedure was explained in detail. Patients were educated on the pain score and asked to rate their average pain using VAS, where 0 is no pain and 10 is the worst pain imaginable. They were instructed to specify the exact site of pain after surgery. All patients received tab Pantocid 40 mg and tab Alprazolam 0.5 mg the night before surgery. Tab paracetamol (650 mg) was given on the morning of surgery at 6 AM with a sip of water.

Anaesthesia Technique

Patients were moved to the pre-op room, and emergency

drugs and airway management equipment were prepared. Vital monitors were attached, and the I/V line secured. The procedure followed strict aseptic precautions. A linear high-frequency probe (Sonosite M-Turbo by Fujifilm Sonosite, United States) was utilized. The block was administered per the randomization number generated. Patients were monitored in the preoperative room until they were taken to the operating room theater.

Details of blocks

Modified 4 in 1 block. The technique of Roy et al was [11]. The Modified 4 in 1 block seeks to target four nerves (saphenous, obturator, nerve to vastus medialis, and sciatic) with one injection point, extending to the adductor canal in the mid-thigh and down to the popliteal area fossa. The patient lay supine with the same-side leg in external rotation, slightly abducted, and knees positioned slightly apart, flexed. Using a high-frequency linear probe, the medial femoral condyle was marked (Figure 1). The probe was placed over the femoral condyle, allowing for the identification and proximal scanning of the vastus medialis muscle. Upon locating the junction of the vastus and sartorius muscles, the probe was moved proximally until the superficial femoral artery became visible in the adductor region hiatus. As we moved proximally, the descending genicular artery, a branch of the superficial femoral artery (SFA), was identified. This point was located 8–10 cm above the femoral condyle, and at this location, the superficial structures were re-visualized. The needle, attached using PNS (Stimuplex DIG, BBraun, Germany), was directed into the Vastus medialis muscle until it stimulated the NVM, causing a contraction at a threshold current of 0.4-0.5 mA. At this stage, 5 mL of the drug was administered following verification of negative blood aspiration and absence of excessive pressure during the injection. The needle was carefully directed in a plane from the lateral to the medial side to access the perivascular area. After confirming negative aspiration again, the remaining 17 mL of the drug was injected, successfully visualizing it pushing against the femoral artery posteriorly. The patient was monitored in the pre-op room till shifted to OT.

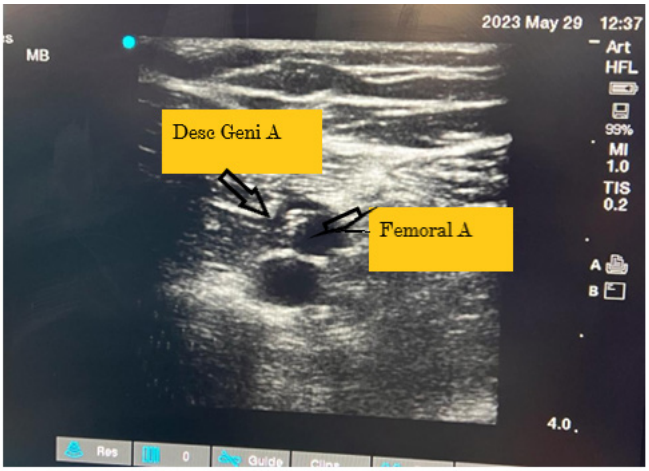

Figure 1 – Sonoanatomy of Modified 4 in 1 block

Adductor canal block (Figure 2). The patient was positioned supine, with the ipsilateral leg externally rotated, slightly abducted, and the knees slightly flexed—a high-frequency linear probe located the Adductor canal at mid-thigh, beneath the sartorius muscle. The probe advanced until the superficial femoral artery (SFA) was visible in the canal, nestled between the vastus medialis and adductor longus muscles. Then,

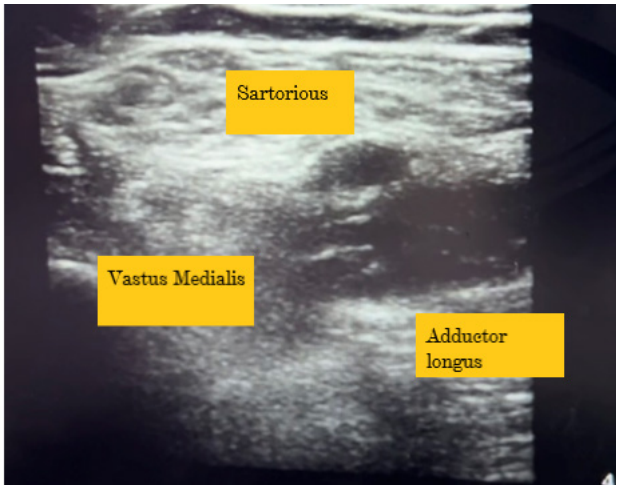

Figure 2 – Sonoanatomy of the Adductor Canal Block

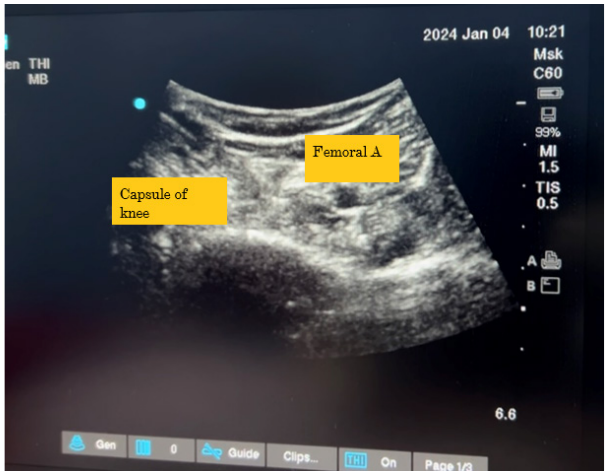

Figure 3 – Sonoanatomy of IPACK

a 22-gauge spinal needle was inserted laterally to medially to access the perivascular area. After negative aspiration, 12 ml of the drug was injected, displacing the femoral artery.

IPACK block (Figure 3). The patient was positioned in the frog leg position with a blanket roll under the thigh for support. The curved transducer was chosen for its depth of penetration and ability to visualize all structures in one window. Following strict aseptic precautions, the transducer was placed on the lower third of the medial thigh to image the femur and femoral vessels in cross-section. It was then moved caudally to view the femoral artery (FA) extending into the popliteal fossa through the adductor hiatus, becoming the popliteal artery. The transducer was adjusted posterior-inferiorly to view the area between the popliteal artery and the femur shaft, just above the femoral condyles. The needle was inserted in-plane from the anterior side, following a medial-to-lateral trajectory and remaining parallel to the femur's acoustic shadow. The needle tip was placed 2 cm past the artery's lateral border. After negative aspiration, 10 ml of the drug was injected in divided doses as the needle was advanced and withdrawn. The patient was moved to OT after 30 minutes of monitoring. Surgery proceeded under SAB.

Patients were moved to recovery. Suppose VAS > 4, Fentanyl 20 mcg IV was administered. For the first three postoperative days, PCM 500 mg was given every 8 hours. Suppose VAS > 4, rescue analgesia with IV PCM (500 mg) and Tramadol 50 mg IV (after a wait of half an hour if pain persisted) was provided. A second dose was repeated if pain persisted.

Patients were encouraged to move the joint after surgery, particularly after 24 hours, to support early physiotherapy and rehabilitation. Physiotherapy was recommended every 6 hours. Patients received 4 mg IV Ondansetron to prevent PONV. Measures were implemented to avoid postoperative falls. Episodes of hypotension, urinary retention, local anaesthetic systemic toxicity (LAST), infection, and 30-day readmission were evaluated and documented.

The following parameters were noted. The primary objective was to achieve a VAS score over 72 hours. The secondary objectives were: Time to perform the block: T1: starting point was lifting the probe before local anaesthetic in the Modified 4-in-1 block. T2a: The process began with selecting the probe and continued to administering local anesthetic ACB. T2b: The starting point was picking up the probe to administer local anaesthetic in the IPACK block. Pain during drug administration was assessed using VAS at 6, 8, 10, 12, 16, 24, 36, 48, and 72 hours after surgery. The exact site of pain (medial, lateral, or upper part of the knee) was noted. The amount and type of rescue analgesia required, as well as the patient satisfaction, were documented.

The quadriceps muscle strength was measured after 24 hours of surgery using the quadriceps strength test (QMST), the thigh of the operated limb was kept at the edge of the bed or table and in sitting position with leg hanging, the patient was asked to extend the knee, where, 1 = complete resistance(normal quadriceps strength) 2 = moderate resistance (mild quadriceps weakness) 3 = poor resistance (severe quadriceps weakness)

Any complications or side effects of the procedure or drug were reported according to the SAE format by ICMR.

Statistical analysis

SPSS version IBM 28.0. was used for statistical analysis of the data. Non-parametric tests were utilized for statistical inference due to the non-normal distribution of the data. The Wilcoxon-Mann-Whitney Test was applied to compare the heart rates, respiratory rates, and VAS of the two groups at each time point. The Friedman test examined the changes in parameters over time within each group. Additionally, Generalized Estimating Equations were employed to assess the difference in parameter changes between the two groups over time.

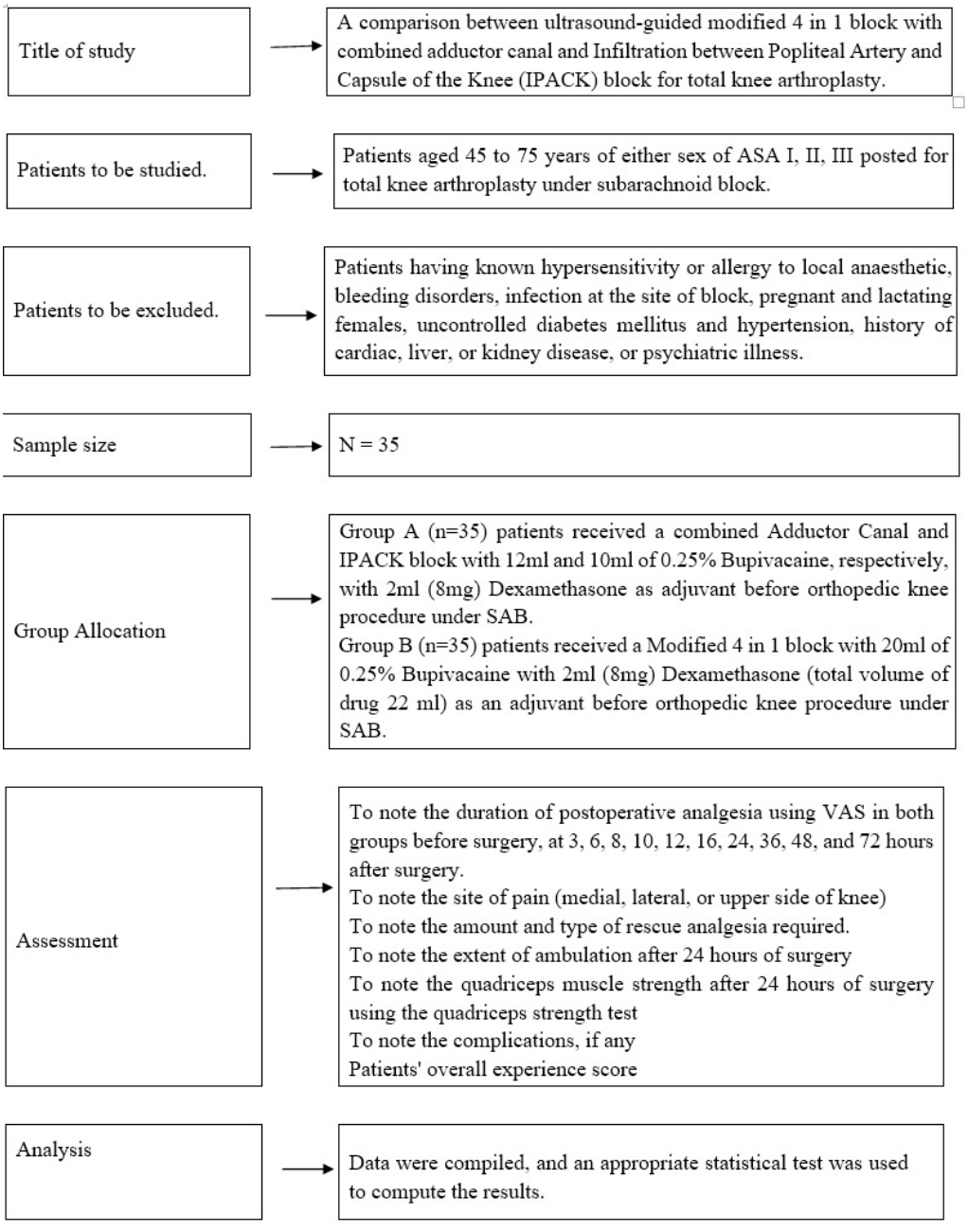

Figure 4 – Flow chart of study

Results

Eighty-six patients were screened for the study, with nine dropouts due to non-compliance with the inclusion criteria. Four patients were lost to follow-up due to early discharge, and three did not consent to the procedure. Seventy patients were included in the statistical analysis, and demographic variables were comparable (Table 1). The Wilcoxon-Mann-Whitney U Test facilitated group comparisons. We used the Generalized Estimating Equations method to compare overall changes in haemodynamic variables over time between the two groups, finding no significant differences (Figure 5, 6).

All participants in both groups were successfully given blocks in the first attempt. There was a significant difference between the two groups in terms of time taken for block (W = 1225.000, p = <0.001), with the median being highest in Group A (range in Group A 6-9 minutes, Group B 3-5 minutes) (Table 2).

VAS changes over time were compared between the two groups using Generalized Estimating Equations. A significant difference in VAS trends was noted (p = <0.001). The groups significantly differed in VAS at 8-, 16-, 24-, and 36-hours post-surgery (Table 3, Figure 7). Therefore, Group A patients experienced better pain relief than Group B (p <0.001).

The two groups differed in QMST at 36 hours (W = 700.000, p = 0.036), with Group A showing better motion (Figure 8). No significant difference in QMST trends over time was noted. Most patients (80%) had no pain. Ten patients received one dose of Inj. PCM 500mg IV, including six from Group B and four from Group A. Only one patient from Group B required a second dose of rescue analgesia. There was no significant difference in Rescue Analgesia distribution (p = 0.513). Group A patients were more satisfied with analgesia, though not statistically significant ($\chi 2 = 4.510$, p = 0.229) (Figure 9). No complications like hypotension, PONV, or LAST occurred.

Table 1 Demographic parameters

Parameter	Group A	Group B	Pvalue
Age	**52.14 5.56**	**51.03 4.82**	**0.524 (Mann-Whitney U test)**
Gender (M/F)	27/8	26/9	0.780 (Fisher's exact test)
ASA grade (I/II/III)	2/26/7	0/23/12	0.193 (Chi Squared test)

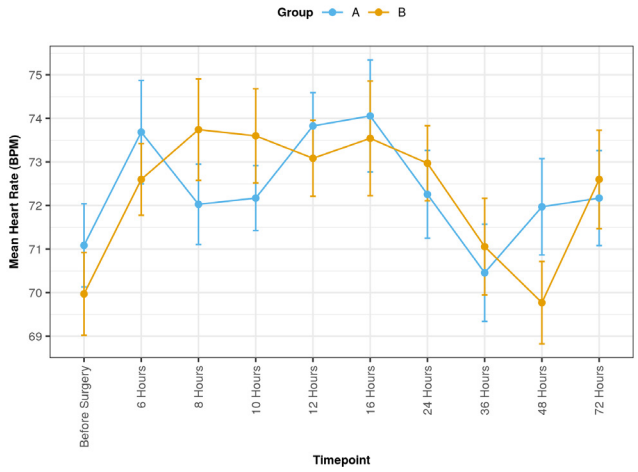

Figure 5 – Change in Heart Rate (BPM) Over Time

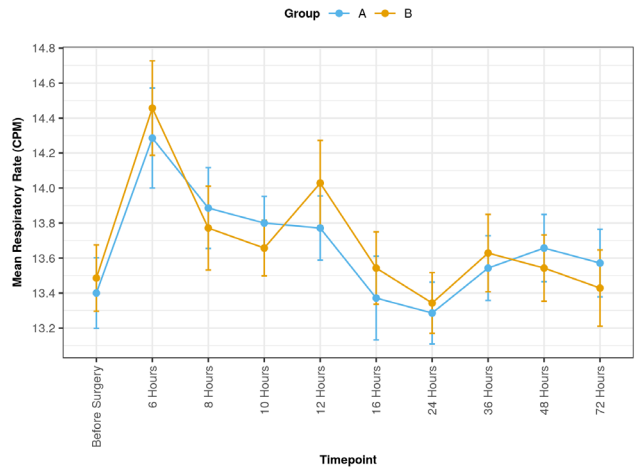

Figure 6 – Line diagram depicting the change in Respiratory Rate (CPM) over time in the two groups.

Table 2 Summary Table for Association between Group and Parameters

Parameters	Group		p value
	A (n = 35)	B (n = 35)	
Number of Attempts for Block (1st attempts)	35 (100.0%)	35 (100.0%)	1.0003
Time Taken for Block (minutes)***	7.09 ± 0.89	4.27 ± 0.65	<0.0011
Total Analgesic Consumption (IV Paracetamol)			0.4982
1000mg	2 (5.7%)	5 (14.3%)	
1500mg	0 (0.0%)	1 (2.9%)	
500mg	2 (5.7%)	1 (2.9%)	
Nil	31 (88.6%)	28 (80.0%)	
QMST (24 Hours)	1.34	1.30	0.8671
QMST (36 Hours)***	1.0	1.12	0.036
QMST (48 Hours)	1.00	1.00	-
QMST (72 Hours)	1.00	1.00	-
Type Of Pain			0.1952
Dull	1 (2.9%)	4 (11.4%)	
None	31 (88.6%)	25 (71.4%)	
Sharp	3 (8.6%)	6 (17.1%)	
Complications: Hypotension	0 (0.0%)	0 (0.0%)	1.0003
Rescue Analgesia			0.5132
None	31 (88.6%)	28 (80.0%)	
Inj PCM 500mg	4 (11.4%)	6 (17.1%)	
Inj PCM 1000mg	0 (0.0%)	1 (2.9%)	
Patients Overall Experience			0.2292
Score 2	0 (0.0%)	3 (8.6%)	
Score 3	2 (5.7%)	4 (11.4%)	
Score 4	2 (5.7%)	3 (8.6%)	
Score 5	31 (88.6%)	25 (71.4%)	

****Significant at p<0.05, 1: Wilcoxon-Mann-Whitney U Test, 2: Fisher's Exact Test, 3: Chi-Squared Test*

Table 3 Comparison of the two Groups in Terms of change in VAS over time

VAS	Group		P value for comparison of the two groups at each of the timepoints (Wilcoxon-Mann-Whitney Test)
	A	B	
	Mean (SD)	Mean (SD)	
Before Surgery	3.63 (1.42)	3.97 (1.50)	0.380
6 Hours after surgery	0.09 (0.28)	0.14 (0.36)	0.462
8 Hours after surgery	0.31 (0.47)	0.63 (0.60)	0.023
10 Hours after surgery	0.66 (0.59)	1.03 (0.89)	0.089
12 Hours after surgery	1.57 (0.98)	1.83 (1.10)	0.312
16 Hours after surgery	1.74 (0.61)	2.74 (1.09)	<0.001
24 Hours after surgery	2.17 (0.62)	2.60 (0.69)	0.015
36 Hours after surgery	2.06 (0.68)	2.43 (0.74)	0.033
48 Hours after surgery	2.49 (0.74)	2.43 (0.78)	0.772
72 Hours after surgery	2.29 (0.57)	2.49 (0.66)	0.168
change in VAS over time within each group (Friedman Test)	<0.001	<0.001	
Comparison of change in VAS over time be-tween the two groups (Generalized Estimat-ing Equations)	<0.001		

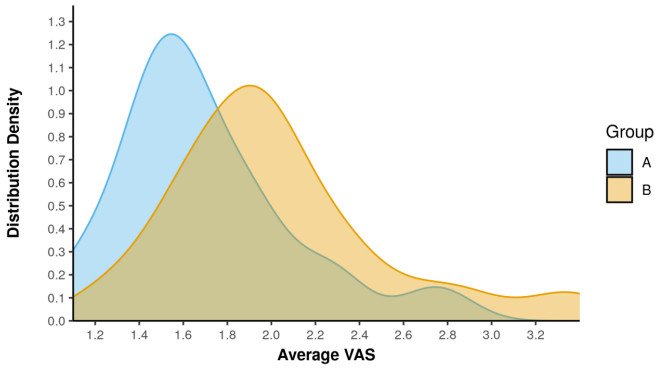

Figure 7 – The density plot below depicts the distribution of Average VAS in the two groups

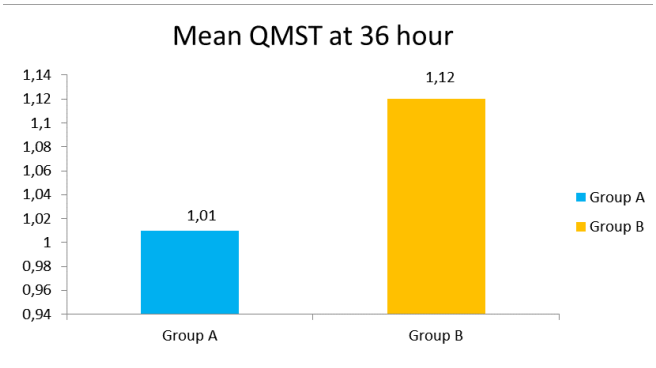

Figure 8 – Line diagram depicting the change in Respiratory Rate (CPM) over time in the two groups.

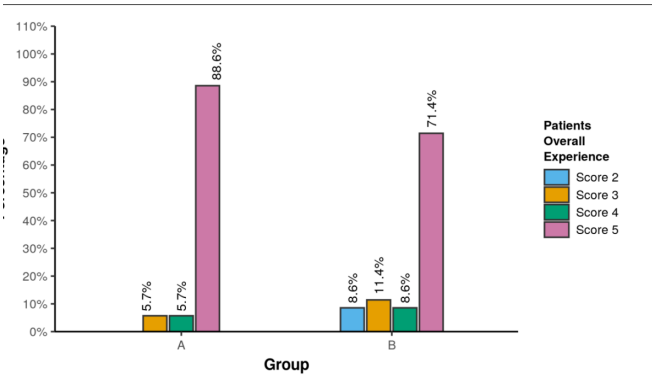

Figure 9 – Association between Group and Patients' Overall Experience

Discussion

This study found that the ACB + IPACK block outperformed the modified 4 in 1 block in terms of VAS scores at 8, 16, 24, and 36 hours, as well as patient satisfaction. The effectiveness of an analgesia regimen is assessed by analyzing the dynamic range of motion (ROM) and the ability to undergo early rehabilitation with minimal complications. While the ACB and IPACK blocks are established techniques, the modified 4 in 1 provides similar analgesic efficacy.

Among various RA techniques, selecting the most appropriate, procedure-specific, motor-sparing, and opioid-sparing options that align with enhanced recovery after surgery (ERAS) protocols is challenging. ACB does not block the articular branches of the femoral nerve innervating the knee joint capsule, so it does not provide adequate pain relief alone [9, 11]. For consistent TKA patient care, ACB + IPACK is attractive. Kandarian et al. 13 emphasized IPACK's importance as part of MMA for TKA. Given its anatomical coverage and simplicity, IPACK should be integral to TKA patient care [12, 13].

NVM's intramuscular, extra muscular, and genicular branches innervate the medial aspect of the knee [14]. The saphenous nerve innervates the knee via the superficial patellar, posterior, and deep genicular nerves. The lateral knee is innervated by the sciatic nerve's genicular branches, primarily from the peroneal nerve division. The popliteal plexus at the back of the knee receives its supply from genicular branches of the obturator and tibial divisions of the sciatic nerve. Cutaneous branches from the femoral and saphenous nerves primarily innervate the skin around the knee [15, 16].

Wong W et al. conducted a cadaveric study. They delineated the exact location of the adductor canal, which was distal to the mid-thigh level (i.e., the point between the ASIS and the base of the patella) [20].

The saphenous nerve exists in the adductor canal. The medial femoral cutaneous nerve runs in the femoral triangle and joins the subsartorial plexus between the vastoadductor membrane and sartorius muscle, just below the apex of the femoral triangle [17, 18]. The adductor canal is covered by the

VAM, making the proximal border the anatomical starting point of the AC. The study revealed that the NVM, which innervates the anteromedial knee, the typical surgical incision site, lies within the VAM's fascial sheath [18–21]. Thus, the modified 4-in-1 block targets NVM along with the saphenous, obturator, and sciatic nerves [11].

All blocks were completed in a single attempt by the same experienced consultant. Group A had two separate injection sites, which took longer to perform, despite the simplicity of the technique (W = 1225.000, p = <0.001). Abdullah FW et al reported similar block procedure times. A successful procedure must be reliable, effective, and executed quickly [17]. The modified 4 in 1 technique is a single injection method that covers relevant knee joint innervations, leading to improved block performance times.

The median Average VAS was highest in Group B; the ACB plus IPACK group showed better postoperative pain control. Roy et al found that at 12 hours post-surgery, VAS scores were significantly lower in the Modified 4 in 1 group compared to the IPACK + ACB group ($p < 0.05$) [13]. These findings contrast with our study. Roy et al4 used Ropivacaine (0.2%) with dexmedetomidine as an adjuvant, with total drug volumes ranging from 32 to 40ml, although the exact dose was undefined, presenting methodological limitations. Yin W et al reported significantly lower pain scores in the ACB + IPACK group than the placebo group, supporting our study. Several others also align with our results [13, 18, 19].

Adequate muscle strength is essential for early discharge and fall prevention. All patients regained quadriceps muscle power, but Group A exhibited better knee joint ROM. None had quadriceps weakness postoperatively, similar to our findings, though their research didn't specify numbers. Other authors reported similar results [19, 20].

There was no significant difference between the two groups regarding pain type and site. No patients in either group had complications in this study. Other authors reported similar results [13, 17, 18]. Most patients (84.3%) did not need rescue analgesia, but about 30% developed significant postoperative pain requiring opioids or rescue blocks [3]. They identified the probable cause by evaluating the pain's type and location. Pain often occurred in the proximal thigh, suggesting tourniquet-related pain due to spinal regression level. Tourniquet pain requires perioperative anti-inflammatory medications. We found no significant correlations between thigh pain and the tourniquet's duration or pressure during surgery. Other authors back our findings [12].

For an adult weighing up to 60kg, approximately 48 ml of 0.25% Bupivacaine corresponds to the safe maximum limit of about 120 mg. In this study, 20 ml of 0.25% Bupivacaine (50 mg) is within the safe dosage. Wong et al. support this, stating that 0.25% LA provides adequate analgesia with minimal side effects in their ACB research with various LA doses (Ropivacaine) [20].

A multidisciplinary approach with multimodal analgesia (MMA) is crucial for surgical success. This approach is key for managing postoperative pain by targeting inflammation and neuropathic pain, reducing pain intensity [13, 21]. This warrants the regular use of PCM postoperatively. We would like to stress the importance of ACB and IPACK in ERAS protocols for TKA, as it has proved to be superior to the modified 4 in 1 block in terms of better pain control, reduced surgical stress, and early rehabilitation by speedy recovery postoperatively (retaining muscle strength). Adequate training of residents is a good option, and the blocks are relatively simple, with a lower learning curve and safer when used with USG guidance.

Our study has several limitations; the absence of a control group may affect the analgesic efficacy of our blocks. We did not assess rebound pain from either block. Additionally, quadriceps strength evaluation was limited to 72 hours post-surgery due to immobilization, preventing the assessment of delayed motor weakness and the length of motor blockade in both groups. Recent findings suggest that post-surgical pain might cause quadriceps weakness, possibly because the analgesic effects of the blocks diminish. Further studies with larger sample sizes are needed to validate our findings. Lastly, our results apply specifically to the groups and type of surgery in this study and may not generalize to other procedures or populations.

Conclusions

This study highlights the promising future of ACB + IPACK and the modified 4 in 1 block, vital for regional analgesia. They improved post-operative analgesia with no motor effects or significant complications. ACB + IPACK provides excellent analgesia, quick rehabilitation, and early ambulation for patients.

The modified 4 in 1 must still prove its efficacy in further surgeries and RCTs with larger populations. Nonetheless, this study suggests its use in TKA patients for effective pain management, as indicated by lower postoperative VAS scores, cumulative rescue analgesia, and early discharge. Both blocks enhanced patient activity without complications.

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Study of the Efficacy of Autologous Cell Therapy in Dilated Cardiomyopathy

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Abstract

Background: Dilated cardiomyopathy (DCM) is one of the main causes of heart failure induction all over the world. In non-clinical trial many authors have demonstrated safety and effectiveness of autologous mesenchymal stem cells (MSCs). However, effectiveness of autologous MSCs in DCM with reduced ejection fraction (HFrEF) (≤40%) has been studied insufficiently.

Objective: To evaluate effectiveness of autologous cell therapy in patients with dilated cardiomyopathy and reduced left ventricular ejection fraction.

Methods: This single center randomized clinical study included 61 patients with DCM, class III-IV of heart failure according to New York Heart Association (NYHA), with HFrEF (≤40%) who made 2 visits every 3 months. Patients included in the study were randomized into two groups: the main group (32 patients) and the control group (29 patients). Patients of the main group received standard optimal medical therapy (OMT) and cell therapy, and patients of the second group received OMT. Data were analyzed using SPSS Statistica 10 and GraphPad Prism 7.

Results: The median patient age in control group was 57.0±12.0 and in the main group was 47.0±10.4 years. On average ~2/3 of the total number of patients were male in both groups. Disease duration in control group was 4.9 ± 3.1, and in main group was 4.8 ± 2.8. Comorbidities: type 2 diabetes mellitus (DM), arterial hypertension (AH) and cardiac arrhythmias (CA) did not differ between groups amounting to 17 to 44% of all patients. After 12 months of observation main group showed significant improvements associated with cell therapy: based on the results of laboratory test: statistically significant decrease in the level of eosinophils, normalization of coagulogram parameters, decrease in urea and increase in blood calcium, furthermore, there was a notable increase in anti-inflammatory cytokines CD73+ and IL-4, alongside reduction in pro-inflammatory CD8+ levels and brain natriuretic peptide (NT-proBNP). Echocardiographic (ECHO) data revealed significant increase in LVEF from 26.1 ± 8.4% to 37.6 ± 7.6% ($p<0.0001$) and reduction in end-systolic volume (ESV) from 146 ± 54 to 113 ± 42 $p=0.0496$) in the main group, whereas no such changes were observed in the control group.

Conclusion: Combined therapy, including OMT and cell therapy, resulted in improved left ventricular function, reduced cardiac chamber dimensions (according to echocardiographic data), decreased pro-BNP levels, and improved routine laboratory parameters. However, these findings require confirmation in large-scale, multicenter randomized clinical trial.

Keywords: heart failure, dilated cardiomyopathy, cell therapy, mesenchymal stem cells.

Introduction

HF is a pathological condition caused by structural or functional disturbances in the heart, which lead to inadequate blood supply to organs and tissues and manifest through characteristic clinical symptoms [1]. One of the causes of HF is considered

to be dilated cardiomyopathy [2]. Dilated cardiomyopathy represents a myocardial disorder of diverse origins, defined by LV or biventricular dilation with systolic dysfunction of the myocardium in the absence of hemodynamic overload (e.g., hypertension, valvular defects, congenital heart anomalies) or coronary artery pathology (e.g., ischemic heart disease)" [3]. Among all types of cardiomyopathy, DCM is the most prevalent accounting for 39% of cases [4,5].

The prevalence of DCM is estimated at 36.5 cases per 100,000 individuals [6]. DCM is an indication for heart transplantation in both adult and pediatric populations of patients with progressive HF [7, 8]. There is no specific etiopathogenetic therapy for DCM. General principles of treatment are no different from those for CHF. In cases of acquired DCM therapy includes treatment of the underlying disease, and all measures to eliminate causes of DCM.

Principles of DCM therapy:

1. Non-drug treatment methods: dietary modifications (reduction of sodium and fluid intake, elimination of alcohol, smoking cessation, adjustment of physical activity), and lifestyle recommendations (restriction of strenuous physical exertion).

2. OMT as recommended by the European Society of Cardiology [9].

In patients with HFrEF including asymptomatic patients OMT aims to prevent HF decompensation and alleviate symptoms rather than to restore LV function [10]. For patients with progressive and decompensated HF unresponsive to OMT heart transplantation and the use of left ventricular assist devices (LVAD) are considered as primary therapeutic options. However, these interventions are not universally available due to high medical costs and LVAD-related complications. Heart transplantation is severely limited by global shortage of donor hearts and is generally reserved for patients with end-stage HF [11]. Major complications include procedural risks, infections, acute organ rejection, cardiac allograft vasculopathy, and malignancies [12]. Cell therapy may be an alternative therapeutic approach for this patient population [13].

Depending on the source of acquisition, stem cells (SC) are divided into:

- allogeneic SCs derived from donors which may be necessary for certain diseases but is associated with the risk of graft-versus-host disease and necessitate careful donor matching [14].
- autologous SCs derived from the patient are regarded as more appropriate for clinical application due to the possibility of repeated harvesting from multiple sources within the body, including bone marrow, circulating blood, and adipose deposits, along with the advantage of lacking ethical or legal constraints. Their genetic compatibility, low risk of immunological rejection, and ease of availability make autologous SCs particularly attractive for clinical application [15, 16]. Among all types of stem cells, mesenchymal stem cells (MSCs) derived from bone marrow, adipose tissue, or umbilical vein have attracted significant attention. MSCs exhibit long-lasting anti-inflammatory effects even after a single administration [17].

MSC clinical appeal lies in several attributes including ease of isolation and expansion in culture as well as their anti-inflammatory and immunosuppressive properties [18]. Bone marrow is a key component of the immune system, responsible for maintaining systemic homeostasis and actively involved in repair mechanisms under pathological conditions. In this regard, the incorporation of bone marrow-derived stem cells—characterized by their pronounced immunoregulatory, anti-inflammatory, anti-apoptotic, and regenerative properties—into the comprehensive treatment of patients with dilated cardiomyopathy may contribute to both clinical improvement and the activation of reparative processes [19]. Importantly, no stem cell type has demonstrated the capacity to differentiate into fully functional cardiomyocytes. The predominant mechanism underlying their regenerative effects is now understood to be paracrine activity and release of extracellular vesicles (EV), particularly nanovesicles known as exosomes which mediate horizontal transfer of genetic information between cells [20, 21].

The discovery of extracellular vesicles as a novel mechanism for intercellular communication, including genetic exchange, represents one of the most important scientific advances of recent decades, and this advancement was recognized by awarding the Nobel Prize in Physiology or Medicine in 2013. The transfer of exosomes into damaged tissue including resident progenitor cells may trigger regenerative programs such as proliferation, differentiation, apoptosis inhibition, immune anti-inflammatory reactions modulation, fibrotic processes, secretory activity [22, 23]. Specifically, exosomes may counteract the processes leading to LV wall thinning and pathological remodeling by suppressing excessive inflammation, inhibiting fibrosis, preventing cardiomyocyte apoptosis, reducing mitochondrial dysfunction and oxidative stress, reducing calcium metabolism disorders in cardiomyocytes, and activating endogenous regeneration mechanisms. These properties make SC exosomes a highly promising tool in contemporary medicine [24].

Excessive and sustained inflammatory response is a key mechanism driving progression of HF with HFrEF and SCs secrete wide array of molecules with therapeutic potential including anti-inflammatory and immunomodulatory factors [25, 26]. These properties may benefit cardiac function not only through local effects of transplanted cells but also through systemic anti-inflammatory mechanisms which may accelerate tissue repair and reduce heart injury [27].

TRIDENT study was the first to demonstrate direct relationship between the effect and number administered cells, providing convincing evidence that higher doses are more effective which allowed to recommend minimum of 100–150 million cells per transplantation [28]. On cell therapy have shown that stem cell-based treatment has an acceptable safety profile, supporting its continued enhancement [29]. Numerous studies confirm high safety profile of stem cell therapy in the treatment of cardiovascular diseases. For instance, meta-analysis comprising 38 randomized controlled trials involving more than 1,900 patients demonstrated that bone marrow cell therapy may reduce mortality for at least 12 months with adverse events occurring infrequently during treatment [30]. These data suggest that cell therapy is a safe and relatively effective approach for treating HF.

Methods

A single-center randomized clinical trial was carried out to assess the therapeutic efficacy of autologous bone marrow-derived cells in patients diagnosed with DCM and HFrEF (≤40%). Patient selection was performed according to inclusion and exclusion criteria. The study protocol (Protocol No. 053/ST-62, December 13, 2021) received approval from the Ethics Committee of the National Scientific Medical Research Center. All patient assessments were conducted in accordance

with Good Clinical and Laboratory Practice standards and the ethical principles set forth in the Declaration of Helsinki and the "Guidelines for Biomedical Research Involving Human Subjects".

Inclusion criteria were: (1) established diagnosis of DCM with LVEF ≤40% based on the results of Echo and cardiac magnetic resonance imaging (MRI); (2) age between 18 and 70 years; (3) disease duration ≥12 months; (4) NTpro-BNP level ≥150 pg/mL; (5) optimal medical therapy for at least 6 months; and (6) patients signed informed consent. Exclusion criteria included: LVEF ≥40% with contraindications to coronary angiography (CAG) or multislice computed tomography (MSCT) of the heart (e.g., iodine allergy), patients with coronary artery stenosis of more than 30%, congenital heart defects, pregnancy and lactation, neoplasmas in past medical history or at the time of the study, presence of acute or chronic autoimmune diseases, infectious diseases (tuberculosis, syphilis, infective endocarditis), and mental illness.

Patients for the study were recruited at the National Scientific Medical Center between April 2022 and December 1, 2023. They were randomized into two groups: the study and the control ones. Patients of the main group received standard optimal medical therapy and autologous MSCs, while the control group patients received optimal medical therapy. The main group included 32 patients (21 male and 11 female; the median age of 47.0±10.4), and the control group included 29 patients (20 male and 9 female; the median age of 57.0±12.0) all with DCM based on the results of Echo and cardiac MRI.

All patients in both groups underwent medical examinations, medication records, 6-minute walk test (6MWT), quality of life assessment using the Kansas City Cardiomyopathy Questionnaire (KCCQ), 12-lead electrocardiography (ECG), Echo, Contrast-enhanced cardiac MRI using gadolinium was performed to quantitatively and visually assess the extent of myocardial fibrosis before and after the transplantation of autologous bone marrow-derived mesenchymal stem cells. MRI was also used to monitor signs of reverse myocardial remodeling, including changes in left ventricular volume and mass, as well as the recovery of contractile function, coronary angiography (CAG), MSCT, serum NTpro-BNP level, complete and biochemical blood tests, thyroid hormones, immunologic tests (CD73+, CD4+, CD8+, IL-4), and tumor marker panels at each follow-up visit (Figure 1).

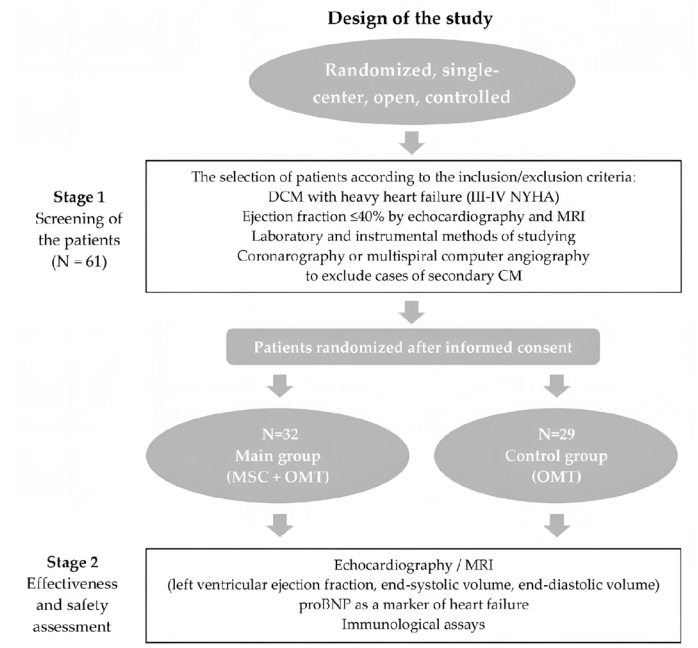

Figure 1 – Design of the study

CM – cardiomyopathy, DCM – dilated cardiomyopathy, OMT – optimal medical therapy, NYHA – New York Heart Association, MRI – magnetic resonance imaging, MSC – mesenchymal stem cells, proBNP – brain natriuretic peptide.

The instruments used for bone marrow stem cells harvesting from the ilium: bone marrow stem cells harvesting needle BME 13/7; 13Gx7 cm, double blood bag 450 mg of anticoagulant solution (India).

For MSC cultivation sterile 50 mL plastic tubes with lids, plastic Petri dishes, sterile pipette tips in racks, DMEM and DMEM-F12 media, L-glutamine, and lysing solution were used. Subculturing was performed with 0.05% Trypsin-EDTA solution (Gibco, USA). Cell quantification and microscopy were conducted using TC20 Automated Cell Counter TC 20 and Olympus binocular microscope. A total of 32 cell samples were processed under biotechnology laboratory conditions. Bone marrow aspirates were cultured in flasks for 24 hours using standard biotechnological methods, cell numbers were assessed microscopically and using automated cell counters.

Procedure performed: Isolation, cultivation and characterization of MSCs: Exfusion of 200-300 ml of bone marrow (BM) was performed by percutaneous aspiration from the posterior iliac spines in a sterile operating room under appropriate anesthesia. If posterior aspiration was not feasible (due to obesity, cachexia, or anatomical variation) the anterior iliac crest was used. The BM aspirate contained hematopoietic and mesenchymal stem cells as well as peripheral blood cells. MSC cultures were obtained by mechanical processing of the aspirate or by cell separation using Ficoll density gradient. The total yield of stem cells per patient ranged from 100×10^6 to 120×10^6. The expansion of autologous bone marrow-derived mesenchymal stem cells (MSCs) was carried out in accordance with established protocols of the cell culture laboratory, using standard culture medium. This approach ensured culture purity, minimized potential external influences, and complied with biosafety and clinical application requirements.

Autologous MSC Transplantation Protocol. Autologous MSC therapy was administered without immunosuppressive protocol following overnight rest of patients under continuous monitoring of vital signs (blood pressure, heart rate, respiratory rate, and body temperature) in intensive care unit. Approximately 100×10^6 cells (mean 120×10^6) were diluted in 200 mL of physiological saline and MSCs were infused intravenously at a rate of 50 mL/hour as previously described. Patients were observed for 24 hours post-infusion. No specific complications such as arrhythmias, hematomas, bleeding, or allergic reactions were observed after surgery.

Statistical analysis. For intragroup (between visit 1 and visit 2) and intergroup (by relevant visits) comparison of average values of indicators ANOVA analysis of variance or Mann- Whitney test were used ignoring paired measurements. The Wilcoxon signed-rank test was applied to assess within-group changes. Spearman's rank correlation coefficient was used to evaluate associations between non-parametric variables Spearman's rank correlation. Statistical analyses were performed using SPSS Statistics 10 and GraphPad Prism 7.

Results

General characteristics of patients in the study and control groups: A total of 61 patients were recruited in the Department

of Interventional Cardiology from 2022 to 2023. The age distribution differed between the groups, with the mean age of patients in the control group being 57.0 ± 12.0 years, compared to 47.0 ± 10.4 years in the main group. Gender distribution was balanced with approximately ~2/3 thirds of the patients in both groups being male.

The duration of the disease did not differ significantly between the groups, with a mean of 4.9 ± 3.1 years in the control group and 4.8 ± 2.8 years in the main group. The mean body mass index (BMI) in both patient groups was ≥30 kg/m², indicating that more than half of the patients in each group were classified as having class I obesity. Identified concomitant diseases revealed no statistically significant difference between the groups in terms of DM, AH, or cardiac rhythm disorders. The etiologies of the disease in the main group included acute respiratory viral infections (ARVI) (50%), coronavirus disease-2019 (COVID-19) (9.2%), heredity (9.2%), endocrine disorders (12.5%), alcohol abuse (3%) , peripartum period (16%), and in the control group, ARVI (51.7%), COVID-19 (21%), heredity (6.8 %), endocrine disorders (10.3 %), peripartum period: (10.3%) (Table 1). The proportion of patients who underwent cardiac resynchronization therapy with defibrillator (CRT-D) implantation for either primary or secondary prevention of sudden cardiac death was comparable between the groups, representing roughly half of the participants in each. However, the number of patients with implanted cardioverter-defibrillators (ICDs) was greater in the main group, indicating a more severe clinical condition in that cohort.

Medical therapy was largely comparable between the two groups. Angiotensin-converting enzyme inhibitors were prescribed to 28% of patients in both groups, while sacubitril/valsartan was administered to approximately three-quarters of patients. Furthermore, nearly all patients in both groups received mineralocorticoid receptor antagonists, beta-blockers, and sodium-glucose transporter inhibitors. Loop diuretics were used in more than half of the patients, and anticoagulants were prescribed to slightly less than half. Accordingly, no statistically significant differences were found between the groups regarding the pharmacological therapies administered (Table 1).

Baseline Echo data, low LVEF, in the main group with values of 26.1 ± 8.4, and in the control group 24.3 ± 8.3, NT-proBNP high levels in both gropus amounting to 3021 ± 6644 in the main group and 2607 ± 2236 in the control group. Twelve-month follow-up outcomes demonstrated that both groups exhibited a statistically significant improvement in quality-of-life scores compared to baseline measurements at visit 2 ($p < 0.0001$; see Table 2 and Figure 2A). Similarly, the 6MWT revealed a significant increase in walking distance at Visit 2 compared with visit 1 in the "Study" group (p=0.0007) and in the "Control" group (p=0.0037) (Table 2 and Figure 2B).

No significant intra- or intergroup differences were

Table 1 General characteristics of patients in the study and control groups

Description	Control group	Main group	p value
Included in RCTs (n)	29	32	
Gender, Men /Women (%)	20 / 9 (69 / 31%)	21 / 11 (66 / 34%)	p=0.781
Age (years)	57.0 ± 12.0	47.0 ± 10.4	p<0.001
Body mass index (kg/m²)	30.3 ± 5.1	30.8 ± 5.2	
Comorbidities			
Diabetes mellitus type 2 (%)	8 (27.6%)	3 (9.4%)	p=0.065
Hypertension (%)	11 (38%)	7 (22%)	p=0.170
Arrhythmia: Atrial fibrillation	13 (44.8%)	14 (43.7%)	p=0.121
Implanted devices			
Cardiac resynchroniza-tion therapy with de-fibrillator	13 (44.8%)	8 (25%)	p=0.088
Implantable cardioverter defibrillator	1 (3.5%)	8 (25%)	p = 0.028
Procedures performed			
Coronary angiography	vessels intact: 21 (72%) stenoses up to 30%: 8 (28%)	vessels intact: 26 (81%) stenoses up to 30%: 6 (19%)	p=0.412
Bone marrow collec-tion and cell transplan-tation (n)	(0)	32	
Ongoing drug therapy for heart failure			
Sacubitril / Valsartan	21 (72.5%)	23 (72%)	p=0.963
ACE inhibitors	8 (28%)	9 (28%)	p=0.963
MRA	29 (100%)	32 (100%)	
Beta blockers	27 (93%)	32 (100%)	p=0.131
SGLT2i	29 (100%)	32 (100%)	
Loop diuretics	27 (93%)	26 (81,2%)	p=0.270
Anticoagulants	13 (44.8%)	14 (43.7%)	
Etiology of the disease	ARVI: - 15 (51.7%) COVID-19: - 6 (21%) Heredity: -2 (6.8 %) Endocrine disorders: -3 (10.3 %) Peripartum period: -3 (10.3%)	ARVI: 16 (50%) COVID-19: 3 (9.2%) Heredity: 3 (9.2%) Endocrine disorders: 4 (12.5%) Alcohol abuse: - 1 (3%) Peripartum period: 5 (16%)	p=0.235

ACE inhibitors – Angiotensin-converting-enzyme inhibitors, ARVI – acute respiratory viral infections, COVID-19 – coronavirus disease 2019, MRA – mineralocorticoid receptor antagonist, RCT – randomized controlled trial, SGLT2i – Sodium-Glucose Transport Protein-2 Inhibitors.

Table 2 Functional parameters and hemodynamic indicators in the main and control groups of patients

Indicators	Control group (n = 29)		Main (n = 32)	
	Visit 1	Visit 2	Visit 1	Visit 2
QOL (index)	72.5 ± 7.5	80.3 ± 6.1 * (p<0.0001)	69.7 ± 6.9	80.8 ± 4.9 * (p<0.0001)
6MWT-(m)	238 ± 75	293 ± 63 * (p=0.0037)	244 ± 81	311 ± 49 * (p=0.0007)
SBP numbers (mmHg)	128.3 ± 26.7	125.7 ± 22.4	120.9 ± 19.0	122.7 ± 16.4
DBP numbers (mmHg)	80.7 ± 10.7	80.7 ± 10.0	79.4 ± 11.1	80.3 ± 9.0
Heart rate (bpm)	84.8 ± 14.1	82.7 ± 16.2	82.9 ± 13.4	77.9 ± 10.4

bpm – beats per minute, DBP – diastolic blood pressure, SBP – Systolic Blood Pressure, HR – Heart rate, QOL – Quality of life assessment, mmHg – millimeters of mercury, 6MWT – 6-minute walk test.

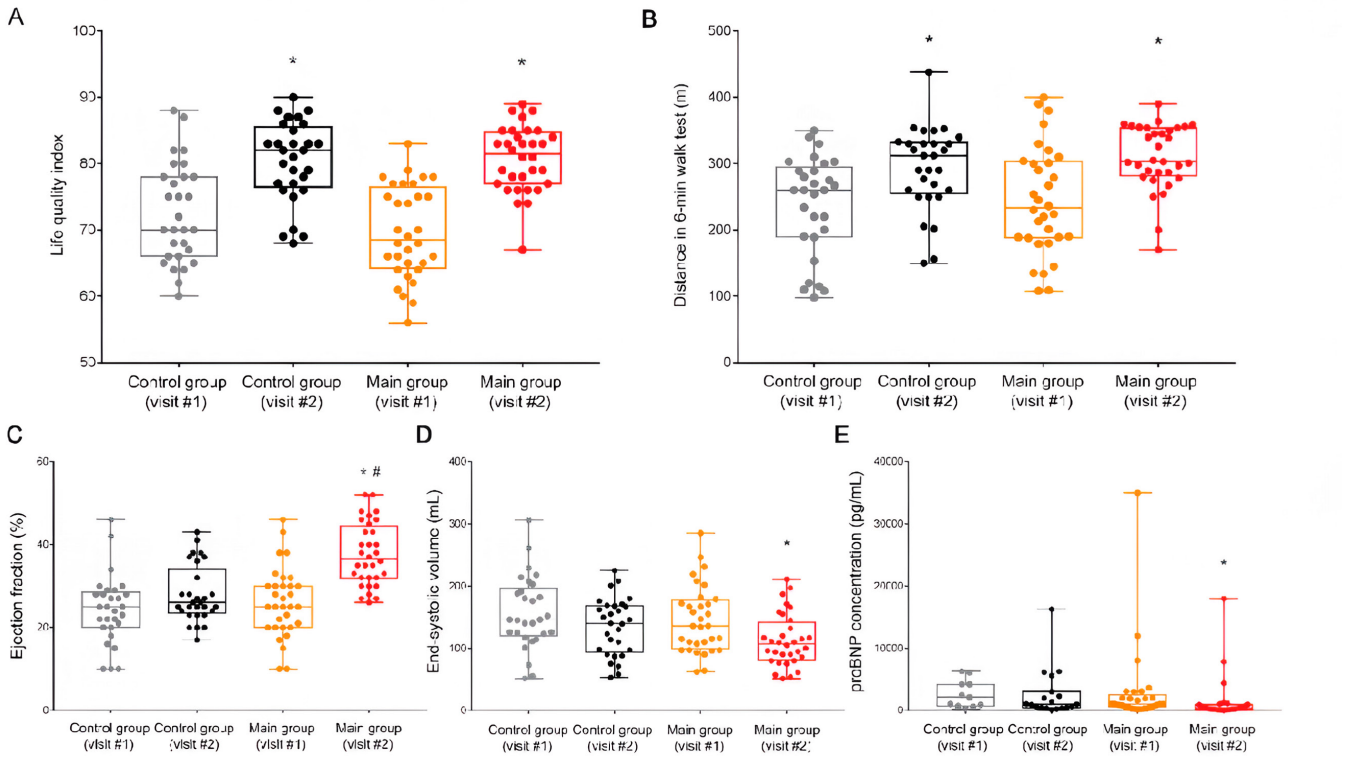

Figure 2 – The effect of cell therapy combined with optimal medication treatment (Main group) vs. optimal medication treatment only (Control group) to physiological, Physico-functional characteristicsin the patients with dilated cardiomyopathy

(A) The life quality index as self-assessed by the patient. (B) The distance walked by the patients in 6-minute walk test. (C) The ejection fraction of the left ventricle. (D) The end-systolic volume of the left ventricle. (E) The concentration of N-terminal prohormone of brain natriuretic peptide (proBNP) in the peripheral blood. Symbol "" indicates the significant difference between mean values measured in visit #2 vs. visit #1 for the same group of patients, i.e. either for Control group or for Main group (as shown in panels A-E). Symbol "#" indicates the significant difference between mean values measured in visit #2 for Control group vs. Main group (panel C).*

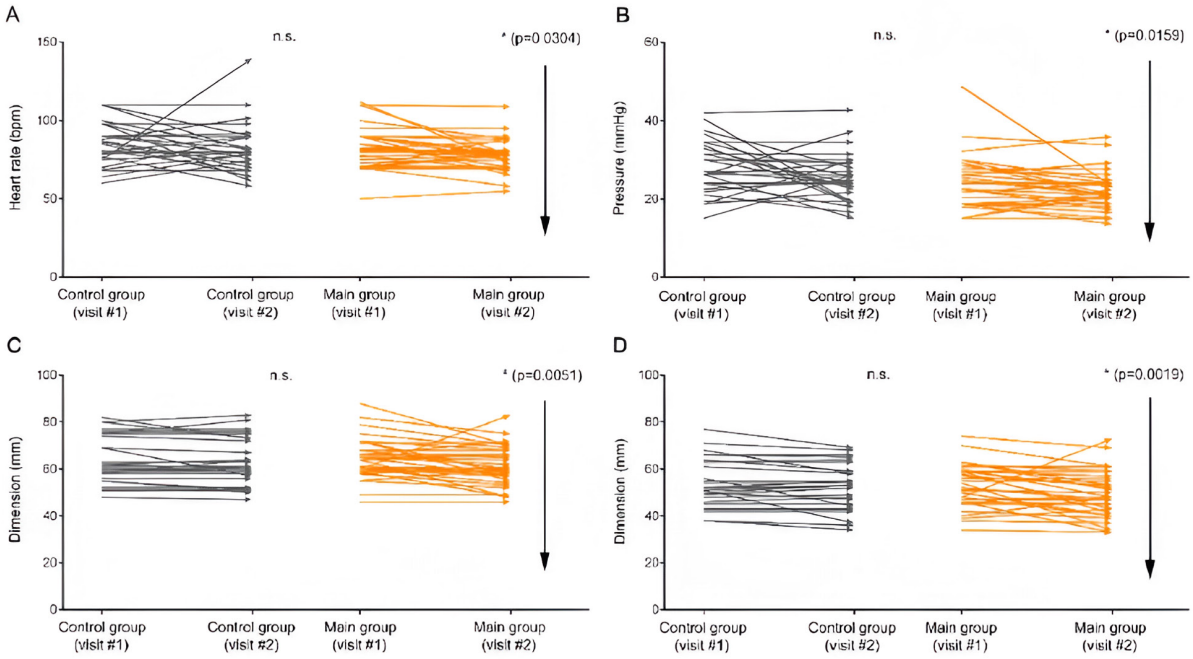

Figure 3 – The time-related prevalence in the effect of cell therapy combined with optimal medication treatment (Main group) vs. optimal medication treatment only (Control group) to the morphofunctional characteristics of the heart in the patients with dilated cardiomyopathy

(A) Heart rate. (B) Estimated pulmonary artery systolic pressure. (C) End-diastolic dimension of the left ventricle. (D) End-systolic dimension of the left ventricle. The 'before-after' plots show individual prevalence in all patients in both groups, as shown by individual arrows. Gray arrows represent Control group, orange arrows represent Main group. Black vertical arrows, if shown, follow the direction of significant prevalence, with exact p value indicated above the arrow. If no significant prevalence is revealed, it is indicated by "n.s." label. In each panel, the Y-scale stays the same for both groups.

Table 3 Comparison of laboratory research data of the main and control groups

Indicators	Control group (n = 29)		Main group (n = 32)	
	Visit 1	Visit 2	Visit 1	Visit 2
Complete Blood Count				
Hemoglobin (g/l)	141.2 ± 17.0	143.1 ± 15.2	147.0 ± 17.6	143.8 ± 18.9
RBC (1012/l)	4.97 ± 0.58	4.72 ± 0.74	4.97 ± 0.74	4.77 ± 0.58
PLT (109/l)	219.7 ± 51.7	231.0 ± 41.2	239.2 ± 60.2	242.6 ± 49.6
ESR	10.89 ± 10.42	12.43 ± 11.05	12.77 ± 10.15	11.81 ± 7.57
Lymphocytes (%)	26.7 ± 7.4	26.5 ± 8.5	30.4 ± 8.5	28.7 ± 6.1
WBC (109/l)	6.18 ± 2.02	6.35 ± 2.72	6.58 ± 1.67	6.60 ± 1.70
Neutrophils (109/l)	4.09 ± 1.31	3.80 ± 2.67	3.97 ± 1.13	3.95 ± 1.18
Monocytes (109/l)	0.470 ± 0.182	0.491 ± 0.235	0.480 ± 0.160	0.526 ± 0.182
Basophils (%)	0.89 ± 1.09	1.02 ± 1.53	0.75 ± 0.61	0.67 ± 0.85
Eosinophils (%)	2.00 ± 1.05	1.99 ± 1.24	1.99 ± 1.19	1.77 ± 1.20 # (p=0.0264)
Immature granulocytes (%	0.257 ± 0.192	0.271 ± 0.334	0.266 ± 0.218	0.310 ± 0.266
Coagulation Profile				
APTT (sek)	34.8 ± 7.8	37.4 ± 9.3	34.8 ± 5.3	35.8 ± 4.4
Fibrinogen (g/l)	4.35 ± 1.19	4.20 ± 1.31	4.16 ± 1.03	4.22 ± 0.70
INR	1.141 ± 0.135	1.206 ± 0.292	1.060 ± 0.180	1.046 ± 0.092 # (p=0.0185)
PT Prothrombin time (sec)	13.43 ± 1.43	13.86 ± 2.92	12.82 ± 2.59	12.38 ± 1.27 # (p=0.0142)
Hematocrit	44.4 ± 5.8	43.1 ± 6.4	45.0 ± 5.5	42.7 ± 5.7
Biochemical blood tests				
Creatinine (mmol/l)	83.5 ± 21.4	83.6 ± 20.8	79.1 ± 22.6	78.1 ± 22.5
Urea (mmol/l)	6.60 ± 2.15	6.76 ± 2.27	5.63 ± 1.56	5.32 ± 1.88 # (p=0.0068)
eGFR	83.6 ± 21.3	83.4 ± 22.2	91.6 ± 19.3	90.8 ± 19.3
Total Cholesterol (mmol/L)	4.49 ± 1.02	4.43 ± 0.87	4.63 ± 0.71	4.59 ± 0.67
LDL	3.08 ± 1.02	2.83 ± 0.75	3.10 ± 0.58	3.04 ± 0.61
Total protein (g/l)	69.0 ± 6.6	68.5 ± 5.5	69.8 ± 5.3	70.2 ± 4.6
Total bilirubin (g/L))	15.2 ± 10.6	14.2 ± 7.4	15.2 ± 8.0	15.0 ± 8.3
Direct bilirubin (g/l)	5.98 ± 2.76	5.89 ± 3.31	6.05 ± 3.26	5.53 ± 2.94
Potassium (mmol/l)	4.18 ± 0.568	4.38 ± 0.47	4.23 ± 0.41	4.34 ± 0.41
Sodium (mmol/L)	141 ± 3	141 ± 3	142 ± 2	142 ± 3
Calcium (mmol/l)	1.26 ± 0.07	1.25 ± 0.08	1.26 ± 0.09	1.33 ± 0.16 * (p= 0.0270) # (p= 0.0493)
ALT (μkat/L)	0.499 ± 0.397	0.502 ± 0.600	0.535 ± 0.782	0.334 ± 0.171
AST (μkat/l)	0.359 ± 0.152	0.377 ± 0.242	0.407 ± 0.308	0.325 ± 0.180
Glucose (mmol/l)	5.98 ± 1.20	6.00 ± 1.16	5.64 ± 0.89	5.60 ± 0.77
Immunological tests				
CD73+	17.5 ± 10.3	19.2 ± 8.4	15.7 ± 7.4	20.2 ± 7.0 * (p = 0.0125)
CD4+	34.5 ± 7.4	37.6 ± 9.7	28.5 ± 9.1	25.0 ± 7.1 # (p = 0.0004)
CD8+	27.3 ± 9.0	29.4 ± 7.3	31.4 ± 9.4	22.4 ± 8.4 * (p = 0.007)
IL-4	1.00 ± 0.45	1.00 ± 0.60	0.93 ± 0.46	1.70 ± 0.70 * (p = 0.0021) # (p = 0.0132)
Thyroid hormones				
Free T3 (pmol/L)	4.18 ± 0.33	4.38 ± 0.39	4.31 ± 1.02	4.73 ± 0.83
Free T4 (pmol/L)	15.9 ± 4.9	14.3 ± 5.9	16.9 ± 3.2	14.8 ± 2.1 * (p= 0.0144)
Thyroid-stimulating hor-mone (μIU/ml)	2.27 ± 1.70	2.14 ± 1.48	2.24 ± 1.40	2.27 ± 1.45
Tumor markers				
Embryonic tumor markers (ng/ml)	1.16 ± 0.65	1.84 ± 1.19	1.63 ± 1.08	1.64 ± 1.04
Prostate specific antigen, free (%)	51.0 ± 25.5	27.4 ± 18.5	9.4 ± 14.5	13.7 ± 12.8
CA 125 (U/mL)	21.6 ± 22.9	34.1 ± 30.7	19.4 ± 16.4	19.4 ± 21.3
CA 15-3 (U/mL)	1.76 ± 0.18	6.47 ± 5.18	9.58 ± 6.12	7.40 ± 3.31
CA 19-9 (U/mL)	7.24 ± 7.08	6.79 ± 4.40	11.34 ± 8.97	11.83 ± 7.02
Cyfra 21-1 (ng/mL)	3.42 ± 3.26	2.25 ± 0.78	2.38 ± 1.01	2.41 ± 0.95

bpm – beats per minute, DBP – diastolic blood pressure, SBP – Systolic Blood Pressure, HR – Heart rate, QOL – Quality of life assessment, mmHg – millimeters of mercury, 6MWT – 6-minute walk test.

observed in systolic or diastolic blood pressure over the study period. Heart rate (HR) showed a tendency toward reduction in the main group at Visit 2 compared to Visit 1 ($p = 0.0304$), however this was not observed in the control group (Figure 3A).

According to laboratory investigations, the following findings were observed at Visit 2 compared to Visit 1 (Table 3): in the main group, complete blood count (CBC) analysis revealed a statistically significant decrease in eosinophil count at Visit 2 compared to Visit 1 ($p = 0.0248$). Coagulation studies demonstrated normalization of the International Normalized Ratio (INR) and Prothrombin Time (PT) at the corresponding visit ($p = 0.0185$ and $p = 0.0142$, respectively). In contrast, the control group showed an increase in both INR and PT values, no significant changes or intergroup differences were noted in other parameters.

The mean level of pro-BNP in the main group showed a statistically significant reduction at Visit 2 compared to Visit 1 ($p = 0.0029$) (Figure 2E), while no comparable change was detected in the control group. Biochemical blood analysis in the main group revealed decrease in urea concentration ($p = 0.0068$) and increase in calcium levels ($p = 0.0270$), in the control group, by contrast, an increase in urea and a decrease in calcium levels were noted.

Comparison of immunological markers of inflammation between the control and main groups demonstrated a statistically significant increase in anti-inflammatory markers CD73+ and IL-4, as well as reduction in the pro-inflammatory marker CD8+ at 12 months following cell therapy (Figure 4), and normalization of thyroid hormone T4 levels ($p = 0.0144$) in the main group, while no such changes occurred in the control group. The safety of the method is supported by the absence of adverse events based on biochemical, immunological, and tumor marker assessments.

Comparison of Echo measurements demonstrated that LVEF was significantly higher in the main group at Visit 2 compared to Visit 1, as well as in the intergroup comparison with the control group ($p < 0.0001$) (Table 4, Figure 2C). In the main group, ESV at Visit 2 was significantly lower compared to Visit 1 ($p = 0.0496$) (Figure 2D).

Although the Control group showed an increase in mean LVEF and a reduction in EDV at Visit 2 relative to Visit 1, these changes were not statistically significant in either intra-group or between-group comparisons. No statistically significant differences were identified between the groups with respect to the remaining echocardiographic parameters. Intra-group analysis of Echo and MRI parameters in the main group revealed significant reduction in both EDD and ESD from Visit 1

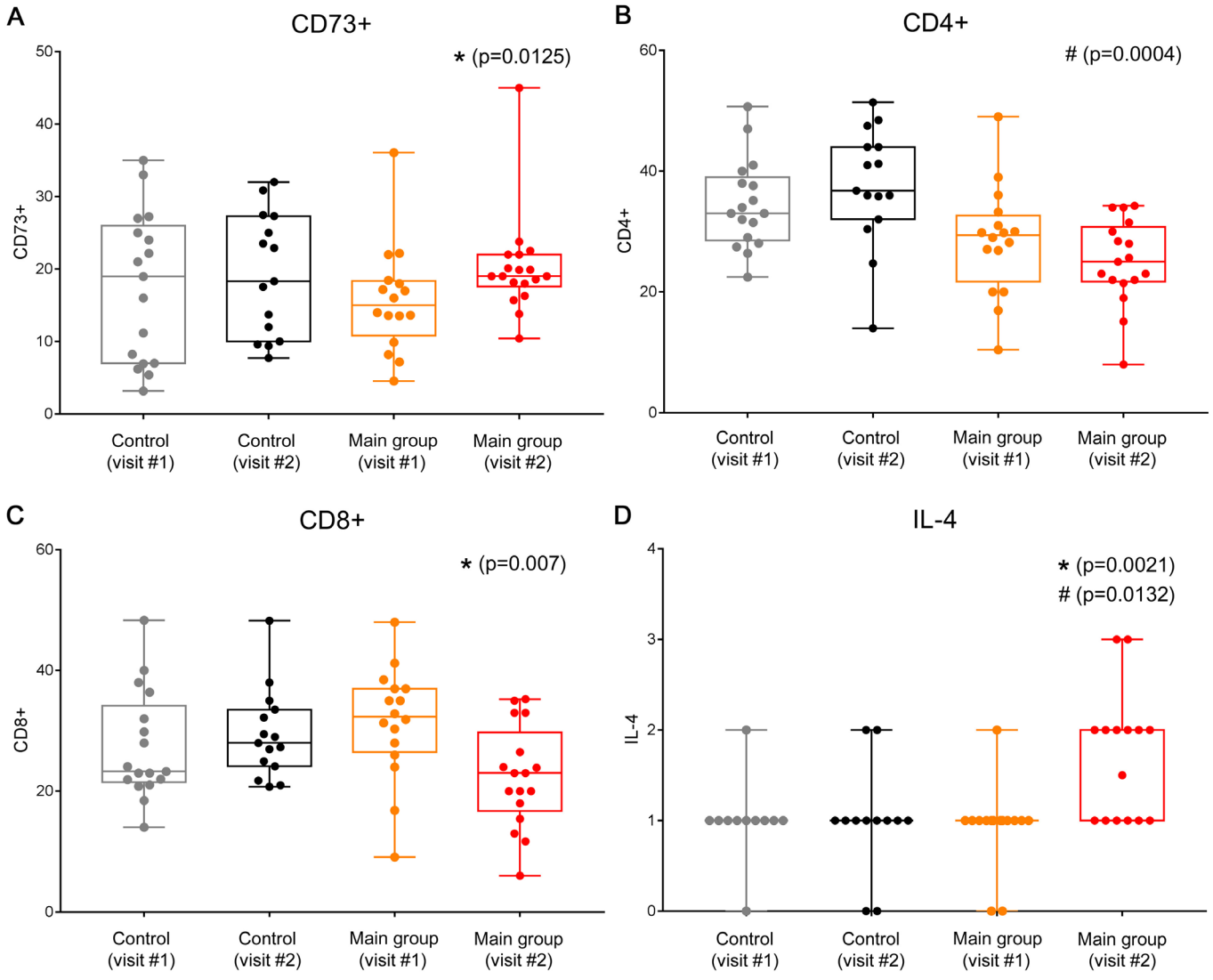

Figure 4 – Comparison of immunological markers between the main and control patient groups

Table 4 Echocardiographic Parameters in Patients from the Main and Control Groups

Indicators	Control group (n = 29)		Main group (n = 32)	
	Visit 1	Visit 2	Visit 1	Visit 2
Echo EF (%)	24.3 ± 8.3	28.0 ± 6.8	26.1 ± 8.4	37.6 ± 7.6 * (p<0.0001) # (p<0.0001)
Base of the aorta (mm)	27.0 ± 2.7	27.2 ± 2.7	27.3 ± 2.8	27.4 ± 2.8
Ascending aorta (mm)	33.3 ± 4.3	34.0 ± 4.7	34.3 ± 4.7	33.7 ± 4.3
Left atrium (mm)	49.5 ± 9.2	49.5 ± 7.3	46.8 ± 6.8	45.6 ± 7.7
Right ventricle (mm)	32.6 ± 6.1	32.3 ± 5.2	32.0 ± 4.8	31.1 ± 5.1
EDD (mm)	65.4 ± 9.9	64.5 ± 10.1	64.7 ± 8.9	61.4 ± 8.6
ESD (mm)	55.4 ± 10.2	53.1 ± 10.0	52.7 ± 9.4	48.9 ± 9.8
EDV (ml)	205 ± 65	187 ± 55	197 ± 61	178 ± 51
ESV (ml)	157 ± 59	135 ± 46	146 ± 54	113 ± 42 * (p=0.0496)
Interven-tricular septum (mm)	10.9 ± 2.5	10.8 ± 2.3	11.3 ± 2.0	11.5 ± 2.3
Posterior wall of the left ven-tricle in diastole (mm)	10.9 ± 2.5	10.7 ± 2.00	11.0 ± 1.9	11.3 ± 2.3
TAPSE (mm)	14.1 ± 2.5	14.8 ± 3.1	14.9 ± 2.7	16.0 ± 3.2
ePASP (mmHg)	37.6 ± 9.2	33.9 ± 8.3	32.2 ± 9.3	29.2 ± 6.7

Echo – echocardiography, EF – ejection fraction, EDD – End-diastolic dimension, ESD – End-systolic dimension, EDV – End-diastolic volume, ESV – End-systolic volume, TAPSE – Tricuspid Annular Plane Systolic Excursion, ePASP – estimated pulmonary artery systolic pressure.

to Visit 2 (p = 0.0051 and p = 0.0019, respectively) (Figures 3C and 3D). Finally, the estimated systolic pulmonary artery pressure showed significant decrease in the main group (p = 0.0159), whereas no such change was observed in the control group (Figure 3B).

Discussion

Previous studies [31] have demonstrated the efficacy of stem cell therapy in heart failure (HF), showing improvements in left ventricular function and mortality in patients with HF and dilated cardiomyopathy (DCM). However, the role of pro-inflammatory and anti-inflammatory cytokines, as well as the systemic effects of stem cells after transplantation, has not been thoroughly investigated. The main findings of this study demonstrate that comprehensive treatment using MSCs combined with OMT compared to standard pharmacotherapy for CHF led to the following outcomes after 12 months: reduction in subjective clinical signs of heart failure, improvement in the clinical status of patients (as measured by KCCQ Questionnaire), decrease in NYHA functional class from IV–III to class II with an increase in physical activity toletolerance (as evidenced by the six-minute walk test).

CBC revealed a statistically significant decrease in eosinophil count and normalization of coagulation parameters INR and PT. Biochemical analysis showed a reduction in blood urea levels and an increase in serum calcium concentration. The reduction in eosinophils may indicate an immunomodulatory effect of cell therapy and a decrease in inflammatory responses. Normalization of INR and PT may suggest improved hepatic blood flow and liver function. The decrease in urea levels may reflect improved renal perfusion, reduced catabolic processes, and less fluid retention. The increase in calcium could be indicative of reduced inflammation and acidosis, and improvement in calcium metabolism. These findings suggest systemic effects of cell therapy, including immunomodulation, improved hepatic and renal function, and normalization of calcium metabolism. Furthermore, there was a significant decrease in serum NT-proBNP levels and immunological assays demonstrated a significant increase in anti-inflammatory cytokines CD73+ and IL-4 and reduction in the pro-inflammatory marker CD8+.

ECHO data showed favorable dynamics: LVEF increasing from 26.1 ± 8.4% to 37.6 ± 7.6%, along with reduction in cardiac dimensions (ESV, EDD, and ESD). These changes were either absent or less pronounced in the control group suggesting that cell therapy is effective and may be considered as an adjunct to standard CHF treatment.

Venous or systemic congestion imposes an additional burden on the heart, resulting in increased ventricular wall tension, valvular insufficiency, myocardial stretching, structural remodeling, myocyte necrosis, and a gradual deterioration of cardiac function [32]. Increased preload on the heart can stimulate cytokine expression in both endothelial and smooth muscle cells. Hypoxia and ischemia have been identified as potent inducers of pro-inflammatory cytokines [33]. The study conducted by Van Tassell BW et al. provided clear evidence of increased inflammatory cytokine levels in patients with heart failure during disease progression, thereby lending support to the "cytokine hypothesis". Within the cytokine family, several proteins require careful consideration. Proteins of the interleukin-1 (IL-1) family exert effects on virtually all components of the innate immune system, including macrophages, neutrophils, eosinophils, and basophils [34]. Cell therapy has been shown to improve LVEF in patients with DCM through paracrine mechanisms and systemic effects of autologous MSCs. This hypothesis is supported by previous clinical studies demonstrating effectiveness of autologous cell CHF [35].

Regenerative medicine is rapidly advancing driven by progress in the generation of specialized cells from pluripotent stem cells, extracellular vesicles, and etc. Novel organoid models such as innervated cochlear organoids and vascularized cardiac microtissues enable precise reproduction of physiological processes and facilitate the testing of genetic and molecular interventions at the tissue and organ levels [36, 37]. Given the observed improvements in cardiac contractile function, as well as objective clinical and laboratory parameters, cell therapy may be considered a promising approach within the comprehensive treatment strategy for CHF. Despite the positive results obtained, the study had several limitations, including a small sample size, single-center design, lack of age stratification, and a limited follow-up period. Future research is planned to expand the study to a multicenter format, incorporate patient stratification, and extend the follow-up duration.

Conclusion

Chronic heart failure is associated with secondary immunodeficiency which further impairs reparative processes in organs, tissues, and systems of the body. Consequently, the bone marrow as the central organ of immunogenesis functions in a state of stress resulting in suppression of the regenerative potential of mesenchymal stem cells. Accordingly, reestablishing the bioregulatory function and regenerative potential of bone marrow-derived cells through in vitro cultivation is considered critically important. The objective of this study was to assess the efficacy of autologous mesenchymal stem cell therapy in patients diagnosed with dilated cardiomyopathy. Comprehensive treatment OMT + cell therapy resulted in improved left ventricular function and reduced cardiac cavity dimensions on Echo, decreased heart failure NT-proBNP levels and improvements in standard laboratory markers. Further multicenter randomized studies with larger sample for evaluation of cardiac function are required to confirm these outcomes. In order to confirm these outcomes large-scale, multicenter randomized trials are required for cardiac functional assessment. A fundamental solution to the problem of heart failure with reduced left ventricular ejection fraction can only be achieved through technologies aimed at replenishing lost cardiomyocyte population.

Author Contributions: Recruitment of patients into the study, M. L., A. M., D. Z. and G. K.; patient hospitalization, B. K.; inclusion of patients and collection of research material, M. L.; bone marrow collection, M. A. and A. T.; cell culturing, A. A.; writing – original draft preparation, M. L.; writing – review and editing, T. S. and M. A.; research director, A. B. All authors have read and agreed to the published version of the manuscript.

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Informed Consent: Written informed consent was obtained from all participants included in the study.

Studies involving human subjects were conducted in accordance with institutional and international ethical standards.

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Traces of Emotional Fluctuations Around the World Regarding the HPV Vaccine: a Netnographic Sentiment Analysis

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Abstract

Background: Human papillomavirus (HPV) is a leading cause of cervical cancer, and vaccination is a key preventive measure. Despite its proven effectiveness, discussions surrounding the HPV vaccine remain polarized on social media, reflecting varying public sentiments.

Aims: The aim of this study is to examine the HPV vaccine discussions on social media platforms netnographically and to analyze sentiment with artificial intelligence.

Methods: The study used data collected from various social media platforms such as Twitter, Tik Tok, YouTube, Facebook, and news sites during a one-month period between April 7 and May 7, 2024. The Brand24® tool was used for data collection, and sentiment analysis of the data was conducted netnographically. The analysis included dimensions such as mention volumes, the most influential sites, trending hashtags, and sentiment analysis.

Results: The analysis showed that 79% of online discussions on HPV vaccination were neutral, 14% were positive, and 7% had negative sentiment. The most influential sites included YouTube, Facebook, Twitter, TikTok, and the CDC. The most popular hashtags included #hpvvaccine, #hpv, and #cervicalcancer. Increases in the volume of mentions were observed, especially on and after World Immunization Day.

Conclusions: The results provide important clues for the development of health communication strategies and vaccination policies. In addition, analyzing the change in online discussions over time contributes to identifying awareness and current trends towards HPV vaccination.

Keywords: HPV vaccine, netnography, sentiment analysis, health communication, vaccine policies.

Introduction

Today, human papillomavirus (HPV) and HPV vaccination are critical issues, especially for women's health. HPV is the leading cause of cervical cancer and can be prevented through vaccination. [1]. The HPV vaccine helps prevent cancer and other serious health problems caused by the virus. Therefore, HPV vaccination is extremely important and is recommended for young girls and boys [2]. Studies have found that vaccinating girls against HPV can reduce the incidence of cervical cancer by about 90% [1, 3–5]. However, HPV vaccination is still a common topic of debate among young girls and women [3]. Much of this debate takes place in digital environments, such as social media platforms. Therefore, studying these discussions using netnographic methods can be an important tool to understand the emotional and behavioral attitudes of the public about the HPV vaccine.

Netnography is a research method that examines interactions that take place in online communities such as social media platforms, forums, and blogs. This method makes it possible to analyze the thoughts, feelings, and experiences that people share in real time [8]. Examining the HPV vaccine debate from a

netnographic perspective is a valuable opportunity to understand the public's emotional reactions and attitudes on this issue. Netnographic analysis of HPV vaccine discussions can reveal different emotional and behavioral themes. For example, some respondents may support the HPV vaccine, while others may express concerns about its safety and efficacy. In addition, a variety of arguments and emotional responses can be observed between vaccine opponents and vaccine proponents [9]. Such analyses can provide important clues to understand the complexity of the HPV vaccine debate and determine the overall public attitude.

Netnographic analyses can also reveal how the HPV vaccine debate has changed over time and what factors have influenced these changes. For example, it may be observed that vaccine discussions intensify following a particular event or news story. In a netnographic study of HPV vaccine debates in Australia using Twitter data, it was found that vaccine hesitancy and anti-vaccination were at the forefront. These themes are seen as an important source of data to understand the general perception and discussions about HPV vaccination on social media platforms [10]. In addition, it can also be examined how a particular piece of content that is popular on social media platforms influences discussions about HPV vaccination. The results of netnographic analyses can also be important for the formulation of public policies on HPV vaccination and the development of health communication strategies. Understanding the emotional and behavioral attitudes of the public can be a valuable source of information to identify the factors affecting vaccination decisions and to guide awareness-raising efforts in this regard. The study examined public perceptions of the resumption of HPV vaccine recommendations in Japan through Twitter analysis and found that negative perceptions were predominant. The researchers suggested that health communication should be strengthened [9]. Netnographic analyses can contribute to an in-depth understanding of the discussions taking place in digital environments such as social media platforms and provide important clues to determine the public's attitude towards general health and vaccination. Therefore, it is important to use netnographic methods more in analyzing HPV vaccine debates and to increase research on this subject. This is because it can help WHO develop targeted communication strategies to increase confidence in the vaccine. For example, by highlighting posts with positive sentiment, the WHO could increase the dissemination of such content. Furthermore, in the development of European Union (EU) health policies, the data from this study can be used to shape campaign strategies by identifying which social media platforms are most effective. By becoming more active on platforms such as YouTube, Facebook, and Twitter, the EU can collaborate to reduce vaccine-related information pollution. For example, Deiner et al. (2019) noted that health institutions should be more active on these platforms. This contributes to the dissemination of information and discussions about vaccination [11].

Previous studies have generally analyzed discussions in a specific country or on a specific social media platform [8,9,10]. However, this study provides a holistic picture of the HPV vaccine debate around the world, using data from a much wider geographical scope and various social media channels. In addition, an important difference is that the study examined a time period coinciding with World Immunization Day. In this way, the study had the opportunity to analyze global awareness and current debates on HPV vaccination. The aim of the study is to understand the public's emotional and behavioral attitudes towards the HPV vaccine and to reveal the changes in debates about the HPV vaccine over time. The results obtained will provide important clues for the formulation of public policies on HPV vaccination and the development of health communication strategies. In this respect, the study aims to make a practical contribution and differs from other static analyses.

Methods

In the study, sentiment analysis of HPV vaccine-related data was conducted using netnography. Netnography is the adaptation of ethnographic research techniques to the online environment to understand the behaviors, attitudes, and social interactions of different human communities or cultures in all digital communication channels.

Data collection

Data was collected online between April 7 and May 7, 2024. It was collected from X (Twitter), Tiktok, Facebook, Youtube, news, and web sites via Brand24® with the mention of #hpvvaccine in English. Brand24® is a tool that enables the daily analysis of internet sources on the basis of relevant keywords and a few mentions and their automatic sentiment analysis as optimistic and pessimistic sentiments. This tool allows for an automated review of online sources. The sentiment analysis methodology used by Brand24® requires the use of a term highly relevant to the results presented in several dimensions, such as the type of sources, the most active sites, the most influential sites, trending hashtags, a summary based on the numerical context of social mentions, and sentiment analysis. Taking this one-month period into account is extremely important, as April 29 is World Immunization Day.

Software Description

Brand24® is a commercial media monitoring and sentiment analysis platform that collects publicly available online data across social media platforms and websites. For sentiment classification, Brand24® uses a proprietary natural language processing (NLP) engine supported by machine learning algorithms. This tool detects the polarity of online mentions—positive, neutral, or negative—based on keyword co-occurrence, syntactic structures, and tone recognition patterns. While the algorithmic infrastructure is not open source, Brand24® provides explainable outputs such as sentiment graphs, keyword clouds, and influencer rankings based on engagement metrics.

Data Analysis

After data collection through Brand24®, a one-month dataset (April 7 – May 7, 2024) was analyzed. The analysis included multiple dimensions: (1) mention volume trends, (2) sentiment breakdown (positive/neutral/negative), (3) keyword frequency, (4) platform distribution, (5) trending hashtags, and (6) identification of most influential websites. Visual outputs (charts, tables, and graphs) were automatically generated by the Brand24® platform. Sentiment fluctuation was examined in relation to key events (e.g., World Immunization Day). No manual annotation or sentiment reclassification was performed post-collection to maintain objectivity.

Ethical consideration

Ethical consideration was not required due to the type of research. The data used in this study are publicly available, as they were published openly on the internet. Nevertheless,

considering the privacy of the users, usernames were not included in the study. Due to the impossibility of obtaining informed consent from all data subjects, no personal or sociodemographic data were analyzed. The data was collected using an automated process. The confidentiality of the data subjects was therefore protected. The research was conducted in accordance with the Declaration of Helsinki.

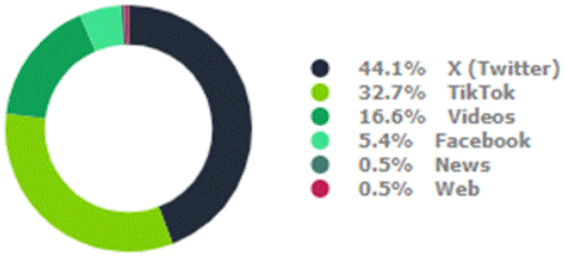

Figure 1 – Mentions by category

Results

The results obtained from the netnographic analysis are comprehensively explained under the following headings:

A. Mentions by category

For sentiment analysis of the data collected through Brand24®, 44.1% of the data was taken from X (Twitter), 32.7% from Tiktok, and 16.6% from sources such as Youtube and various video platforms (Figure 1).

B. Volumes of social mentions

Figure 2 shows the volume of HPV vaccine mentions between April 7 and May 7, 2024. It can be seen that this volume was 25 on April 29, 2024, which is attributed to World Immunization Day, and reached a peak value of 27 on May 1, 2024.

C. Contexts of discussion

The online sentiment analysis tool also provides information on contexts of discussion. The analysis of the top 100 result keywords revealed the hashtags #cancer, #HPV, #cervicalcancer, #cancersucks, and the terms hpv, prevent, cancer, cervical, and vaccine. These words give an idea of the

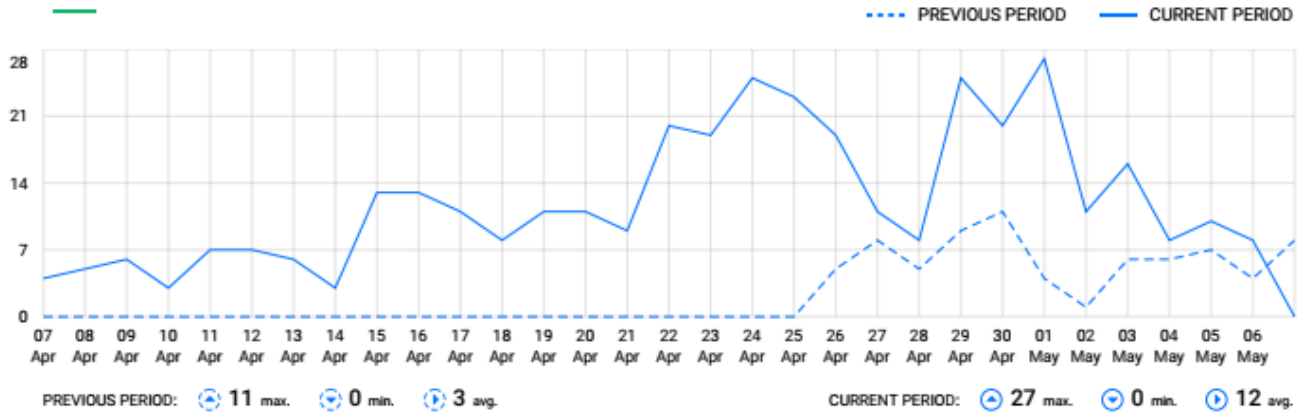

Figure 2 – One-month volume of mentions related to HPV vaccines

Source (s): Brand24®

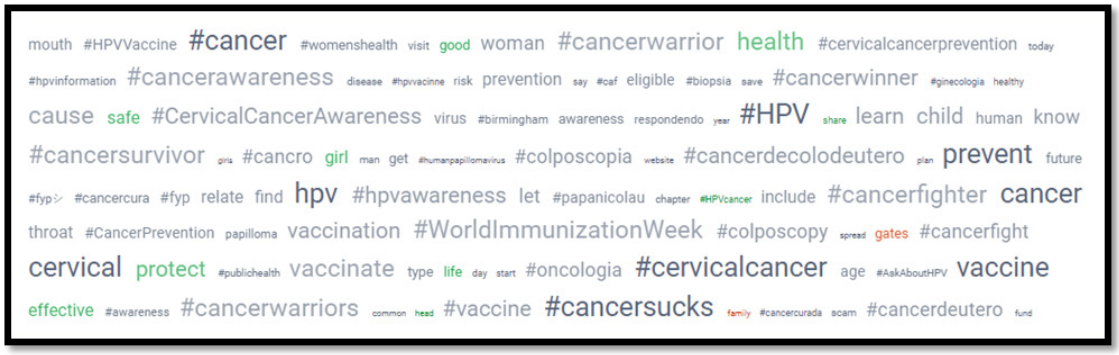

Figure 3 – Context of the discussion on HPV vaccines

Source (s): Brand24®

topics searched and researched by users about HPV vaccines around the world (Figure 3).

D. Most influential sites

The sentiment analysis also provided numerical data on the most influential websites (Table 1). According to these metrics, youtube.com, facebook.com, twitter.com, tiktok.com, and cdc.gov emerged as the most influential websites with an influence score of 10/10. When the websites in Table 1 are analyzed, it is seen that youtube.com has 33 million visits, facebook.com has 16 million, twitter.com has 6 million, tiktok.com has 2 billion, and cdc.gov has 50 million visits. With a good score of 8/10, suckhoedoisong.vn ranked in the list with 9 million visitors.

Table 1 Most influential sites

No	Web site	Visits	Influence score
1	youtube.com	33B	10/10
2	facebook.com	16B	10/10
3	twitter.com	6B	10/10
4	tiktok.com	2B	10/10
5	cdc.gov	50M	10/10
6	suckhoedoisong.vn	9M	8/10
7	accypn.org.au	4 244	1/10

Source (s): Brand24®

E. Trending hashtags related to HPV vaccines

Table 2 shows the 20 trending hashtags related to HPV vaccines within a one-month period. According to the table, the most mentioned hashtags were #hpvvaccine (308 mentions) and #hpv (141 mentions). The least mentioned hashtags were #colposcopia (24 mentions), #cancerfight (23 mentions), and #cancerdeutero (23 mentions).

F. Numerical Summary

Brand24® also provided access to a numerical summary of mentions on social media. According to the online report, there were a total of 367 mentions covering the one-month period between April 7 and May 7, 2024. Of these, 363 mentions were from social media, and 4 mentions were from non-social media. Social media reach was 751K, and non-social media reach was 94666. In addition, interactions and 8382 likes were determined for 9998 data points (Table 3).

G. Sentiment Analysis

When the sentiment analysis about HPV vaccines in a one-month period was performed, it was determined that the highest positive value was on April 22, 2024, with 7 points, and the highest negative value was on May 1, 2024, with 6 points (Figure 4).

In terms of sentiment distribution, 14% of the data accessed through Brand24® was positive, 79% was neutral, and 7% was negative polarity (Figure 5).

Some examples of data on HPV vaccines with positive, neutral, and negative polarities are shown in Table 4.

Table 2 Trending hashtags related to HPV vaccines

No	Hashtag	Mentions
1	#hpvvaccine	308
2	#hpv	141
3	#cervicalcancer	59
4	#cancer	58
5	#cancerawareness	38
6	#cancersucks	37
7	#cancerfighter	33
8	#vaccine	33
9	#cancerwarrior	32
10	#cancersurvivor	31
11	#cancerwarriors	31
12	#hpvawareness	30
13	#cervicalcancerawareness	30
14	#hpvvaccines	29
15	#worldimmunizationweek	28
16	#cancerwinner	27
17	#cancerdecolodeutero	27
18	#colposcopia	24
19	#cancerfight	23
20	#cancerdeutero	23

Source (s): Brand24®

Table 3 Numerical summary

Features	Number
Mentions	367
Social media mentions	363
Non-social mentions	4
Social media reach	751 K
Non social media reach	94666
Interactions	9998
User generated content	363
Likes	8382
Mentions from X (Twitter)	162

Source (s): Brand24®

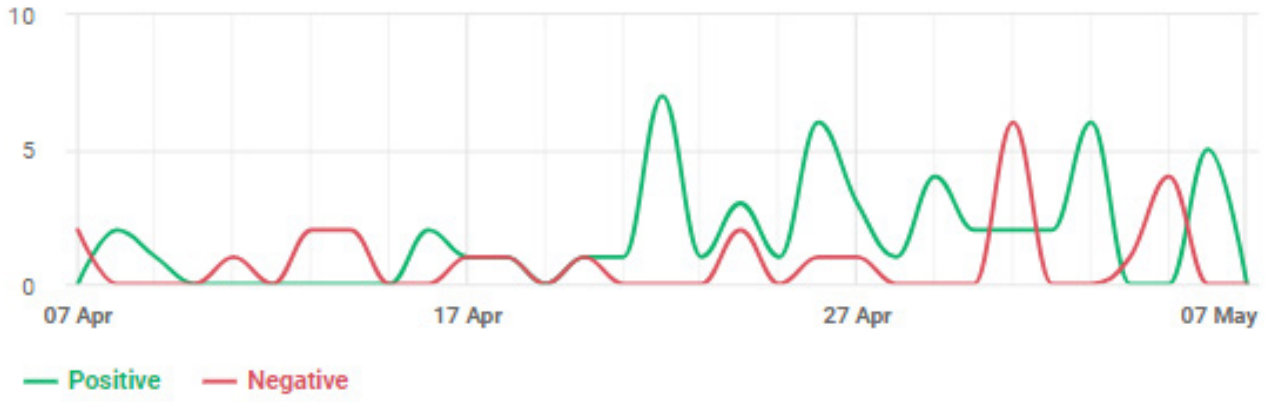

Figure 4 – Sentiment analysis on HPV vaccines

Source (s): Brand24®

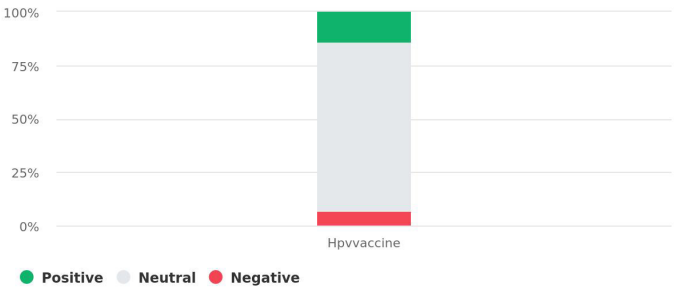

Figure 5 – Sentiment breakdown

Source (s): Brand24®

Table 4 Examples datas and polarities

Sentiment	Text
Positive	... [...] Nigeria's proactive approach to public health, includ-ing the introduction of the #HPVvaccine into our EPI, was greatly influenced by the advocacy efforts of the First Ladies Against Cancer (FLAC), led by H.E Dr [...] Zainab Shinkafi Bagudu. No doubt! ...
Positive	#HPVVaccine is the best defense against cervical cancer and diseases caused by HPV. Girls aged 9-14 are to be vaccinated to be protected. #HPVVaccine #CancerPrevention #Vaccine-goodoh
Neutral	HPV is a sexually transmitted infection affecting millions in the US. 14 million new cases occur yearly, with 19,000 peo-ple getting infected daily, mostly those aged 15 to 24. Check the link below to learn more.
Neutral	... With over 3,500 new #CervicalCancer cases recorded an-nually in #Morocco, Dar Zhor Association urged better #HPVvaccine access in a recent press conference in Casa-blanca, as part of the national efforts to eliminate the diseases by 2030 ...
Negative	Lights are still hurting her eyes, heavy ... [...] I want to say i think shes over the worst of it. Some sun and swimming has certainly helped! #hpvinformation #vaccine #awareness #HPV #hpvvaccine ...
Negative	Shes ... [...] But why arent we made fully aware of what could happen? Huge lack of information around this drug is being provided! #fyp #fyp #hpvvaccine #HPV #awareness #vaccine #hpvinformation #hpvawareness ...

Source (s): Brand24®

Discussion

As part of the in-depth analysis of online discussions about the HPV vaccine, we examined Twitter, TikTok, YouTube, and other platforms. We also focused on the change in the volume of mentions of the HPV vaccine over time. We observed that there was a particularly high volume of mentions on April 29th, World Immunization Day, and May 1st. Within the scope of the analysis, we examined the websites preferred by users looking for information about the HPV vaccine. In this analysis, we determined which websites attracted more attention based on the number of site visits and influence scores. The findings obtained provide important data on which websites are more effective in terms of health communication. Through this analysis, we found that online discussions about HPV vaccination were largely neutral (79%) in terms of emotional content. However, the presence of positive (14%) and negative (7%) emotional posts is also noteworthy. In particular, the highest levels of positive sentiment were observed on April 22, and the highest levels of negative sentiment were observed on May 1, indicating how specific events influence these discussions. Such analyses play an important role in determining public health policies and raising public awareness [8].

Polarization between those who trust and distrust vaccines

This study showed that online discussions about HPV vaccination were largely neutral (79%) in terms of emotional content. However, the presence of positive (14%) and negative (7%) emotional posts is noteworthy. This reflects the polarization between those who trust the vaccine and those who do not. And it reveals the complex nature of social attitudes towards the HPV vaccine. The polarization between those who trust and those who do not trust the vaccine is a theme also highlighted by Katz et al. (2020) and should be taken into account in the development of health communication strategies [10]. In particular, the observation of the highest levels of positive sentiment on April 22 and the highest levels of negative sentiment on May 1 shows how certain events can influence the HPV vaccine debate. Boucher and colleagues' (2023) study of HPV vaccine discourse during the COVID-19 pandemic highlights such event-driven shifts. [8]. In another study, trust in vaccines and the system that delivers them was the most frequently cited reason for vaccine acceptance or rejection [12]. However, after April 29, the use of hashtags such as #hpvvaccine and #cervicalcancer increased (Cervivor (@iamcervivor), 2024). This has helped to increase public awareness of the vaccine [13]. Gholami et al. (2022) highlighted the importance of vaccine-related content management on social media platforms [14]. Therefore, the change in HPV vaccine discussions over time is an issue that should be taken into account when developing health communication strategies. In the study, it was observed that discussions about the vaccine increased on certain dates (e.g., World Immunization Day). WHO can adjust the timing and content of its campaigns by taking such events into account. For example, it could organize intensified information activities on certain days.

According to this study, the most effective social media platforms are YouTube, Facebook, Twitter, TikTok, and the CDC. Therefore, having insights into the most influential social media platforms can significantly influence a policymaker's communication strategy. This is a critical element for reaching the target audience, using resources efficiently, managing crises, and gaining a competitive advantage. The study by Terada et al. (2023) in Japan highlights the role of these platforms in spreading anti-vaccine rhetoric [9]. A different study examining issues of mistrust and uncertainty about the HPV vaccine in Europe shows how attitudes and concerns about the vaccine vary across different European countries. The study points to a decline in vaccine coverage rates in European countries where confidence in the HPV vaccine has been shaken. [15]. Sources that provide general information on the use and effects of HPV vaccines examine practices in different countries and public attitudes towards the vaccine. Such information can help inform the development of vaccination strategies [16]. This study provides important data to understand hesitancy toward the vaccine. Increased participation in the HPV vaccine debate is an important opportunity for policymakers. Feedback from different segments of society can help improve vaccine policies. In addition, policymakers can continuously update their communication strategy based on feedback from followers.

Opponents' concerns about the safety of the HPV vaccine stand out as a critical issue in the development of health communication strategies. Sabeena et al. (2017), in

their study on low socioeconomic groups in India, emphasized that concerns about vaccine safety have a negative impact on vaccination rates [3]. Similarly, Gürsoy and Sağtaş's (2022) study on university students in Turkey revealed that vaccine safety concerns constitute a significant barrier. [4]. Concerns of vaccine opponents about HPV vaccine side effects, long-term risks, and a lack of transparent information point to the need for health authorities to strengthen their communication strategies. Hakimi et al. (2023) reported that vaccine safety concerns were also prominent among the barriers faced by HPV vaccination programs in the Eastern Mediterranean region [5]. This shows that similar concerns are shared globally. In addition, the World Health Organization (WHO) also draws attention to the preventive effect of the HPV vaccine [2]. In short, the safety concerns of those who do not trust the HPV vaccine and the emphasis on its benefits by those who trust it stand out as key differences that should be considered in shaping health communication strategies. This study provides a comprehensive overview of the global debate on the HPV vaccine. Using this data, WHO can organize global awareness-raising campaigns on the vaccine and develop effective strategies in different cultural contexts. Knowing which platforms receive more engagement can therefore help develop quick and effective communication strategies to deal with negative perceptions. A strategy without insight can also fall short in times of crisis.

This study has shown that online discussions on HPV vaccination have largely neutral emotional content, but there are also posts with positive and negative emotions. This suggests that the content management policies of social media platforms are a critical factor in formulating health communication strategies. It is important for health authorities to closely monitor the content management policies of social media platforms and develop effective communication strategies in this regard. On the other hand, the identification of the most effective social media platforms, such as YouTube, Facebook, Twitter, TikTok, and the CDC, may be a guide for health authorities in directing awareness and information activities on HPV vaccination. In addition to the current study, there are examples in the literature that address the HPV vaccine debate on social media platforms from different perspectives. For example, Malik et al. (2020) conducted a qualitative analysis of HPV vaccine-related posts on Facebook and Twitter in India. Their study revealed “vaccine safety," “vaccine ambivalence,” and “vaccine advocacy” as the main themes of HPV vaccine discussions on social media platforms [17]. Similarly, Katzman et al. (2021) conducted a visual analysis of HPV vaccine content on Instagram. Their findings showed that both positive and negative HPV vaccine content was widely shared on Instagram [18]. On the other hand, Smith et al. (2019) examined HPV vaccine discussions in Facebook groups. The researchers emphasized that large-scale groups on these platforms play an important role in spreading vaccine ambivalence. [19]. All these studies show that social media platforms influence HPV vaccine debates in different ways. In particular, the prominence of themes such as vaccine safety, ambivalence, and advocacy suggests that the role of these platforms should be considered in the development of health communication strategies. The content management policies of social media platforms are a critical factor that directly shapes the HPV vaccine debate and should be taken into consideration when developing health communication strategies.

This study analyzed in-depth online discussions on HPV vaccination on a global scale. The findings provide important clues for the development of health communication strategies and vaccination policies. The results of the analysis revealed that online discussions on the HPV vaccine have largely neutral emotional content, but there are also posts with positive and negative emotions. In particular, it was found that the safety concerns of opponents of the vaccine were effectively disseminated. This emphasizes the need to strengthen health communication strategies. In addition, the identification of the most effective social media platforms, such as YouTube, Facebook, Twitter, TikTok, and the CDC, has guided health authorities in directing awareness and information activities on HPV vaccination. It is recommended that health authorities focus primarily on these platforms while conducting awareness and information activities on HPV vaccination. On the other hand, the fact that the most popular hashtags focused on cancer prevention and vaccination revealed that health communication strategies should focus on these areas. In conclusion, the findings provide important clues for the development of health communication strategies and vaccination policies.

Limitaions

The data collection period is limited to a one-month period. However, similar data collected at other times of the year or in other years would also provide useful insights. The study focused only on data collected in English. Analyzing online discussions in other languages may better reflect global dynamics. The study did not include qualitative content analysis on social media platforms. In addition to sentiment analysis, an in-depth analysis of the content and context of posts can contribute to the development of health communication strategies. This study only analyzed discussions in online environments. Sentiments may differ substantially between countries, so the results here cannot be generalized to every country without additional insights or assumptions.

Additionally, due to the lack of consistent geolocation metadata in Brand24® outputs, disaggregating sentiment data by specific countries or income levels was not feasible in the current study. Future research could benefit from using advanced geotagged datasets or manually annotated sources to compare attitudes towards the HPV vaccine across World Bank income groups or WHO regional classifications.

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Comparative Analysis of Diagnostic Imaging Modalities for Anatomical Instability in Patellofemoral Pain Disorders: a Systematic Review

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Abstract

Patellofemoral pain disorders (PFPD) are characterized by pain around or behind the patella, commonly aggravated by activities such as squatting, running, or stair climbing. Accurate diagnosis of anatomical instability is essential in managing PFPD. This systematic review evaluates the diagnostic accuracy and clinical utility of various imaging modalities—X-ray, magnetic resonance imaging (MRI), and computed tomography (CT)—in detecting key anatomical risk factors associated with patellofemoral instability, including trochlear dysplasia, patellar height, tibial tubercle–trochlear groove (TT-TG) distance, and medial patellofemoral ligament (MPFL) integrity.

A comprehensive literature search was conducted in accordance with PRISMA guidelines across databases including PubMed, Scopus, ScienceDirect, Google Scholar, and the Cochrane Library, focusing on studies published from 2013 to 2023. Only comparative studies assessing imaging modalities in PFPD were included, while case reports and non-English articles were excluded. Two reviewers independently screened articles, extracted data, and evaluated study quality using the QUADAS-2 tool. A qualitative synthesis was conducted due to the heterogeneity of study designs.

Results show that MRI and CT are superior to X-ray for evaluating trochlear dysplasia and patellar height, with MRI demonstrating high sensitivity (90–97%) and specificity (93–98%). CT is the gold standard for assessing TT-TG distance (sensitivity 95–98%, specificity 93–96%), while MRI is preferred for evaluating MPFL integrity, showing near-perfect diagnostic accuracy.

The review underscores the need for standardized imaging protocols and diagnostic criteria to improve comparability and reliability across studies. Future research should investigate the role of dynamic imaging techniques, larger study populations, and cost-effectiveness to refine and optimize diagnostic strategies for PFPD.

Keywords: Patellofemoral pain or patellar instability, Imaging, Anatomical instability, Radiographic imaging, Sensitivity, Specificity.

Introduction

Patellofemoral pain disorders (PFPD) involve discomfort around or behind the patella, often exacerbated by activities like squatting, running, or stair climbing [1]. Anatomic instability significantly influences patellar alignment and tracking dynamics, contributing to patellar femoral pain syndrome (PFPS) [2]. Notably, females are 2-3 times more likely to develop patellofemoral instability (PFI) compared to males [3].

The patellofemoral joint (PFJ) is geometrically complex, and both soft tissue and bony surgical

procedures are available to address PFI [4]. While non-operative management is recommended for most cases following an initial dislocation without osteochondral fractures, these patients often experience high recurrence rates and ongoing activity limitations [5].

Diagnosing PFPD typically involves patient history, physical examination, and imaging [6]. Imaging is vital in assessing anatomic instability, patellar alignment, and tracking abnormalities, with different modalities offering unique strengths and limitations. Conventional radiography, including skyline, lateral, and Merchant views, is commonly used due to its accessibility and ability to assess bony morphology and alignment [7]. The skyline view is particularly useful for detecting patellar tilt and subluxation, while the lateral and Merchant views help evaluate patellar height and trochlear morphology [8]. However, radiography has limitations, such as its inability to assess soft tissue structures or dynamic patellar tracking [9]. Advanced imaging modalities like CT and MRI offer better visualization of soft tissues, ligamentous structures, and dynamic tracking, with CT being ideal for bony abnormalities and MRI for soft tissue assessment [10].

This systematic review aims to thoroughly analyze and compare the effectiveness of various diagnostic imaging modalities in evaluating anatomical instability in PFPD. We will examine the strengths and limitations of each technique, focusing on their ability to detect the following critical instability risk factors:

- Trochlear Dysplasia: Abnormal development of the trochlear groove, the bony indentation on the femur that guides patellar tracking. A shallow or dysplastic groove can predispose the patella to subluxation or dislocation [11].
- Patellar Height: An abnormally high-riding patella (patella alta) disrupts its normal tracking within the trochlear groove, increasing the risk of instability [12].
- Tibial Tubercle-Trochlear Groove Distance (TT-TG Distance): This distance reflects the alignment between the tibial tubercle (where the patellar tendon inserts) and the trochlear groove. An increased TT-TG distance suggests a tendency for patellar maltracking [13].
- Medial Patellofemoral Ligament (MPFL) Integrity: The MPFL is a key stabilizer of the patella that can be disrupted due to injury or insufficiency, leading to patellar instability [14].

Objectives of the Review:

This systematic review aims to address these knowledge gaps by critically evaluating the existing evidence on the diagnostic performance of various imaging modalities in assessing anatomical instability in PFPD. The objectives of this review are

- To identify and critically appraise the current literature on the diagnostic accuracy of different imaging modalities (X-ray, MRI, CT) for evaluating anatomical instability risk factors in PFPD patients.
- To comparatively analyze the strengths and limitations of each modality in visualizing key instability factors such as trochlear dysplasia, patellar height, TT-TG distance, and MPFL integrity.
- To synthesize the findings and identify the most effective imaging approaches for diagnosing anatomical instability in PFPD, considering factors like diagnostic accuracy, cost-effectiveness, and radiation exposure.

Methods

This systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure a comprehensive and unbiased synthesis of the available evidence on diagnostic imaging modalities for anatomic instability in patellofemoral pain disorders.

1. Search Strategy

A comprehensive electronic search was conducted across PubMed/MEDLINE, Google Scholar, Scopus, Science Direct, and Cochrane Library. The search utilized a combination of controlled vocabulary terms (MeSH terms) and keywords specific to each database's search syntax. The search string included the terms related to patellofemoral pain or patellar instability, anatomic instability factors (such as mal tracking, trochlear dysplasia, patellar height, TT-TG distance, and MPFL integrity), diagnostic imaging modalities (X-ray, MRI, CT scan), and diagnostic accuracy (diagnosis, accuracy, sensitivity, specificity). Furthermore, a manual review of the reference lists of pertinent and incorporated studies has been conducted. This search was limited to articles published in English between January 1, 2013, and December 31, 2023, to ensure the inclusion of recent and pertinent data.

2. Selection Criteria

2.1. Inclusion Criteria

- Studies evaluating imaging modalities (MRI, CT, ultrasound, X-ray) for diagnosing anatomic instability in patients with PFP disorders.
- Comparative studies, including randomized controlled trials (RCTs), review studies, cohort studies, and case-control studies.
- Studies published in English.
- Full-text articles available.

2.2. Exclusion Criteria

- Studies focusing on non-anatomic causes of PFPD, such as tibiofemoral joint pathology, and post-surgical populations.
- Case reports, case series, editorials, and conference abstracts.
- Animal and cadaveric studies.
- Studies published in languages other than English.

3. Study Selection Process

3.1. Screening

All retrieved citations were imported into a reference management software, Mendeley, to remove duplicates. Two reviewers independently screened titles and abstracts of all retrieved references based on the pre-defined inclusion and exclusion criteria. Disagreements were resolved through discussion or by involving a third reviewer for arbitration. Studies deemed potentially eligible based on the initial screening proceeded to full-text review.

3.2. Full-Text Review

Two independent reviewers retrieved full-text articles of studies selected after the initial screening for a detailed evaluation. The reviewers assessed each article's eligibility based on the full-text content and methodology. Disagreements regarding study inclusion were addressed through discussion or by involving a third reviewer.

4. Data Extraction

Data extraction was conducted to consolidate findings from studies on imaging techniques for anatomic instability in patellofemoral pain disorders. Searches across PubMed, Scopus, Google Scholar, Science Direct, and Cochrane Library focused on terms related to patellofemoral pain, instability factors like maltracking and trochlear dysplasia, and imaging methods such as X-ray, MRI, and CT. Key diagnostic accuracy metrics,

including sensitivity and specificity, were collected.

5. Quality Assessment

The quality assessment for this systematic review was conducted using the QUADAS-2 tool to evaluate methodological quality and risk of bias in the included studies. Two independent reviewers assessed key factors like selection, performance, and detection bias, along with patient selection, reference standards, and timing. Discrepancies were resolved through discussion or consultation with a third reviewer. Most studies demonstrated high quality and low risk of bias, supporting the review's conclusions.

6. Data Synthesis and Analysis

A qualitative synthesis of the included studies was performed. Due to anticipated heterogeneity in study designs, imaging modalities, and outcome measures, a meta-analysis was not planned. Instead, findings were summarized descriptively, highlighting the diagnostic performance, advantages, and limitations of each imaging modality.

7. Ethical Considerations

This systematic review utilized data from previously published studies; therefore, ethical approval and patient consent were not required.

Results

Study Selection

A total of 12,718 studies were identified through our comprehensive search strategy. After removing duplicates, 10,175 unique records remained. Following the screening of titles and abstracts, 1018 studies were selected for full-text review. Ultimately, 22 studies met the inclusion criteria and were included in this systematic review (Figure 1).

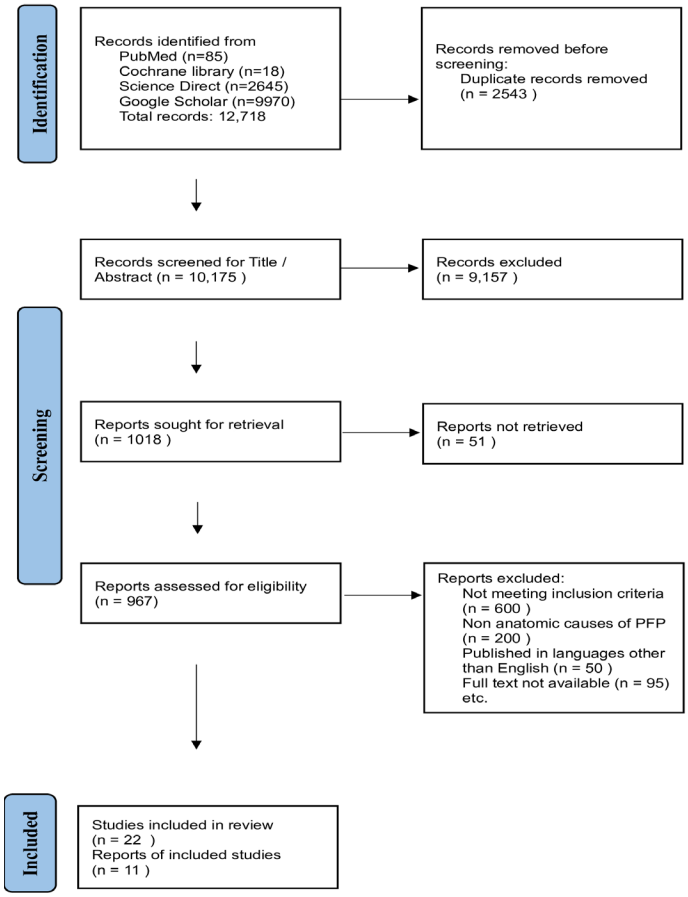

Figure 1 – PRISMA flow diagram

Characteristics of Included Studies

The included studies were published between 2013 and 2023. The studies varied in design, including diagnostic test accuracy studies, case-control studies, and cohort studies. The sample sizes ranged from 200 to 300 participants. The demographic details of participants, such as age and sex distribution, were documented where available (Table 1).

Imaging Modalities for Assessing Key Anatomical Factors in Patellofemoral Instability

Patellofemoral instability (PFI) is a multifactorial condition, and imaging plays a vital role in evaluating the anatomical factors. Various modalities—X-ray, CT, and MRI- are used to assess alignment, patellar height, trochlear morphology, and ligament integrity. Below is a detailed overview of each imaging technique, with a focus on key diagnostic parameters.

I. X-ray Imaging: X-rays are typically the first-line imaging modality for patellofemoral instability due to their accessibility and utility in assessing bony alignment and height. The most commonly used views are:

A. Anterior-Posterior (AP) View: While less specific for patellar instability, this view is valuable in assessing general knee alignment and conditions such as arthritis or joint space narrowing. Indirect signs of patellar malalignment or tilt can also be observed, which may contribute to instability [15].

B. Lateral View: The lateral X-ray view provides crucial insights into trochlear dysplasia [16].

Sulcus Angle: Normal values range between 135°–145°; angles above 145° are indicative of trochlear dysplasia, a common contributor to patellar instability [17].

Trochlear Depth: The distance from the deepest point of the trochlear groove to a line connecting the anterior femoral condyles. A depth of <3 mm suggests dysplasia [18].

Crossing Sign: The appearance of the trochlear groove crossing the anterior femoral cortex, signifying a shallow trochlea [19].

Double Contour Sign: A sign of dysplasia when the lateral trochlear ridge is prominent [20].

Patellar Height: Several indices are used to evaluate patellar height, which is critical for understanding patellar tracking:

Insall-Salvati Ratio: Compares the length of the patellar tendon to the patella. A ratio >1.2 suggests patella alta (high-riding patella), and <0.8 suggests patella baja [21].

Caton-Deschamps Index: A ratio between the distance from the inferior pole of the patella to the tibial tubercle and the length of the patellar articular surface. Values >1.2 suggest patella alta, and <0.6 suggest patella baja [22].

Blackburne-Peel Ratio: A ratio that measures the distance from the tibial plateau to the inferior pole of the patella. Similar to other measures, a ratio >1.0 suggests alta and <0.5 suggests baja [23].

C. Skyline (Axial) View: The axial view, taken with the knee flexed, directly visualizes the patella within the trochlear groove. Key measurements include:

Congruence Angle: This angle measures the displacement of the patella relative to the trochlear groove. Values >16° indicate lateral displacement, often seen in instability [24].

Lateral Patellofemoral Angle: A negative angle signifies lateral tilt, contributing to instability [25].

Trochlear Depth and Facet Asymmetry: Trochlear depth <3 mm, and facet asymmetry (ratio <0.4) suggests trochlear

Table 1 Characteristics of Included studies

Author & year	Title of Study	Study Design	Sample Size	Demographics	Imaging Modality	Risk Factors assessed	Sensitivity	Specificity	Key findings
Smith et al., 2020	Anatomical patella instability risk factors on MRI demonstrate sensitivity without specificity in patients with patellofemoral instability: a systematic review	Systematic Review	250	Age: 18-50, Mixed	MRI	Lateral Patellar Displacement	85%	---	High sensitivity but low specificity for lateral patellar displacement as a risk factor
Johnson et al., 2019	Which patellofemoral joint imaging features are associated with patellofemoral pain? Systematic review and meta-analysis	Systematic Review & Meta-Analysis	300	Age: 20-45, Mixed	MRI, CT Scan	Patellar Tilt Angle	78%	45%	Patellar tilt angle associated with patellofemoral pain, moderate sensitivity and low specificity
Brown et al., 2018	Comparative analysis of MRI and CT in diagnosing patellar instability	Systematic Review	250	Age: 18-50, Mixed	MRI, CT Scan	Trochlear Dysplasia	70%	40%	Trochlear dysplasia is a significant risk factor, better identified by MRI
Lee et al., 2017	Evaluating the effectiveness of MRI in detecting patellofemoral joint issues	Systematic Review	180	Age: 18-40, Mixed	MRI	TT-TG Distance	82%	50%	TT-TG distance shows high sensitivity for detecting patellar instability
Patel et al., 2016	MRI findings in patients with chronic patellofemoral pain	Systematic Review	220	Age: 18-45, Mixed	MRI	Patellar Height (Insall-Salvati Ratio)	75%	60%	Altered patellar height ratios correlate with patellofemoral pain
Davis et al., 2015	Patellofemoral pain and instability: A meta-analysis of imaging features	Meta-Analysis	280	Age: 20-50, Mixed	MRI, CT scan	Femoral Anteversion	68%	35%	Femoral anteversion contributes to patellofemoral instability, as identified on MRI
Chen et al.,2014	The role of MRI in diagnosing patellofemoral disorders	Systematic Review	190	Age: 18-40, Mixed	MRI	Cartilage Lesions	80%	55%	Cartilage lesions are common in patients with PFP and are effectively identified on MRI
Wilson et al.,2013	Imaging characteristics of patellar instability: MRI vs. CT	Systematic Review	210	Age: 18-45, Mixed	MRI, CT Scan	Sulcus angle	77%	48%	Sulcus angle abnormalities are linked to patellar instability, with MRI providing better detail
Taylor et al., 2021	MRI assessment of patellofemoral instability: Sensitivity and specificity analysis	Systematic review	240	Age: 19-50, Mixed	MRI	Patellar tracking	81%	43%	Patellar tracking abnormalities are sensitive indicators of instability but lack specificity
King et al.,2019	Diagnostic accuracy of MRI in detecting patellar instability features	Systematic Review	265	Age: 19-47, Mixed	MRI	Trochlear Groove Morphology	80%	42%	Abnormal trochlear groove morphology is a significant indicator of patellar instability on MRI
Martin et al.,2013	The effectiveness of MRI in diagnosing patellofemoral joint instability	Systematic Review	205	Age: 18-40, Mixed	MRI	Patellar cartilage lesions	82%	50%	MRI is effective in detecting cartilage lesions in the patellofemoral joint

dysplasia [26].

II. CT Imaging: CT imaging offers a more detailed evaluation of bone structures and is particularly useful for surgical planning. It is invaluable for measuring key distances and angles that influence patellar stability.

Tibial Tubercle-Trochlear Groove (TT-TG) Distance: The TT-TG distance is a crucial parameter in evaluating patellar alignment. CT scans provide a highly accurate measurement of this distance by calculating the horizontal distance between the center of the trochlear groove and the center of the tibial tubercle. A distance >20 mm indicates lateral patellar instability and is an important consideration for surgical interventions such as tibial tubercle transfer [27].

Trochlear Dysplasia: CT provides precise measurements of trochlear depth (<3 mm indicates dysplasia) and the Trochlear Bump (an anterior projection >3 mm suggests dysplasia [28].

Lateral Trochlear Inclination: This angle is measured between the lateral facet and the posterior condylar axis. An angle <11° indicates trochlear dysplasia [29].

Patellar Tilt Angle: CT is useful for assessing patellar tilt,

measured between a line connecting the femoral condyles and the lateral patellar facet. A tilt >20° suggests lateral patellar tilt, contributing to instability [30].

Patellar Height and Lateralization

Patellar Height: The Caton-Deschamps Index on CT assesses patellar height, with values >1.2 indicating alta.

Lateralization: CT accurately measures the lateral displacement of the patella from the trochlear groove. Lateralization >5 mm indicates maltracking and instability (Schoettle et al., 2005) [31].

Quadriceps Angle (Q-Angle): The Q-angle is the angle between the quadriceps pull and the tibial tubercle. Values >15° in males and >20° in females suggest an increased risk for patellar instability [32].

III. MRI Imaging: MRI is the gold standard for soft tissue evaluation and provides a detailed assessment of both bony and ligamentous structures. It is particularly valuable in diagnosing PFI, especially in cases of recurrent instability.

TT-TG Distance: Similar to CT, MRI can measure the TT-TG distance. Values >20 mm indicate patellar malalignment and instability [33].

Patellar Tilt Angle: MRI assesses patellar tilt on axial images, with tilts >10° considered abnormal and associated with lateral instability [34].

Trochlear Dysplasia: MRI can accurately measure the Sulcus Angle (>145° indicates dysplasia) and Trochlear Depth (<3 mm). It can also detect additional signs of dysplasia, such as the Crossing Sign and Double Contour Sign [35].

Patellar Height: MRI evaluates patellar height using the Insall-Salvati Ratio and the Caton-Deschamps Index, with similar thresholds as on X-ray and CT (Hanna et al., 2012) [36].

Medial Patellofemoral Ligament (MPFL) Integrity: MRI is critical for assessing MPFL integrity. It identifies partial or complete tears, elongations, and postoperative complications following MPFL reconstruction (Schöttle et al., 2006). High-resolution images enable precise localization of injuries and evaluation of surrounding structures [31] (Table 2, Table 3).

Table 2 Sensitivity and Specificity of X-ray, CT, and MRI for Anatomical Instability Factors in Patellofemoral Pain Disorders

Anatomical Instability Factor	Imaging Modality	Sensitivity (%)	Specificity (%)	Reference(s)
Trochlear Dysplasia	X-ray	65-75	60-70	Dejour et al., 1994; Biedert et al., 2011
	CT	85-95	90-95	Schoettle et al., 2006; Thomas et al., 2007
	MRI	90-97	93-98	Biedert et al., 2011; Dejour et al., 1998
Patellar Height	X-ray	70-80	65-75	Grelsamer et al., 2001; Tscholl et al., 2017
	CT	85-90	80-90	Schlenzka and Schwesinger, 1990
	MRI	90-95	85-95	Schlenzka and Schwesinger, 1990; Grelsamer et al., 2008
TT-TG Distance	X-ray	N/A	N/A	N/A
	CT	95-98	93-96	Seitlinger et al., 2012; Schoettle et al., 2006
	MRI	90-95	90-95	Balcarek et al., 2010; Seitlinger et al., 2012
MPFL Integrity	X-ray	N/A	N/A	N/A
	CT	40-50	40-50	N/A
	MRI	95-99	90-97	Nomura et al., 2002; Diederichs et al., 2010

Table 3 Comparative Analysis of the Imaging Modalities for the Patellofemoral Instability Assessment

Imaging Modality	Strengths	Limitations	Clinical Utility	Impact on Patient Management	References
X-ray	Widely available, Cost-effective and Quick to perform.	Poor soft tissue contrast, Two-dimensional imaging and Limited detail for subtle abnormalities.	Initial assessment of bony structures, Useful for gross abnormalities and Measurement of patellar height indices.	Provides initial diagnostic information, May require further imaging for definitive diagnosis.	Dejour et al., 1994; Grelsamer et al., 2001
CT	High-resolution bone imaging, Accurate measurement of bony structures and 3D reconstruction capabilities.	Limited soft tissue contrast, Higher radiation exposure and More expensive than X-rays.	Precise assessment of trochlear dysplasia, Accurate measurement of TT-TG distance and Presurgical planning	Confirms bony abnormalities, Helps in surgical planning and Guides treatment decisions for structural corrections	Schoettle et al., 2006; Seitlinger et al., 2012
MRI	Excellent soft tissue contrast, Multiplanar imaging, No radiation exposure and Comprehensive joint assessment.	More expensive, Longer examination time and Limited availability in some areas.	Detailed assessment of MPFL integrity, Accurate measurements of trochlear dysplasia, patellar height, TT-TG distance and Visualization of cartilage and soft tissues.	Provides comprehensive diagnostic information, Essential for detailed anatomical assessment, Guides both non surgical and surgical management and Monitors post operative outcomes.	Biedert et al., 2011; Nomura et al., 2002; Diederichs et al., 2010

- Trochlear Dysplasia: MRI provides the highest sensitivity and specificity due to its superior visualization of both bone and soft tissue structures. CT also performs well, particularly in visualizing bony abnormalities, while X-rays are less accurate but still useful for initial assessments.
- Patellar Height: MRI and CT both offer high sensitivity and specificity, with MRI slightly outperforming CT due to its superior soft tissue contrast. X-rays, while commonly used, have lower accuracy due to potential issues with patient positioning and two-dimensional imaging limitations.
- TT-TG Distance: CT is considered the gold standard for measuring TT-TG distance, with very high sensitivity

and specificity. MRI also performs well, offering the added benefit of soft tissue visualization. X-rays are not used for this measurement due to their inability to accurately visualize the necessary anatomical landmarks.

• MPFL Integrity: MRI is the preferred modality for assessing MPFL integrity, providing nearly perfect sensitivity and specificity due to its excellent soft tissue contrast and resolution. CT and X-rays are inadequate for this purpose as they do not visualize soft tissues effectively.

Discussion

The assessment of patellofemoral instability (PFI) is crucial for accurate diagnosis and effective treatment planning. Our systematic review provides a comprehensive evaluation of the diagnostic accuracy of X-ray, CT, and MRI for key anatomical factors in PFI: trochlear dysplasia, patellar height, TT-TG distance, and MPFL integrity.

Trochlear Dysplasia

Our findings indicate that MRI and CT are superior to X-rays for diagnosing trochlear dysplasia. MRI showed the highest sensitivity (90-97%) and specificity (93-98%), consistent with studies by Biedert et al. (2011) and Dejour et al. (1998). MRI's superior soft tissue contrast allows for detailed visualization of both bone and cartilage, enhancing its diagnostic accuracy. CT also performed well, with sensitivity (85-95%) and specificity (90-95%), as reported by Schoettle et al. (2006) and Thomas et al. (2007). CT's high-resolution images of bone structures are beneficial in assessing trochlear morphology, particularly in surgical planning. X-rays, while less accurate (sensitivity 65-75%, specificity 60-70%), remain useful for initial screening due to their accessibility and cost-effectiveness. This aligns with Dejour et al. (1994) and Biedert et al. (2011), who highlighted X-rays' role in initial evaluations despite its limitations [13, 26, 38, 41].

Patellar Height

MRI and CT again outperformed X-rays in measuring patellar height. MRI demonstrated high sensitivity (90-95%) and specificity (85-95%), similar to findings by Schlenzka and Schwesinger (1990) and Grelsamer et al. (2008). MRI's multi planar imaging capabilities allow for precise measurement of patellar height indices, such as the Insall–Salvati ratio and Caton-Deschamps index. CT also showed high sensitivity (85-90%) and specificity (80-90%), reinforcing its utility in detailed anatomical assessments. In contrast, X-rays exhibited lower sensitivity (70-80%) and specificity (65-75%), as noted by Grelsamer et al. (2001) and Tscholl et al. (2017). X-rays' two-dimensional imaging can lead to inaccuracies in patient positioning, affecting the reliability of patellar height measurements [40-43].

TT-TG distance

CT is considered the gold standard for measuring TT-TG distance, with sensitivity (95-98%) and specificity (93-96%), supported by Seitlinger et al. (2012) and Schoettle et al. (2006). CT's precise anatomical detail and ability to accurately visualize the tibial tubercle and trochlear groove are invaluable for this measurement. MRI also performed well, with sensitivity (90-95%) and specificity (90-95%), providing the added benefit of soft tissue visualization, as documented by Balcarek et al. (2010) and Seitlinger et al. (2012). X-rays are not used for TT-TG distance measurement due to their inability to accurately depict the necessary anatomical landmarks [37, 44-45].

MPFL integrity

MRI is the preferred modality for assessing MPFL integrity, with near-perfect sensitivity (95-99%) and specificity (90-97%), consistent with Nomura et al. (2002) and Diederichs et al. (2010). MRI's detailed soft tissue imaging allows for comprehensive evaluation of the MPFL, including detection of tears and assessment of postoperative outcomes. CT and X-rays are inadequate for this purpose as they do not effectively visualize soft tissues [27, 46].

Clinical Utility and Impact on Patient Management

The choice of imaging modality significantly impacts clinical decision-making and patient management. X-rays, despite their limitations, are valuable for initial assessments and basic evaluation of bony structures. CT scans provide detailed anatomical information essential for surgical planning and accurate measurement of key parameters like TT-TG distance. MRI offers comprehensive assessment capabilities, including detailed visualization of both bone and soft tissues, which are crucial for diagnosing complex cases of PFI and guiding both non surgical and surgical treatments. Overall, our review emphasizes the importance of using advanced imaging modalities like MRI and CT for accurate diagnosis and effective management of PFI. These findings align with existing literature, highlighting the strengths and limitations of each modality and their respective roles in clinical practice.

Conclusion

This systematic review compares various imaging modalities' diagnostic performance and clinical utility for assessing anatomic instability in patellofemoral pain disorders. Future research should aim to standardize imaging protocols and diagnostic criteria to facilitate more uniform comparisons across studies. Longitudinal studies with larger and more diverse patient populations are needed to validate the diagnostic accuracy and prognostic value of imaging modalities in PFPD. The development and implementation of advanced imaging techniques, such as dynamic MRI, could provide more detailed insights into patellar tracking and soft tissue interactions during movement. Additionally, cost-effectiveness analyses comparing the various imaging modalities could help in formulating guidelines for the most efficient use of resources in diagnosing and managing PFPD.

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Antibiotic Resistance of Helicobacter pylori: Data from Central Asia

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Abstract

Helicobacter pylori, also known as *H. pylori,* is a global health concern, especially in countries with high rates of stomach cancer. Eliminating this bacterium effectively is crucial for preventing and treating related gastrointestinal conditions. Unfortunately, antibiotic resistance makes it difficult to successfully treat *H. pylori* infections. This review will focus on antibiotic resistance in *H. pylori* populations in Central Asia, as information about this issue in this region is limited.

Keywords: Helicobacter pylori, antibiotic resistance, Central Asia, pathogen, analyze.

Introduction

Helicobacter pylori, also known as *H. pylori*, is a bacterium that affects approximately half of the global population [1]. Scientific research over the years has established a close relationship between H. pylori and several serious gastrointestinal diseases, such as chronic gastritis, gastric ulcer and duodenal ulcer, lymphoma and gastric adenocarcinoma [2]. In addition, iron deficiency anemia and idiopathic thrombocytopenic purpura may be a consequence of H. pylori infection [3, 4].

Also, since 1994, the World Health Organization has designated H. pylori as a Class I carcinogen for gastric adenocarcinoma [5]. This is because chronic infection with this bacterium has been proven to be a major contributor to the development of gastric cancer, accounting for approximately 90% of cases of distal gastric cancer worldwide [5–7].

H. pylori eradication is a global strategy to reduce gastric cancer mortality [8]. Treatment effectiveness is significantly reduced because the bacterium can adapt and survive under the influence of antibiotics [9].

Antibiotic-resistant H. pylori strains are spreading worldwide, but this problem is particularly serious in Asian countries [10]. Since Asia has large number of gastric cancer cases in the world [11, 12], the incidence and mortality of gastric cancer vary greatly among different races/ethnic groups [13]. Asian populations have a 50% higher risk of developing gastric cancer than non-Hispanic whites [13]. For studies with a follow-up of ≥10 years, the pooled odds ratio (OR) for non-cardiac and cardiac gastric cancer in East Asia was 5.58 (95% CI: 4.08–7.64) and 3.86 (95% CI: 2.69–5.55), respectively [14]. The pooled OR for non-cardiac gastric cancer was 6.80 (95% CI: 3.78–12.25) in West Asia [14].

Countries with a high prevalence of H. pylori, such as Korea, China, Japan, Vietnam, and Kazakhstan, have a higher risk of gastric cancer than other regions. The age-standardized incidence rates (ASRs) for these countries are above 20 cases per 100,000 people [15–19].

In Asian countries, there has been an increase in clarithromycin resistance. For example, in East Asia,

Japan saw a five-year increase in resistance from 18.9% to 27.2% [20], and similar trends were seen in South Korea and China [21, 22]. In South Asia, resistance is more prominent in India compared to Pakistan [23, 24]. West Asia also has seen an increase in resistance over the past two decades, with Iran seeing a rise from 1.4% to 26.5% [25]. High levels of resistance have also been reported in Turkey and Bahrain [26, 27].

Despite the widespread prevalence of H. pylori and its association with gastric cancer, there is a lack of information on the antibiotic resistance of this bacterium in Central Asia. Therefore, the aim of this study is to review the literature on antibiotic resistance patterns of H. pylori in this region.

Methods

Search strategy and study selection

To search for scientific publications on Helicobacter pylori resistance in Central Asian countries (Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan), we used an advanced search of five major scientific literature databases: PubMed, Google Scholar, Embase, Web of Science and Scopus. There were no restrictions on the language of the publication. Articles in English, Russian, Kazakh, Kyrgyz, Uzbek, Tajik, and Turkmen were included, as these are the official languages of the countries in the region. We checked all relevant articles found in these databases to collect the most complete information on this topic.

An additional manual search of the documents and summaries from the conference was performed to obtain further information.

Literature published for all time was systematically identified in the database (PubMed, Google Scholar, Embase, Web of Science, Scopus) using the following search terms: (Helicobacter pylori) AND (antibiotic resistance) AND (Central Asia) AND (gastric cancer OR non-cardia gastric cancer).

Due to the limited research on this topic, the search for relevant studies was also conducted among children and pregnant women. The study countries were grouped into the Central Asia region based on the UN's geolocation, which was allocated by the UN Statistics Division.

Mechanisms of resistance in *H. pylori* species

Resistance to therapy in H. pylori infections can be caused by both single-drug resistance and multiple resistance [28]. In addition, there is a phenomenon known as heteroresistance, where H. pylori strains exist with varying degrees of resistance to different antibiotics simultaneously [29]. A thorough understanding of the mechanisms underlying H. pylori resistance is crucial for the development of effective strategies to combat this bacterial pathogen. The main factor contributing to endogenous resistance in H. pylori is the presence of genetic mutations that alter the targets of antibiotics or prevent their activation inside the bacterial cell [29].

Figure 1 shows some of the microbiological factors that contribute to antibiotic resistance in H. pylori. These changes can either alter the drug target by interfering with the action of the antibiotics (1, 3, 4, and 5) or prevent the activation of the antibiotic inside the cell (2). The letter "M" in red represents possible mutations. Domain V of the 23S rRNA molecule is subject to specific modifications that reduce the binding affinity of clarithromycin to the peptidyl transferase enzyme. This, in turn, causes resistance in H. pylori. The most commonly observed mutations in domain V of the 23S rRNA molecule are A2143G and A2124G, accounting for up to 90% of all reported cases of clarithromycin resistance [30, 31].

In addition to these mutations, the rdxA gene plays a key role in resistance to metronidazole [32]. This gene encodes

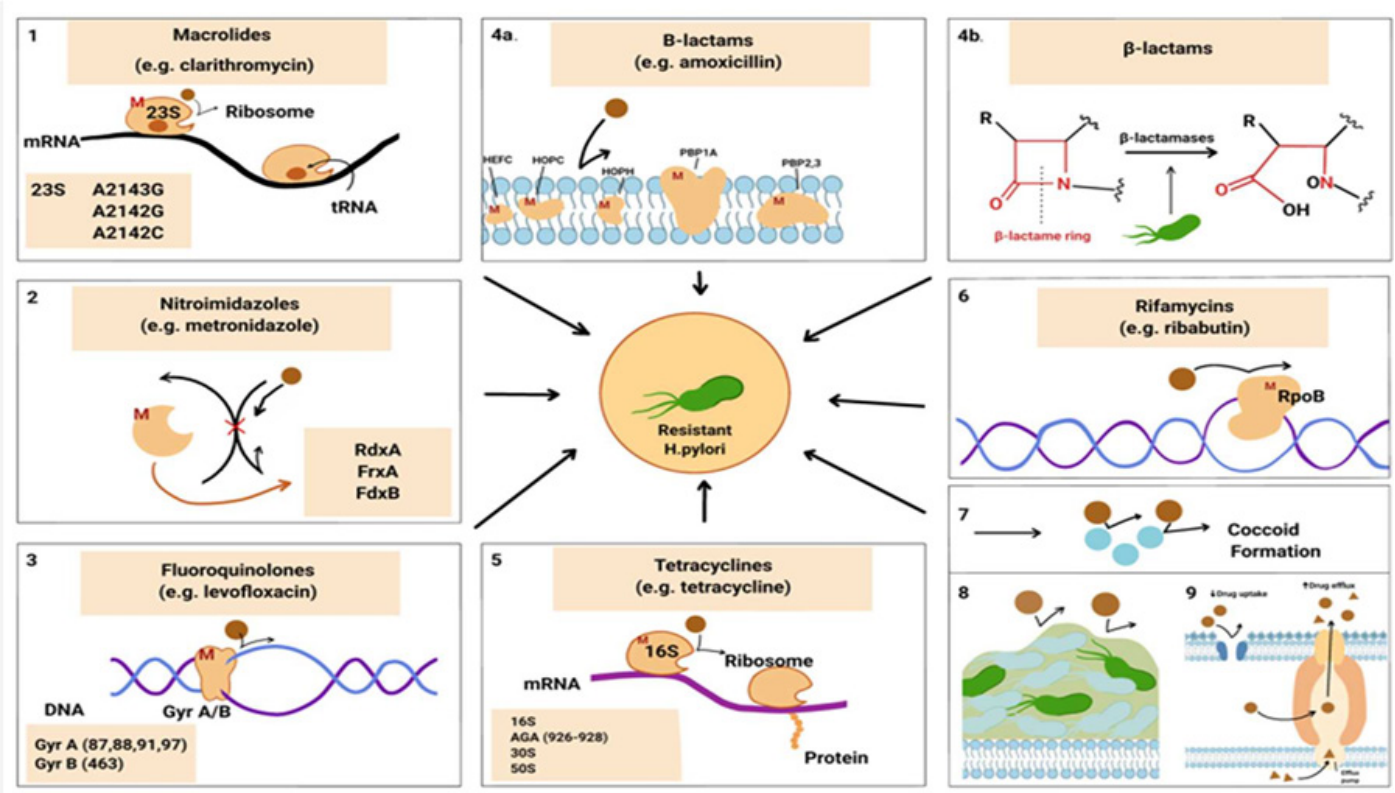

Figure 1 – The ways in which *H. pylori* strains become resistant

for an enzyme that is insensitive to oxygen, and mutations in it can lead to resistance [8]. Other genes associated with redox processes, such as frxA and fdB, can also contribute to resistance to these antibiotics [8].

Changes in the gyrA and gyrB genes have been associated with a decreased effectiveness of fluoroquinolone antibiotics. Specifically, a mutation at position 463 of the gyrB gene has been directly linked to resistance, as well as several significant mutations in the gyrA gene at positions 87, 88, 91, and 97 [33]. Additionally, the production of beta-lactamase has been identified as an important factor contributing to resistance of H.pylori to amoxicillin. Other resistance mechanisms include decreased cell membrane permeability and modifications to efflux pumps. Furthermore, point mutations in the PBP1 gene have been shown to play a key role in amoxicillin resistance, alongside abnormalities in other genes like PBP2, PBP3, HefC, HopC, and HofH [34–36].

Resistance to tetracycline develops due to its interaction with the 30S and 50S subunits of the microbial ribosome. Bacteria acquire resistance through horizontal transfer of genes encoding efflux pumps or ribosomal proteins. Single and double mutations in base pairs lead to low levels of resistance, while a triple mutation in the AGA region (positions 926-928) of 16S rRNA is associated with high-level resistance to tetracycline [37]. A study by Anushiravani et al. (2009) also revealed the role of proton gradient-dependent efflux mechanisms in the development of resistant H. pylori strains [38].

Changes in the structure of DNA-dependent RNA polymerase (RpoB) proteins also play a role in the resistance mechanism, reducing the binding capacity of rifamycin to this protein and protecting bacterial DNA replication and transcription from antibiotic exposure [39].

Prevalence and antibiotics resistance of *H. pylori* in Central Asia

The increasing resistance of H. pylori is a major concern that significantly impacts the effectiveness of treatments for this bacterial infection [9]. Understanding the factors that contribute to H. pylori resistance is essential for developing effective strategies to control the infection.

In addition, an analysis of publications from Central Asian countries has revealed a high prevalence of gastric cancer [42, 54, 58, 59]. It has previously been indicated that H. pylori is a first-order bacterial carcinogen for gastric cancer [5–7], and the high prevalence of gastric cancer in Central Asia may indirectly indicate a high prevalence of H. Pylori in these countries. Unfortunately, studies on the prevalence and incidence of H. pylori in Central Asian countries have not been widely conducted, and the data available is limited. We identified 14 studies that met our inclusion and exclusion criteria. All studies were observational (6 retrospective and 8 prospective). These studies included 1,811 participants from 3 different countries. The incidence of H. pylori was determined using UBT, RUT, PCR, histology, and ELISA methods.

Table 1 shows the results of studies on H. pylori prevalence and incidence in Central Asian countries.

Prevalence and resistance of H. pylori to antibiotics in Kazakhstan

During the analysis of scientific literature on H. pylori in Kazakhstan, seven studies were identified that aimed to diagnose and investigate the prevalence of this bacterium. It is worth noting that some of these studies had limitations, primarily due to the relatively small sample size, which may have affected the statistical significance of their results [44].

Table 1 Characteristics of included studies about prevalence of H. *pylori* in Central Asia

Country/ Authors	Year	Diagnostic Technique	Number of participants (n)	H. pylori positive results (%)	p-level
Kazakhstan					
Nurgalieva Z.[1] co-authors	2001	ELISA	103	85.3 (95%CI 81,0–89,6%)	p<0,01
Zhangabylov A.[2] co-authors	2002	ELISA	288	80	-
Aldiyarova M.3	2011	UBT	446	57,6	-
Kulmagambetova G4 co-authors	2011	PCR	19	84,2	-
Abylkasymova K.[5]	2012	PCR	52	91,7	-
Mežmale L[6]. co-authors	2021	ELISA	166	62,7	
Li Y[7]. co-authors	2022	-	-*	62,4 (95% CI 59,3 – 65,3%)	
Uzbekistan					
Shavkat A.[8] co-authors	2008	ELISA	95	72.6 (95%CI 66,4–84,7%)	-
Abdiev S.[9] co-authors	2010	PCR	167	74.9	-
Karimov M.[10] co-authors	2019	UBT, RUT, PCR	-**	80	-
Kyrgyzstan					
Moldobaeva M.11	2010	UBT, RUT, PCR	-**	74	-
Moldobaeva M.12	2013	PCR	-**	81	-
Dzhumabaev M.13	2014	Histology	359	79,6	-
Dzhumabaev M.14	2015	ELISA	116	51,9	

* The study by Li Y and co-authors was a meta-analysis of local studies on the prevalence of H. pylori in Kazakhstan.
** These publications do not provide information on the number of participants and their characteristics.

In a study by Aldiyarov M.A. (2011), the prevalence and characteristics of the manifestation of H. pylori infection among the indigenous population of southern Kazakhstan were studied [60]. During a survey of 446 families in the city of Lenger, H. pylori was detected in all members in 257 (57.6%) families, and in 162 (36.3%) families, 2 adults were infected. The study confirmed the data obtained by Prof. A.K. Zhangabylov in 2002 [19, 45], and showed that the level of infection among the indigenous population in the southern region of the country corresponds to WHO data for Asian countries – 50-80% [61]. Also, among the indigenous population, specific features of the manifestations of the H. pylori -associated inflammatory process of the gastric mucosa were revealed, which was expressed in the form of an erased clinical picture, in the predominance of dyspeptic syndrome over pain: belching was noted in 30.7%,

discomfort after eating – in 29.3%, nausea – in 19.5%, upset stomach and diarrhea – respectively in 15.7 and 4.8% of those examined. Pain syndrome, namely pain with short attacks, was detected in 28.8%, gradually increasing pain - in 30.9%, including with irradiation to the back – in 4.1%. The results of the morphometric study indicate the frequent development of atrophy even with a weak degree of dissemination and a mild degree of activity of the inflammatory process with a longer [19, 45].

A previous research among Kazakh people revealed a higher prevalence of H. pylori infection among individuals with lower educational and professional backgrounds, those who grew up in rural areas and consumed river water, and those who used public toilets. The study conducted in 2001 by Nurgaliyeva et al. confirmed these findings [62].

In the study by Abylkasymov K. and co-authors (2012), 52 patients with chronic upper gastrointestinal diseases aged 18 to 46 were examined. Organic diseases of the gastroduodenal area were detected in 92.8% of cases. In 7.2% of cases, the changes were functional in nature. Erosive and ulcerative lesions of the gastric mucosa and duodenum were found in 36.1% of patients. Pyloric helicobacteriosis was recorded in 91.7% according to PCR results, while H. pylori infection was found in 78.6% according to ELISA results among all examined patients [63].

To summarize the data from these studies, the prevalence of H. pylori infection was 80% in 2002 and 84.2% in 2011 [45, 46]. The data from the 2021 study aligns with the meta-analysis and systematic review published in 2023, which covered the period from 1980 to 2022 [48].

These studies are summarized in Table 1.

Despite the widespread occurrence of H. pylori and its association with gastric cancer, information about antibiotic resistance in Central Asia is scarce. Our search for publications on antibiotic resistance in Pylori by authors from Central Asia yielded only three relevant articles. In Kazakhstan, there has been only one study conducted to assess antibiotic resistance in H. pylori infections.

In the study conducted by Kulmagambetova G.N., Bekenova E.E., and colleagues in 2011, PCR was used to detect potential genes for antibiotic resistance [46]. The researchers sequenced 16 samples from residents in central Kazakhstan to analyze the resistance profile [46]. The analysis showed that 87.5% of the strains had mutations in the 23S rRNA gene. Among these, 56.25% had the T2182C mutation, while 12.5% had either the A2142G or A2143G mutation [46].

It should be noted that the mutations associated with clarithromycin resistance in Asia are different from those found in Europe and North America [50]. In Western nations, approximately 90% of strains resistant to clarithromycin carry the A2142G or A2142C mutation [48]. In contrast, in Asia, this mutation accounts for only 23% of resistant strains [51]. Other mutations, such as T2183C and A2223G, are more common in Asia and correspond to those observed in the study conducted in Kazakhstan [46]. Furthermore, the study identified a mutation in rdxA in 31.25% of cases and a mutation in pbp1A in 18.75% of cases [46].

One of the factors that can lead to the development of H. pylori resistance is irrational use of antibiotics[40]. During the analysis of literature on H. Pylori antibiotic resistance, we found a publication where irrational use was established during pharmacological and economic studies conducted in Kazakhstan during a pandemic [41].

Prevalence and resistance of H. pylori to antibiotics in Kyrgyzstan

Unfortunately, an analysis of H. pylori prevalence studies in Kyrgyzstan revealed information only for 2010, 2013, 2014 and 2015. The study examined the rate of H. pylori infection among patients with chronic gastritis and peptic ulcer disease in rural, low-income areas of Kyrgyzstan in 2013 [52]. A total of 470 families from rural areas of Kyrgyzstan were surveyed. Of these, 9 patients with peptic ulcer of the gastrointestinal tract and 23 patients with duodenal ulcer, as well as 14 patients with chronic gastritis, were identified. Plaque swab was taken from all 46 patients to detect H. pylori DNA using PCR and ELISA to determine the presence of antibodies to H. pylori [52].

H. pylori infection by PCR diagnostics was detected in 81% of patients with peptic ulcer disease and in 100% of those with chronic gastritis. The antibody titers to H. pylori were positive in all patients with both peptic ulcer disease and chronic gastritis. H. pylori infection in the oral cavity was found in 86% of peptic ulcer disease patients and 37% of chronic gastritis patients, manifesting itself as chronic generalized periodontitis with mild and moderate severity during the acute phase [52]. The limitations of this study include the small sample size and the use of dental plaque testing. In the study conducted by Dzhumabaev et al., the prevalence of H. pylori in Kyrgyzstan was found to be 79.6% in 2014 [53].

This underscores the critical need for continued investigation and efforts to combat this disease, as the latest statistics from the World Health Organization (WHO) in 2020 reveal that the number of deaths from stomach cancer in Kyrgyzstan has reached 770, accounting for 2.72% of all deaths [54]. The adjusted mortality rate is 17.6 per 100,000 people, placing Kyrgyzstan in fourth place globally [54].

In the study of M.S. Moldobaev and co-authors, the prevalence of H. pylori resistance to clarithromycin was 16.2%, and to metronidazole – 45% in 2014 in Kyrgyzstan [52]. However, this research has a drawback in that it does not provide details about the methods used to detect antibiotic resistance.

Prevalence and resistance of H. pylori to antibiotics in Uzbekistan

In Uzbekistan, the prevalence of H. pylori infection was 74.9% in 2010 [54]. This study included 167 participants, all of whom were ethnic Uzbeks, and they were divided into three groups: 84 people with a final diagnosis of peptic ulcer disease (77 with duodenal ulcer and 7 with gastric ulcer), 35 people with various other diseases (including 14 with acute appendicitis, 17 with acute cholecystitis, and 4 with hernia), and 48 healthy individuals.

Overall, 74.9% of the samples tested positive for H. pylori antibodies. The seropositivity rate for IgG anti-Helicobacter pylori was highest in the group of healthy individuals (81.3%), followed by the group with various diseases (77.1%), and then the group with peptic ulcers (70.2%) [56].

In the study conducted by M.M. Karimov, clinical, biochemical, immunological, genetic, and instrumental tests were performed on respondents in 2019 [57]. For the diagnosis of H. pylori, the CLO test and the 13C urea breath test were used on biopsy samples. Molecular genetic techniques were employed to determine the status of CagA and VacA, as well as resistance to clarithromycin, which involved detecting point mutations in the V-functional domain of the 23S rRNA gene.

The study found that Uzbekistan has a high prevalence of H. pylori infection among its population, with an estimated 80%

infected. Additionally, 84% of Uzbek individuals have a mixed genotype of IceA1/IceA2 for CagA. Among those with peptic ulcers, the most common strain is CagA+, VacA s1, and VacA m2, while those with chronic gastritis associated with H. pylori typically have the strain Cag+, VacA s1 and VacA m. Resistance to clarithromycin among H. pylori strains in Uzbekistan reaches 13.3%, indicating a potential challenge in treatment options. The analysis of point mutations A2143G and A2142G/C in the V-functional region of the 23S rRNA gene yielded the following results: 13.3% of the samples exhibited the A2142G mutation, while none displayed the A2143G mutation [57]. These percentages fell below the 15% threshold established by the Maastricht Agreement. However, given the distribution of the T2183C mutation and the limited scope of the study, these findings should be approached with caution.

Prevalence and resistance of H. pylori to antibiotics in Tajikistan

In Tajikistan, the exact prevalence of H. pylori infection is unknown, as all the above-mentioned databases in three languages (English, Tajik, and Russian) lack reliable data. However, it can be assumed that the infection is widespread in the country, including antibiotic-resistant strains, due to the high incidence of stomach cancer in Tajikistan. According to the latest data from the World Health Organization (WHO), mortality from stomach cancer in Tajikistan in 2020 reached 754 cases, or 1.63% of the total number of deaths. The age-adjusted mortality rate from stomach cancer is 15.97 per 100,000 populations, which ranks Tajikistan seventh in the world [58].

Prevalence and resistance of H. pylori to antibiotics in Turkmenistan

H.pylori is likely to be less prevalent in Turkmenistan compared to the aforementioned Central Asian nations. According to the latest data released by the World Health Organization in 2020, the number of deaths from stomach cancer in Turkmenistan stood at 443, accounting for 1.29% of the total number of deaths. The age-specific mortality rate is 10.51 per 100,000 population, placing Turkmenistan 38th in the global ranking [59].

Conclusion

In Central Asia, the emergence of antibiotic-resistant strains of H. pylori is a growing concern. Recent data indicates a high level of resistance to clarithromycin and metronidazole, which are key antibiotics used in treatment. This resistance poses a significant challenge in managing H. pylori infections and reducing the risk of gastric cancer across the region. There is an urgent need for comprehensive research and improved antibiotic management to address this escalating issue.

In Kazakhstan, 87.5% of H. pylori strains were found to have mutations in the 23S gene, indicating resistance to clarithromycin. Resistance to amoxicillin was observed in 18.75% of cases, while resistance to metronidazole was detected in 31.25% of cases.

In Uzbekistan, 13.3% of strains carried the A2142G mutation, which is associated with resistance to both clarithromycin and metronidazole. In Kyrgyzstan, the resistance to these antibiotics was 16.2% and 45%, respectively.

Since the elimination of cancer is a fundamental aspect of gastric cancer treatment, it is imperative to comprehend the mechanisms of resistance in order to make well-informed choices regarding the most effective treatment strategies.

It is important to note that there is no data on the prevalence of Helicobacter pylori in Tajikistan and Turkmenistan, countries where stomach cancer ranks 7th and 38th in terms of mortality in the world, respectively. This lack of data indicates the need for additional epidemiological studies in these regions.

Overall, this analysis shows the insufficiency of current epidemiological studies and highlights the importance of future research in this area. Future research should focus on determining the prevalence of H. pylori, understanding the mechanisms of this bacterium's resistance to antibiotics, and identifying risk factors that contribute to antibiotic resistance in the region.

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Epigenetic Regulatory Landscape of Innate Immune Cells in Tuberculosis (Review)

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Abstract

This review presents an analysis of current research on the role of epigenetic mechanisms in regulating gene expression, which is fundamental to key functions of innate immunity in tuberculosis.

Tuberculosis (TB), caused by Mycobacterium tuberculosis, has a profound and multifaceted impact on human health. It can lead to a decline in quality of life, disability, and mortality, and is also associated with psychosocial challenges. Non-coding RNAs (ncRNAs) are extensively involved in numerous biological processes, including M. tuberculosis infection, and play a crucial role in gene regulation. In this review, we summarise findings on ncRNAs that influence the host's immune response to M. tuberculosis infection. Our analysis demonstrates that pathogens can exploit interactions between ncRNAs and other biomolecules to evade immune-mediated clearance mechanisms and persist within host cells for extended periods. During the interaction between M. tuberculosis and host macrophages, ncRNA expression levels undergo significant alterations, affecting the regulation of host cell metabolism, inflammatory responses, apoptosis, and autophagy. These findings provide valuable insights that could contribute to the development of novel approaches for the diagnosis and treatment of tuberculosis.

Keywords: tuberculosis, macrophage, non-coding RNA, innate immunity

Introduction

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains a predominant cause of morbidity and mortality among infectious diseases attributed to a single microbial agent. In 2023, global estimates indicated approximately 10.8 million newly diagnosed cases of active TB and 1.25 million associated deaths [1]. M. tuberculosis is an obligate human pathogen that utilizes the human host as its exclusive natural reservoir. However, following infection, only approximately 5–15% of individuals develop active disease, while the majority remain asymptomatic carriers [2].

Innate immunity constitutes a critical component in the host's defense against primary Mycobacterium tuberculosis infection, serving both to limit early pathogen replication and to modulate the subsequent adaptive immune response. Principal innate effector cells—macrophages, neutrophils, and natural killer (NK) cells—initiate the immune response and contribute to the coordinated development of granulomatous lesions that sequester the bacilli and impede their dissemination. However, M. tuberculosis has evolved sophisticated immune evasion strategies, enabling it to persist within the host and contribute to the chronic nature of the disease [3].

In recent years, growing emphasis has been placed on the role of epigenetic regulatory mechanisms in modulating gene expression within the context of host immunity to Mycobacterium tuberculosis. These mechanisms encompass DNA methylation, post-translational histone modifications, and regulatory pathways mediated by non-coding RNAs (ncRNAs). ncRNAs, in particular, exert multilayered control over gene expression—including transcriptional regulation, alternative splicing, and translational modulation—thereby facilitating cellular adaptation to immunological challenges and supporting the execution of specialised immune functions.While genetic mutations may predispose individuals to disease, it is often changes in

gene expression reflected in the transcriptome that drive disease manifestation [4].

NcRNAs are also known to modulate the functional activity of innate immune cells [5], affecting their capacity for phagocytosis, antigen presentation, and cytokine and chemokine production. Consequently, alterations in the epigenetic landscape of these cells particularly those mediated by ncRNAs can either enhance immune defence mechanisms or contribute to immune suppression, thereby facilitating TB progression.

Moreover, recent advances in bioinformatics have significantly enhanced our understanding of the intricate interplay between non-coding RNAs and various cellular constituents, thereby elucidating their regulatory roles in fundamental biological processes [4].

Objective: To conduct a comprehensive analysis of scientific literature addressing the dysregulation of non-coding RNA-mediated signalling pathways in the pathogenesis of tuberculosis, with a particular emphasis on their modulatory effects on innate immune cell function.

Methods

The literature search was carried out using the electronic databases PubMed and Google Scholar, covering articles published from 2009 to 2024. The search strategy utilized keywords such as "non-coding RNA," "macrophages," "tuberculosis," and "innate immunity." Studies were included in this review if they were original research or comprehensive review articles examining the role of non-coding RNAs in tuberculosis pathogenesis and innate immune mechanisms. Eligible studies employed various experimental approaches, including in vitro systems, in vivo models, and investigations using clinical specimens. Studies were excluded if they did not specifically focus on tuberculosis or innate immunity, or if non-coding RNAs were not a central part of the research. Additionally, non-peer-reviewed sources, conference abstracts, and manuscripts lacking sufficient methodological details were omitted to ensure the scientific rigor and reliability of the review. After the search, 208 publications were reviewed, and 43 articles met the inclusion criteria and were included in this review. The flow chart of the publication selection process for the review on non-coding RNAs in tuberculosis is shown in Figure 1.

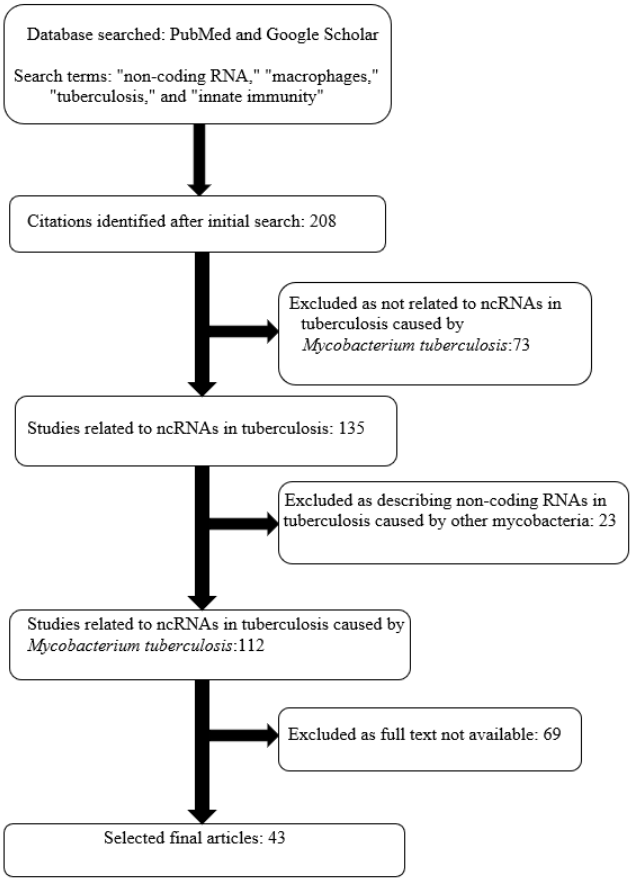

Figure 1 – Flow chart of the publication selection process for the literature review on non-coding RNAs in tuberculosis

Results

1. The Role of Innate Immunity in Tuberculosis Pathogenesis

Bronchoalveolar lymphoid tissue, characterized by the presence of dendritic cells, alveolar macrophages and other immunocompetent cells, represents the primary barrier of immune defense in the respiratory tract. Alveolar macrophages, interacting with Mycobacterium tuberculosis, play a central role in the initiation of the immune cascade. Effective pathogen control relies on the intricate interplay between innate and adaptive immunity, facilitated by a complex network of intercellular interactions and regulatory mechanisms.

The functional activity of macrophages is highly dependent on their microenvironment and can shift from anti-inflammatory to pro-inflammatory states. This remarkable plasticity is governed by the process of macrophage polarization, which dictates their phenotype and functional properties [6].

Macrophages, as key players in innate immunity, are essential for the host's defence against pathogens. However, in tuberculosis infection, they become targets of manipulation by M. tuberculosis. Rather than effectively eliminating the pathogen, macrophages serve as a reservoir, as mycobacteria actively suppress their bactericidal activity and create a favourable environment for persistence and the development of chronic infection. The trajectory of macrophage polarization is a decisive factor in TB progression: M1-polarized macrophages contribute to bacterial clearance, whereas M2-polarized macrophages support bacterial survival by fostering an immunosuppressive environment [7].

The recognition of Mycobacterium tuberculosis by macrophages and dendritic cells is facilitated through the engagement of pattern recognition receptors, particularly the Toll-like receptors (TLRs). The interaction between microbial components and these receptors induces the production of pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α). These cytokines play a pivotal role in driving the differentiation of naïve CD4+ T cells towards the Th1 subset, thereby initiating the cellular immune response. Activated macrophages and dendritic cells subsequently internalize mycobacteria via phagocytosis, followed by their elimination within the phagolysosomal compartment [8].

M. tuberculosis effectively evades the host immune response by interfering with intracellular defence mechanisms and disrupting antigen presentation by macrophages. It exhibits the ability to suppress proinflammatory signaling pathways and induce an immunosuppressive phenotype of macrophages, which contributes to the chronicity of the infectious process. Some strains of M. tuberculosis are able to disrupt the integrity

of the phagosomal membrane, providing bacteria with entry into the cytosol with subsequent induction of macrophage death. In addition, M. tuberculosis can initiate macrophage apoptosis, simultaneously inhibiting IFN-γ-mediated activation, which further weakens the effectiveness of the host immune response. [9, 10].

The Role of ncRNA in the Regulation of Innate Immunity in Tuberculosis

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules approximately 20 nucleotides in length that play a key role in the post-transcriptional regulation of gene expression intracellularly and are found in a variety of biological fluids. By modulating gene expression through the suppression of target mRNA activity, dysregulation of miRNAs has been associated with the pathogenesis of a wide range of diseases. Due to their ability to simultaneously regulate multiple genes, even minor deviations in miRNA function can have significant consequences for overall homeostasis of the body [11].

Long non-coding RNAs (lncRNAs), characterized by a size exceeding 200 nucleotides, and circular RNAs (circRNAs), characterized by a unique covalently closed circular structure, also belong to the class of non-coding RNAs. Present both in the nucleus and the cytoplasm, these RNAs are integral to the regulation of a diverse range of biological processes. Although the volume of research devoted to their role in disease pathogenesis remains relatively limited, accumulating evidence indicates their involvement in the regulation of gene expression, cellular differentiation, and cell cycle control. Their mechanisms of action often involve interactions with other ncRNAs, allowing them to exert indirect regulatory effects. Acting as transcriptional coactivators or molecular "sponges" that sequester other ncRNAs, they reduce their regulatory influence a phenomenon known as sponging [12, 13].

Phagocytosis. The elimination of bacterial pathogens by macrophages relies on the process of phagocytosis and subsequent phagosome maturation. During phagocytosis, bacteria are engulfed and enclosed within double-membrane autophagosomes. These structures then fuse with lysosomes, initiating the degradation of the pathogen through a series of enzymatic and antimicrobial mechanisms.

miRNA and Phagocytosis

Pathogenic Mycobacterium tuberculosis (Mtb) circumvents phagosome maturation, in part, by disrupting actin polymerization. Notably, it has been demonstrated that miRNA-142-3p modulates the expression of N-WASP, an actin-binding protein essential for the phagocytic uptake of M. tuberculosis. Experimental overexpression of miRNA-142-3p resulted in decreased N-WASP levels, leading to impaired mycobacterial phagocytosis. Conversely, the loss of miRNA-142-3p function augmented phagocytic activity. Furthermore, M. tuberculosis was shown to trigger the early expression of miRNA-142-3p, which partially reduced N-WASP protein levels in both the murine J774A.1 cell line and primary human macrophages [14].

The study by Feng Deng and colleagues showed a decreased level of miRNA-215-5p in TB patients. At the same time, experimental increase in miRNA-215-5p led to suppression of autophagic flux, which most likely reflects the complexity of compensatory-adaptive regulatory mechanisms in TB. At the same time, the fact of decreased expression of miRNA-215-5p in complex with other miRNAs, such as miRNA, allowed the authors to propose a potential diagnostic model for tuberculosis [15].

Der-Yuan Chen and colleagues demonstrated that miRNA-889 impedes autophagy, thereby facilitating the survival of Mycobacterium tuberculosis in individuals with latent tuberculosis infection. This effect is mediated through the regulation of TWEAK, a cytokine belonging to the TNF superfamily. TWEAK is known to stimulate autophagy and promote the maturation of mycobacterial autophagosomes via activation of AMP-activated protein kinase (AMPK). However, miRNA-889 inhibits autophagy by post-transcriptionally repressing TWEAK expression [16].

Furthermore, a study by Kunmei Liu and colleagues revealed that miRNA-106a suppresses both autophagy and antimicrobial responses by targeting critical autophagic proteins, including ULK1, ATG7, and ATG16L1, during mycobacterial infection. Using the non-replicating M. tuberculosis strain H37Ra, the study demonstrated that overexpression of miRNA-106a markedly inhibited autophagy in human macrophages in response to H37Ra. In contrast, the inhibition of miRNA-106a enhanced autophagy during H37Ra infection [17].

circRNA and Phagocytosis

A study by Qinghong Shi and colleagues demonstrated that circRNA AGFG1 modulates both autophagy and apoptosis in macrophages infected with M. tuberculosis (Mtb) through the Notch signaling pathway in tuberculosis patients. The researchers found that circAGFG1 promotes an increase in Notch protein levels by suppressing miRNA-1257, which in turn enhances autophagy while reducing apoptosis [18].

Another investigation into circRNA revealed that circRNA TRAPPC6B inhibits intracellular Mtb growth while simultaneously inducing autophagy in macrophages by targeting miRNA-874-3p. Mtb infection in monocytes/macrophages led to a significant reduction in circTRAPPC6B levels, which normally suppress intracellular Mtb growth. Upregulation of circTRAPPC6B expression was shown to activate autophagy and increase the production of the autophagy-related protein LC3-II in Mtb-infected macrophages. Moreover, circTRAPPC6B enhanced autophagy in Mtb-infected macrophages by preventing miRNA-874-3p from inhibiting ATG16L1, a key protein essential for autophagy [19].

lncRNA and Phagocytosis

A study by Kamlesh Pavar and colleagues demonstrated that the expression levels of lncRNA MEG3 are downregulated following M. tuberculosis (Mtb) infection. lncRNA MEG3 is implicated in the regulation of the mTOR and PI3K-AKT signaling pathways, both of which are critical for autophagy regulation. In infected macrophages, IFN-γ-induced autophagy resulted in persistent downregulation of MEG3, whereas the absence of IFN-γ led to the opposite regulatory effect on MEG3 expression. Knockdown of MEG3 in macrophages promoted autophagy and enhanced the intracellular clearance of M. bovis BCG [20].

In addition, it was shown that the suppression of lncRNA-EPS inhibits apoptosis and promotes autophagy by activating the JNK/MAPK signaling pathway in RAW264.7 macrophages infected with BCG, thereby facilitating Mtb survival within macrophages [21].

The study by Li and colleagues demonstrated that PCED1B-AS1 levels are decreased in patients with active TB, leading to reduced apoptosis and increased autophagy. Experiments confirmed that downregulation of PCED1B-AS1

directly causes these changes. It is suggested that PCED1B-AS1 acts as a "sponge" for miR-155, suppressing it. In contrast, upregulation of miR-155 target genes, FOXO3/Rheb, abolishes the effects of PCED1B-AS1. Thus, PCED1B-AS1 regulates macrophage apoptosis and autophagy in TB via the miR-155 axis [22].

An experimental study by Fang Jiang and colleagues found that lncRNA MIAT is upregulated in Mtb-infected macrophages (THP-1 cells) while miR-665 is downregulated. This overexpression of MIAT suppresses apoptosis and reduces autophagy, which increases Mtb survival. It is proposed that MIAT does this by acting as a "sponge" for miR-665, which in turn regulates ULK1. Thus, MIAT is a negative regulator of macrophage antimicrobial effects via the miR-665/ULK1 axis and may be a novel target for TB treatment [23].

Toll-like Receptors (TLRs)

The involvement of Toll-like receptors (TLRs) in Mycobacterium tuberculosis (Mtb) infection has been extensively investigated [3]. TLR activation initiates a cascade of intracellular signaling events, primarily through the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and mitogen-activated protein kinase (MAPK) pathways. This signaling cascade ultimately leads to the induction of an inflammatory response and the clearance of pathogens via the production of various pro-inflammatory mediators. n response to activation of Toll-like receptors (TLRs), a spectrum of proinflammatory cytokines is released, including TNF-α, IL-1, IL-6, and IL-8, each of which plays a significant role in the initiation and maintenance of the inflammatory process. The IL-1 cytokine family acts as key regulators of innate immunity. Moreover, while most members of this family, such as IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ, are characterized by proinflammatory activity, others, including IL-37 and IL-38, exhibit anti-inflammatory properties.

miRNA and TLRs

Several microRNAs (miRNAs) have been identified as key regulators of Toll-like receptor (TLR) activation, thereby modulating inflammatory responses. Notably, TLR4 plays a crucial role in the pathogenesis and survival of Mycobacterium tuberculosis (Mtb). A body of evidence indicates that Mtb infection significantly increases the expression of miRNA-1178 in human macrophages. This upregulation of miRNA-1178 exhibits a clear dependence on both bacterial concentration and the duration of infection. Functional studies have further elucidated the role of miRNA-1178, demonstrating that its overexpression enhances intracellular Mtb growth, whereas its knockdown results in a marked suppression of Mtb survival [24].

Another investigation demonstrated that mimicking miRNA-708-5p enhanced intracellular survival of Mycobacterium tuberculosis in macrophages, whereas inhibition of miRNA-708-5p reduced bacterial survival. Moreover, the secretion of pro-inflammatory cytokines, including IFN-γ, IL-6, IL-1β, and TNF-α, was significantly elevated in Mtb-infected macrophages, while the miRNA-708-5p mimic attenuated their secretion. TLR4 was identified as a direct and functional target of miRNA-708-5p, with miRNA-708-5p exerting a negative regulatory effect on TLR4 expression in macrophages [25].

In a study by Wenyi Niu and colleagues, it was demonstrated that upregulation of miRNA-125a negatively regulates the NF-κB pathway by directly targeting TRAF6 (TNF receptor-associated factor 6). This inhibition of TRAF6 synthesis weakens the immune response and promotes Mtb survival in macrophages [26].

Macrophage Glycolysis

Glycolysis is the central catabolic pathway responsible for glucose degradation, occurring in nearly all biological cells under both aerobic and anaerobic conditions. During Mycobacterium tuberculosis (Mtb) infection, glycolysis becomes the predominant metabolic pathway in immune cells, playing a critical role in the inflammatory response. Infected macrophages undergo a metabolic reprogramming, transitioning from oxidative phosphorylation to aerobic glycolysis, thereby ensuring rapid ATP production. This process, referred to as the "immunometabolic shift," is essential for the production of IL-1β, a key pro-inflammatory cytokine critical for the host's defense against the infection [27].

miRNA and Glycolysis

It has been shown that miRNA-21 modulates intracellular glycolysis and limits macrophage metabolic reprogramming during Mycobacterium tuberculosis (Mtb) infection. miRNA-21 regulates the expression of phosphofructokinase muscle-type (PFK-M), inhibiting glycolysis in infected macrophages. In contrast, IFN-γ, a key cytokine orchestrating the host's immune response against Mtb, downregulates miRNA-21 expression. This suppression leads to a shift in the PFK isoenzyme complex, sustaining the expression of PFK-M following Mtb infection. Therefore, miRNA-21 targets PFK-M to modulate the immunometabolic functions of macrophages [28].

The role of miRNA-26a in modulating macrophage glycolysis has also been explored. A study by Joyoti Basu and colleagues examined the miRNA-26a/SIRT6/HIF-1α signaling pathway, which governs glycolysis and macrophage immune responses to Mtb infection. SIRT6 (sirtuin 6) is a protein involved in various molecular pathways, including DNA repair, telomere maintenance, glycolysis, and inflammation. Hypoxia-inducible factor 1-alpha (HIF-1α) plays a key role in the progression of malignant neoplasms and the development of various pathological conditions, in particular through the regulation of vascularization, angiogenesis, metabolism and cell survival factor. Mtb infection was found to suppress miRNA-26a, while simultaneously increasing its target, SIRT6, which in turn inhibited glycolysis and the expression of HIF-1α-dependent glycolytic genes [29]. *Macrophage Apoptosis*

In the context of tuberculosis, the programmed cell death of macrophages harboring Mycobacterium tuberculosis (Mtb) emerges as a pivotal host defense mechanism. This process curtails bacterial expansion during the initial phases of infection and concurrently initiates downstream immunological responses. The apoptotic vesicles formed during the death of infected cells activate CD8+ cytotoxic lymphocytes, which then eliminate the infected cells, contributing to the control of the infection [30].

miRNA and Macrophage Apoptosis

Several miRNAs involved in macrophage apoptosis following Mtb infection have been identified. Evidence indicates that decreased levels of miRNA-20a-5p correlate with reduced expression of the pro-apoptotic factor Bim. This, in turn, promotes mycobacterial clearance and diminishes macrophage apoptosis. In contrast, miRNA-125b-5p expression is elevated in macrophages from tuberculosis patients. Targeting miRNA-125b-5p has been shown to fortify macrophages against Mtb-

induced damage by promoting apoptosis, upregulating pro-apoptotic gene expression, and dampening the secretion of pro-inflammatory cytokines IL-6 and TNF-α. Mechanistically, this inhibition reduces intracellular Mtb burden in human macrophages in vitro, at least in part, by targeting DNA damage-regulated autophagy modulator 2 (DRAM2), thereby favoring apoptosis and suppressing inflammation [31].

In macrophages infected with the H37Rv strain of M. tuberculosis, Xiue Xi et al. observed a significant increase in the expression levels of miRNA-223. Transfection of miRNA-223 into human macrophages inhibited apoptosis, with miRNA-223 directly suppressing the synthesis of FOXO3, a key apoptosis regulator. Intriguingly, the pro-apoptotic effects suppressed by elevated miRNA-223 levels were reversed by the overexpression of FOXO3 [32].

A study by Qin Sun et al. in human macrophages demonstrated that MTB infection triggers programmed necrosis through mitochondrial cyclophilin D (CypD), leading to mitochondrial depolarization and cell death. Inhibition, downregulation, or knockout of CypD significantly attenuated both MTB-induced programmed necrosis and apoptosis, while overexpression of CypD enhanced cytotoxicity. Furthermore, miR-1281 was identified as a direct negative regulator of CypD. Ectopic overexpression of miR-1281 decreased CypD expression and protected macrophages from MTB-induced programmed cell death, while inhibition of miR-1281 increased CypD and enhanced cytotoxicity. Notably, the protective effects of miR-1281 were dependent on CypD, as its manipulation had no impact on CypD-knockout macrophages [33].

In a study by Beibei Fu et al., miRNA-325-3p expression increased following Mtb infection. It was shown that miRNA-325-3p directly interacts with and inhibits LNX1, thereby stabilizing NEK6 by preventing its proteasomal degradation. NEK6 is recognized as a crucial regulator of the cell mitotic cycle. Abnormal accumulation of NEK6 activates STAT3 signaling, thereby suppressing apoptosis and promoting intracellular Mtb survival [34].

miRNA–155: A "Double Agent" in Infection

An intriguing aspect of miRNA-155 lies in its dual role, acting as a "double agent" with opposing effects at different stages of infection and in various immune cells. During the early phase of infection, miRNA-155 suppresses the activity of SHIP-1, an enzyme involved in regulating apoptosis. This suppression triggers the Akt signaling pathway, which is crucial for the survival and proliferation of macrophages infected with M. tuberculosis. As a result, the inhibition of SHIP-1 may contribute to cell survival. However, in the later stages of infection, miRNA-155 shifts its role to enhance T-cell production of IFN-γ, consequently promoting the clearance of Mtb. This staged, bidirectional action underscores the complexity and multifaceted nature of the immune response to infection [35].

An overview of key non-coding RNAs and their described mechanisms of influence on macrophage function in the pathogenesis of tuberculosis is systematized and demonstrated in Table 1.

Table 1 Effects of non-coding RNAs on innate immune functions in tuberculosis

ncRNA	Type	Target/ Pathway	Effect on macrophage function	Consequence for M. tuberculosis
miR-142-3p	miRNA	N-WASP	Suppresses phagocytosis	Increased Mtb survival
miR-215-5p	miRNA	Autophagy	Inhibits autolysosome fusion	Facilitates persistence
miR-889	miRNA	TWEAK → AMPK	Suppresses autophagy	Supports latent Mtb survival
miR-106a	miRNA	ULK1, ATG7, ATG16L1	Suppresses autophagy and antimicrobial activity	Increased Mtb survival
circAGFG1	circRNA	miR-1257 → Notch	Enhances autophagy, reduces apoptosis	Macrophage protection, infection control
circTRAPPC6B	circRNA	miR-874-3p → ATG16L1	Activates autophagy	Suppresses Mtb growth
lncRNA MEG3	lncRNA	mTOR, PI3K/AKT	Regulates autophagy, enhances Mtb clearance	Reduced bacterial load
lncRNA-EPS	lncRNA	JNK/MAPK	Inhibits apoptosis, promotes autophagy	Facilitates Mtb survival
PCED1B-AS1	**lncRNA**	**miR-155**	**Reduces apoptosis, promotes autophagy**	**Supports immune defense**
MIAT	lncRNA	miR-665 → ULK1	Suppresses autophagy	Increased Mtb survival
miR-1178	miRNA	TLR4	Suppresses inflammatory response	Supports Mtb survival
miR-708-5p	miRNA	TLR4	Reduces cytokine production	Enhances intracellular survival
miR-125a	**miRNA**	**TRAF6 → NF-κB**	**Weakens macrophage activation**	**Supports Mtb survival**
miR-21	miRNA	PFK-M	Suppresses glycolysis	Reduces immune energy response
miR-26a	miRNA	SIRT6 → HIF-1α	Inhibits glycolysis	Reduces inflammatory response

lncRNA and Macrophage Apoptosis

A study investigating the interplay between lncRNA NEAT1 and miRNA-373 demonstrated that individuals with tuberculosis (TB) exhibited increased NEAT1 levels and decreased miRNA-373 levels in comparison to healthy controls. Further investigation showed that increased NEAT1 expression amplified inflammatory responses in macrophages, diminished their phagocytic capacity and ability to clear bacteria, and promoted cell proliferation while inhibiting apoptosis in infected cells. Thus, NEAT1, by suppressing miRNA-373, modulates several key cellular processes in macrophages, including inflammation, proliferation, and apoptosis [36].

Exosomes

Ranging in diameter from 30 to 150 nm, exosomes are small membrane vesicles that are secreted into the extracellular space by almost all cell types. They participate in immune response regulation, underscoring their significance in intercellular interactions. Recent discoveries have identified

numerous miRNAs and messenger RNAs (mRNAs) transported via exosomes to target cells, leading to a growing interest in these extracellular vesicles [37].

miRNA and Exosomes

Zhicheng Sun and colleagues identified several differentially expressed non-coding RNAs (ncRNAs) in the exosomes of bone macrophages from tuberculosis (TB) patients. Among these, miRNA-125b-5p emerged as a particularly significant finding. Not only was it proposed as a potential diagnostic biomarker for tuberculosis, but it also highlighted the involvement of critical signaling pathways, including Ras, Rap1, MAPK, TNF and PI3K-Akt, in driving disease progression [38].

This in vitro study, using RAW 264.7 macrophages, investigated the previously unknown role of miR-20b-5p in Mtb infection. It was found that the expression levels of miR-20b-5p decreased over time in infected macrophages and that miR-20b-5p was present in exosomes released from these cells. Upregulation of miR-20b-5p significantly suppressed Mtb survival in macrophages, while decreasing macrophage viability and inducing apoptosis in infected cells. In contrast, downregulation of miR-20b-5p enhanced Mtb survival, increased macrophage viability, and attenuated apoptosis. Bioinformatic analysis and subsequent experimental validation identified Mcl-1, an anti-apoptotic member of the Bcl-2 family, as a direct target of miR-20b-5p. Thus, miRNA-20b-5p acts as a negative regulator of Mcl-1 expression, thereby modulating key processes of cell survival and apoptosis in Mtb-infected macrophages [39].

Macrophage Polarization

Macrophage polarization is a driving process by which immune cells differentiate into different phenotypes in response to microenvironmental cues. Among the identified types of macrophage polarizations, two subtypes have been extensively studied. M1 macrophages are characterized by their pronounced phagocytic activity, which effectively internalizes and kills bacteria, viruses, and other pathogens. They play a critical role in initiating the inflammatory process and mediate the host immune response to infectious agents. In contrast, M2 macrophages are primarily involved in wound healing, resolution of secretion, and maintenance of tissue homeostasis. They monitor tissue regeneration and respond to adverse effects [40].

Recent investigations have explored the molecular pathways through which circular RNA hsa_circ_0003528 and certain microRNAs influence macrophage polarization in the context of TB. The research employed both clinical samples, specifically plasma from TB patients and healthy controls, as well as in vitro cell models, namely THP-1 and U937 cells. Expression analysis revealed that hsa_circ_0003528 was significantly elevated in the plasma of TB patients. In cell line experiments, hsa_circ_0003528 expression negatively correlated with miR-224-5p, miR-324-5p, and miR-488-5p, while positively correlating with miR-192 and miR-217. Notably, miR-224-5p, miR-324-5p, and miR-488-5p were significantly down-regulated in active pulmonary TB patients compared to healthy controls. Functional studies demonstrated that hsa_circ_0003528 acts as a competing endogenous RNA for miR-224-5p, miR-324-5p, and miR-488-5p, with CTLA4 identified as a shared target gene. Upregulation of circRNA-0003528 has been shown to promote TB-associated macrophage polarization by increasing CTLA4

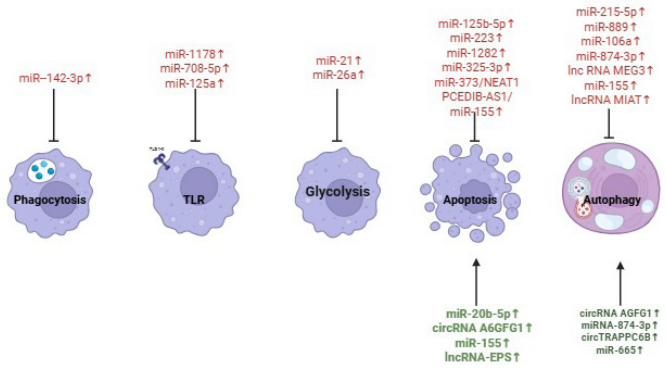

Figure 2 – Changes in non-coding RNA expression in macrophages infected with Mtb

expression, which is mediated by the downregulation of specific miRNAs [41, 42].

In another study, the role of lncRNA XIST in tuberculosis pathogenesis was explored. To simulate a model of negative pressure therapy in vitro, a clinical negative pressure bottle was connected to Mtb-infected RAW264.7 cells and a culture of macrophages derived from human monocytes. The application of negative pressure resulted in a reduction of XIST levels and an increase in miRNA-125b-5p levels. This change led to the suppression of A20 protein, an inhibitor of NF-κB, thereby influencing the polarization of macrophages. As a result, negative pressure promoted a shift in macrophages toward a more active pro-inflammatory M1 state [43].

Figure 2 of this study presents a detailed illustration, created by the authors, that summarizes the changes observed in non-coding RNA expression in Mycobacterium tuberculosis-infected macrophages.

Conclusion

To sum up, the findings presented in this review highlight the pivotal role of non-coding RNAs in regulating innate immune cells during tuberculosis progression. These molecules exhibit diverse functions, including the modulation of gene expression crucial for pathogen detection and immune response initiation, as well as the regulation of immune cell differentiation and polarization.

Among them, microRNAs stand out for their remarkable ability to fine-tune signaling pathways, ensuring a balanced response that efficiently eliminates M. tuberculosis while preventing excessive inflammation, which could otherwise lead to tissue damage. In contrast, long non-coding RNAs act as "orchestrators" of gene expression, coordinating gene clusters essential for an adequate immune reaction to Mtb.

Circular RNAs, although not yet fully understood, also appear promising. Preliminary data indicate that they might function as "molecular sponges" by sequestering microRNAs, thereby safeguarding mRNAs from degradation. This protective mechanism could influence the expression of key proteins involved in the immune defense against tuberculosis.

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Combined Use of Inhaled and Intravenous Colistin in a Patient with Acute Lymphoblastic Leukemia Complicated by Multi-Organ Failure and Sepsis: A Case Report

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Abstract

Thyroid nodules are highly prevalent, particularly among women. Palpation detects nodules in 2–7% of cases, whereas high-resolution ultrasound (US) identifies them in 20–76% of individuals. In most cases, thyroid nodules are benign and incidentally detected, causing no clinical symptoms. But when clinical symptoms begin to appear, it is necessary to make a decision on the tactics of further management. Surgical treatment is often used, including hemithyroidectomy or total thyroidectomy, poses several limitations, such as prolonged hospital stay, airway obstruction, recurrent laryngeal nerve paralysis, iatrogenic hypothyroidism, and challenges associated with reoperation. Microwave ablation is used today, is a minimally invasive method used to treat various benign and malignant tumors, including those of the thyroid, liver, and lungs.

Keywords: thyroid, microwave ablation, benign nodules.

Introduction

Thyroid nodules are highly prevalent, particularly among women. Palpation detects nodules in 2–7% of cases, whereas high-resolution ultrasound (US) identifies them in 20–76% of individuals [1]. In most cases, thyroid nodules are benign and incidentally detected, causing no clinical symptoms. However, symptomatic nodules [2] may lead to cosmetic concerns, dysphagia, coughing, and esophageal compression, ultimately reducing the patient's quality of life [3].

In the diagnostic evaluation of patients, ultrasound imaging and fine-needle aspiration biopsy (FNAB) are commonly used to differentiate between benign and malignant thyroid lesions [4–9]. Additionally, the presence of clinical symptoms—such as dyspnea, choking, or a sensation of air hunger—as well as cosmetic concerns, are considered indications for nodule ablation.

Currently, in addition to traditional surgical treatments like hemithyroidectomy or total

thyroidectomy—which are associated with various limitations including extended hospital stays, airway obstruction, recurrent laryngeal nerve paralysis, iatrogenic hypothyroidism, and complications with revision surgeries [10] — minimally invasive techniques have been developed.

The following types of minimally invasive techniques are available: ethanol ablation, laser ablation, radiofrequency ablation and microwave ablation. Ethanol ablation is primarily indicated for cystic nodules [11]. It is also used in cystic-solid nodules as a preparatory step before thermal ablation. Laser ablation is rarely used in modern clinical practice due to technical limitations: the electrode must be placed centrally within the nodule, which results in the treatment of a restricted zone, often leaving peripheral areas untouched and thereby increasing the likelihood of recurrence. Furthermore, the effective treatment area is constrained by the fiber's size (300–400 mm), making it suitable only for small nodules. Considering that, to date, the ablation of benign thyroid nodules is indicated only in the presence of clinical symptoms, small nodules suitable for laser ablation are generally not subject to removal [12–15].

Thermal techniques—radiofrequency ablation (RFA) and microwave ablation (MWA)—are used for solid thyroid nodules as well as those with a cystic component [16,17].

Microwave ablation is a minimally invasive method used to treat various benign and malignant tumors, including those of the thyroid, liver, and lungs. The principle of microwave ablation is based on the use of heat energy, where a needle electrode generates thermal energy, leading to thermal coagulation of the nodular tissue [18].

RFA and MWA are highly effective due to the use of the "moving-shot" technique under ultrasound guidance, which enables comprehensive treatment of the entire nodule. However, radiofrequency ablation is typically applied to nodules measuring up to 3–4 cm, whereas microwave ablation is used for larger nodules, including very large ones (9–10 cm), as the microwave field covers a wider area, resulting in more effective tissue ablation.

Case Presentation

A 49-year-old female patient presented with complaints of a lump sensation in the throat, a palpable mass on the anterior neck, and a cosmetic defect. According to the patient, these symptoms had been present for three years, with worsening clinical symptoms and a more pronounced cosmetic defect over the past year.

On palpation, a round, firm-elastic nodule up to 3 cm in diameter with a smooth surface was detected on the anterior neck.

Ultrasound findings: The left thyroid lobe contained a solid-cystic nodule measuring 4.5 × 2.8 × 3.7 cm (V = 27 cm³). The nodule exhibited a heterogeneous structure, with a 30% cystic component, well-defined smooth margins, and horizontal growth orientation. Color Doppler imaging (CDI): Increased perinodular vascularization. Thyroid echotexture - heterogeneous. TIRADS classification- Category 3 (probably benign). Cervical lymph nodes- not enlarged (Figure 1).

Following ultrasound imaging, a fine-needle aspiration biopsy (FNAB) was performed. Cytological analysis revealed cytological features of autoimmune thyroiditis with follicular epithelial proliferation and areas of cystic degeneration. According to the Bethesda System for Reporting Thyroid

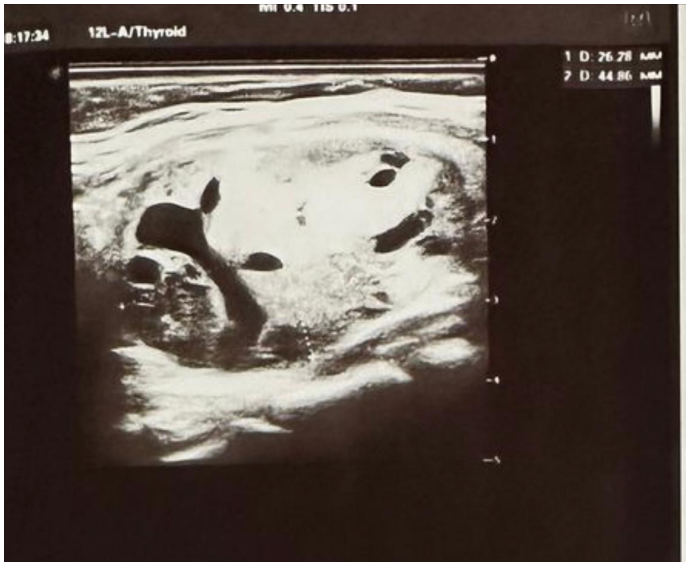

Figure 1 – Nodule before microwave ablation

Cytopathology (TBSRTC 2017), the nodule was classified as Category II (benign).

Subsequent laboratory evaluation of thyroid-stimulating hormones (TSH, T3, T4) confirmed euthyroid status.

Based on the comprehensive ultrasound, cytological, and hormonal assessment, microwave ablation (MWA) was selected as the preferred treatment. In August 2024, the patient underwent microwave ablation of the solid-cystic nodule in the left thyroid lobe. The procedure was performed under local anesthesia, without incisions, highlighting the minimally invasive nature of the technique. Another key advantage was the absence of a rehabilitation period, allowing the patient to be discharged the following day and immediately return to work.

A six-month follow-up evaluation demonstrated complete resolution of cosmetic concerns and full remission of clinical symptoms. Ultrasound imaging revealed a 9-fold reduction in nodule volume, measuring 2.4 × 1.5 × 1.5 cm (V = 3 cm³), with complete absence of vascularity (Figures 2, 3).

Discussion

Thyroid nodules are a common condition, occurring in 68% of patients undergoing thyroid ultrasound examination [19]. Clinical symptoms typically appear when nodules extend

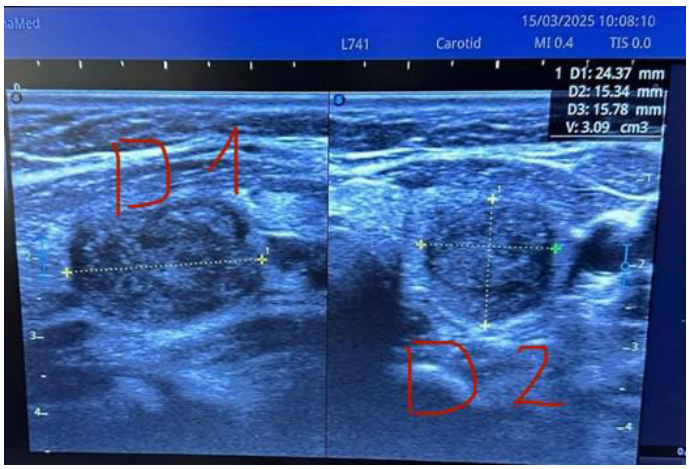

Figure 1 – Nodule 6 month after microwave ablation

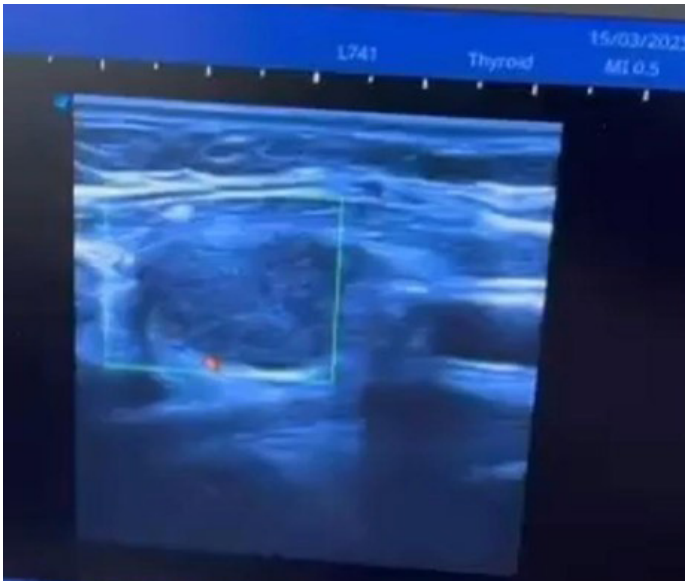

Figure 3 – Color doppler imaging 6 month after microwave ablation (absence of vascularity)

beyond the thyroid contours. In 95–98% of cases, nodular, cystic-solid, solid-cystic formations, and thyroid cysts are benign [20].

Unfortunately, a significant proportion of surgical treatments for benign thyroid nodules results in underestimation of risks and benefits [21], leading to economic burden on the healthcare system and increased complications.

Today, the development of minimally invasive surgery is an integral part of modern medicine. Microwave ablation is a proven effective and safe method for treating symptomatic benign thyroid nodules [22–24]. The minimally invasive approach of microwave ablation has several advantages over thyroidectomy, including preservation of thyroid function, no scarring, no rehabilitation period, and no need for hormone replacement therapy.

Reported side effects of MWA include hematoma formation (3.8%), transient or permanent voice changes (4.6%), and pain during the procedure (2.2%) [24].

Currently, MWA is considered a first-line therapy for benign thyroid nodules in many countries and is included in clinical guidelines for the management of thyroid nodular disease [25–26]. In addition, an increasing body of evidence supports the use of MWA in the treatment of papillary thyroid microcarcinomas. The proven efficacy and safety of this method open up significant opportunities for improving treatment strategies for lesions in various anatomical locations, including the thyroid gland.

The presented clinical case demonstrates both the effectiveness and safety of this technique. The application of the moving-shot technique allowed for complete thermal ablation of the nodule under real-time ultrasound guidance, while ensuring protection of the "danger triangle" area from thermal injury. According to Vuong et al., the volume reduction rates of thyroid nodules at 3, 6, and 12 months after MWA were 66.8%, 74.3%, and 81%, respectively [27]. In our case, a volume reduction of nearly 90% was observed at 6 months post-procedure. Furthermore, the MWA procedure took 34 minutes, whereas conventional open thyroidectomy typically requires up to 120 minutes to perform [28].

Microwave ablation is an operator-controlled procedure, ensuring predictable ablation of the nodule volume, and is considered a first-line treatment for solid and solid-cystic thyroid nodules.

A limitation of this case is the absence of follow-up data at 3 months post-ablation, due to the patient residing in another country. A major strength of the presented case is that microwave ablation is routinely performed not only for thyroid nodular disease, but also for lesions in the liver, lungs, uterus, and other organs.

Conclusion

Microwave ablation is an effective and safe method for treating solid and solid-cystic thyroid nodules, allowing for the management of symptomatic benign nodules.

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Early Outcomes of Aortic Valve Bicuspidization Using Autologous Pericardium in Pediatric Aortic Stenosis: a Preliminary Case Series

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Abstract

Aortic valve bicuspidization using autologous pericardium is a promising valve-sparing technique for treating aortic stenosis in pediatric patients. However, literature describing this procedure in children remains limited.

To present early clinical outcomes of aortic valve bicuspidization with autologous pericardium in a small case series of pediatric patients with aortic stenosis.

We conducted a retrospective analysis of five pediatric patients who underwent aortic valve bicuspidization using autologous pericardial patches in January 2025. Data were collected on surgical technique, echocardiographic findings, and postoperative complications.

All five patients had successful surgical outcomes, with significant reductions in transvalvular gradients and no intraoperative or early postoperative complications. Valve function remained stable during follow-up (up to three months), and no reintervention was required.

This preliminary case series demonstrates the technical feasibility and short-term safety of aortic valve bicuspidization using autologous pericardium in children. Larger, longer-term studies are needed to validate these findings.

Keywords: Aortic stenosis, pediatric cardiac surgery, aortic valve repair, valve-sparing techniques.

Introduction

Aortic stenosis (AS) in children is a congenital or, less frequently, acquired condition characterized by obstruction to blood flow from the left ventricle to the aorta, typically caused by malformations of the aortic valve such as unicuspid, bicuspid, or dysplastic valves [1, 2]. The condition leads to increased left ventricular pressure, concentric hypertrophy, and, if left untreated, may result in heart failure, arrhythmias, or sudden cardiac death [3, 4].

Surgical intervention remains the mainstay of treatment for moderate to severe AS in the pediatric population. Traditional approaches include balloon valvuloplasty, surgical commissurotomy, or valve replacement. However, each method has limitations: valvuloplasty may be associated with restenosis, valve replacement introduces the need for lifelong anticoagulation or reoperation due to somatic growth, and valve repair techniques often vary in reproducibility and durability [5–7].

Valve-sparing techniques, such as leaflet augmentation or bicuspidization using autologous pericardium, have emerged as promising alternatives, particularly in young patients. These approaches aim to restore valve competence while preserving native tissue and avoiding prosthetic replacement [8].

This study aims to contribute to the growing body of literature on valve-sparing techniques by presenting early clinical outcomes of aortic valve bicuspidization with autologous pericardium in a pediatric population. Given the limited number of published case series, especially in children, our findings may offer valuable insights into the feasibility, safety, and potential benefits of this reconstructive approach.

Case presentation

This is a retrospective, single-center case series conducted at the National Scientific Medical Centre. It includes five pediatric patients diagnosed with congenital aortic stenosis who underwent aortic valve bicuspidization using autologous pericardium. Inclusion criteria were: Age under 18 years, isolated congenital aortic stenosis, and no prior aortic valve surgery. Patients with complex congenital heart defects, severe valve regurgitation, or connective tissue disorders were excluded. All patients underwent preoperative transthoracic echocardiography to evaluate valve morphology, transvalvular gradients, and ventricular function.

Table 1 Patient demographics and clinical characteristics

Patient	Age (years)	Weight (kg)	Pre-op gradient (mmHg)	Post-op gradient (mmHg)	CPB time (min)	Compli-cations	Follow-up (months)
1	1 year 11 months	12.7	103\53	28\15	130\107	None	3
2	3 years 8 months	14.0	77\40	21\10	73\35	None	3
3	4 years	16.6	75\38	18\8	157\101	None	3
4	10 years	51.8	120\75	58\34	120\97	None	3
5	10 years	47	83\45	46\20	104\69	None	3

Pre-op gradient, Preoperative gradient; Post-op gradient, Postoperative gradient; CPB time, Cardiopulmonary bypass time.

All patients tolerated the procedure well. There were no intraoperative or early postoperative complications. Postoperative echocardiography demonstrated a significant reduction in transvalvular gradients and satisfactory leaflet motion. No significant aortic regurgitation was observed. During follow-up, valve function remained stable, and no patient required reintervention.

Surgical Technique

Following the induction of general anesthesia, a standard median sternotomy was performed. Cardiopulmonary bypass was established via aortic and bicaval cannulation. Myocardial protection was achieved through the antegrade administration of cold blood cardioplegia. During surgical inspection, the aortic valve was found to be morphologically unicuspid. The non-coronary cusp was aplastic, while the right coronary cusp was hypoplastic and rudimentary. Both cusps were fused at the commissures and appeared thickened and fibrotic (Figure 1A). The valve opening was significantly restricted. Fibrotic tissue at the valve margins was sharply dissected to the required depth. The rudimentary (false) cusp was excised. A decision was made to reconstruct coapting leaflets of optimal height using autologous pericardium. Partial excision of the native cusps was performed along the commissures down to the fibrous annulus. A neocommissure was created from autologous pericardium, followed by augmentation of the left coronary cusp and subsequently the right coronary cusp. The autologous pericardial patch was secured to the neocommissure, and leaflet height was adjusted to ensure adequate coaptation (Figure 1B).

A bougie No. 11 was passed through the aortic valve annulus; the calculated average annular size was 11, with a minimum acceptable size of 8. The aortic wall was reconstructed using a continuous double-layer suture with 6/0–10 Prolene. Rewarming of the patient was initiated, and standard measures were undertaken to prevent air embolism. The aortic cross-clamp was removed, and spontaneous return of cardiac activity was achieved. Cardiopulmonary bypass was subsequently discontinued.

Intraoperative transesophageal echocardiography (TEE) confirmed preserved coaptation of the aortic valve. The valve was bicuspid in morphology. The transvalvular pressure gradient was measured at 28/15 mmHg (preoperative gradient 103\53 mmHg). Left ventricular contractility remained intact.

Two temporary epicardial pacing electrodes were sutured to the right ventricle. Decannulation of the aorta, inferior vena cava, and right atrium was performed. Meticulous hemostasis was achieved. Both pleural cavities were drained using No. 20 chest tubes. The sternum was reapproximated, and the surgical wound was closed in anatomical layers. A sterile dressing was applied.

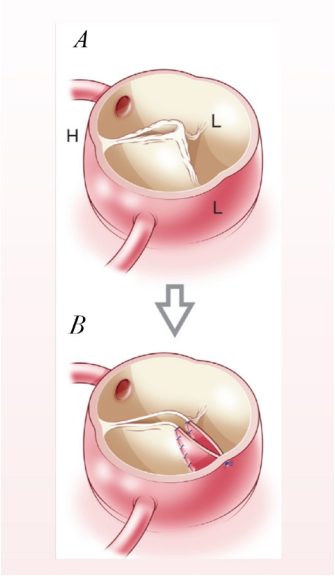

Figure 1 – A – right cusp fibrosis, and B – leaflet augmentation [8]

H, high; L, low.

Discussion

Aortic valve bicuspidization using autologous pericardium represents a novel valve-sparing technique that offers an alternative to prosthetic valve replacement, particularly in the pediatric population, where the need to preserve native valve tissue and accommodate somatic growth is critical. This case series demonstrates the technical feasibility and short-term safety of this approach in five children with congenital aortic stenosis.

All patients in our series underwent successful surgical reconstruction, with no intraoperative or early postoperative complications. The significant reduction in peak and mean transvalvular gradients, combined with preserved valve function and the absence of reintervention during early follow-up,

suggests that bicuspidization can offer effective hemodynamic relief while avoiding the downsides of prosthetic implantation.

The use of glutaraldehyde-treated autologous pericardium for leaflet augmentation has been widely reported in adult populations undergoing aortic valve reconstruction, particularly within the Ozaki procedure framework. However, data in children remain scarce, partly due to anatomical variability, technical demands, and concerns about long-term durability in a growing population. Notably, Ozaki et al. excluded pediatric patients in their foundational studies, citing unpredictability in valve geometry and tissue remodeling [9, 10].

Recent pediatric studies, including reports by Schäfers et al. and d'Udekem et al., have begun to explore valve-sparing strategies using pericardium in children, particularly in the context of unicuspid or dysplastic aortic valves [11, 12]. Our findings support these preliminary observations, reinforcing that anatomical remodeling of the aortic valve using autologous tissue can restore competence without introducing foreign materials or requiring anticoagulation.

One technical point of emphasis in our series is the careful sizing of the annulus and reconstruction of the leaflets to achieve symmetric coaptation. Intraoperative TEE was instrumental in evaluating leaflet mobility and confirming adequate repair, aligning with previous recommendations for real-time functional assessment [12].

While our outcomes are encouraging, the study has limitations inherent to small case series. The three-month follow-up duration is insufficient to assess durability, the risk of calcification, or reoperation rates. Longitudinal studies with larger cohorts and extended echocardiographic monitoring are necessary to determine the long-term efficacy and structural integrity of pericardial patches in growing children.

Additionally, this technique requires a steep surgical learning curve, and reproducibility across centers may vary. Establishing standardization in patient selection, patch sizing, and leaflet configuration will be essential for broader adoption and consistent outcomes.

Conclusion

Aortic valve bicuspidization using autologous pericardium is a technically feasible and short-term safe reconstructive option in children with unicuspid aortic valves. This technique may serve as a valuable alternative to valve replacement in selected pediatric patients. Further multicenter studies with larger populations and long-term follow-up are needed to assess durability and functional outcomes.

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Pathogenic Variant BRCA2 c.7008-2A>T in a 66-year-old Man with Metastatic Castration-resistant Prostate Cancer: a Case Report from Kazakhstan

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Abstract

Prostate cancer has a high association with disruption in DNA repair genes. In this case report, we describe a 66-year-old man diagnosed with advanced prostate cancer from Kazakhstan. Genetic testing identified a likely pathogenic BRCA2 c.7008-2A>T variant (rs81002823), which is known to disrupt nuclear DNA repair mechanisms and has a clinical association with prostate cancer with familial predisposition. We used computational tools, including SpliceAI, to predict the molecular impact of the variant on the gene expression and disease pathogenesis. We discuss the clinical significance of this variant and the role of targeted therapies such as Poly ADP-ribose polymerase (PARP) inhibitors for familial cancer patients with germline variants affecting BRCA1 and BRCA2. We want to demonstrate the role of genetic testing in prostate cancer management to inform personalized treatment strategies and improve family outcomes.

Keywords: Prostate cancer; Advanced stage; BRCA2 variant; Lutetium-177 PSMA; PARPi; DNA sequencing; Genetic testing; Kazakhstan.

Introduction

Prostate cancer (PC) is the most common malignancy in men in 112 countries, with an expected global rise two-fold in 2040 [1]. The complex etiology of PC involves both environmental and genetic factors [2]. While age, race, and family history remain key risk factors, advances in genetic research have guided the role of inherited mutations in the pathogenesis of the disease [3]. Among the most significant are mutations in DNA homologous recombination repair (HRR) genes such as BRCA1, BRCA2, and ATM, which are not only associated with hereditary breast and ovarian cancer but are also increasingly linked to PC, particularly in more aggressive forms of the pathology [4]. BRCA2 pathogenic variants are believed to increase the risk of early-onset PC, characterized by its rapid progression with abundant metastatic involvement, and patients in risk groups are recommended screening at the age of 40 [5]. Personalized diagnosis and treatment have been included in the care guidelines for patients and their family members affected by the pathogenic variants in high-risk genes [6]. Genetic testing allows clinicians to tailor better treatment strategies, such as utilizing targeted therapies like poly (ADP-ribose) polymerase inhibitor (PARPi) recommended for advanced prostate cancer patients having germline mutations in HRR

genes, including BRCA1, BRCA2 [7], while providing genetic counseling for at-risk relatives carrying the pathogenic variants [8], potentially improving outcomes through early detection and intervention.

In this case report, we describe the first documented metastatic castration-resistant prostate cancer patient in Kazakhstan, caused by a splicing-disrupting variant in the BRCA2 gene (NM _ 000059.4(BRCA2):c.7008-2A>T). This variant is located within the intronic splice-acceptor site, and it has been demonstrated to disrupt mRNA splicing experimentally, leading to an abnormal transcript [9]. The affected patient had a family history of early breast cancer diagnosed in his younger sister, suggesting the germline familial effect of the variant. This case report demonstrates the rare pathogenic genetic variant in a PC patient from Kazakhstan.

Clinical Case Presentation

A 66-year-old male presented in May 2019 to the Kazakh Institute of Oncology and Radiology in Almaty City with chief complaints of moderate dysuria, weak urinary stream, and unilateral lower limb edema. Initial evaluation revealed an elevated prostate-specific antigen (PSA) of 1,000 ng/mL. Pelvic MRI demonstrated locally advanced PC based on Prostate Imaging Reporting and Data System (PIRADS-5) with invasion into the bladder, seminal vesicles, left ureter, and mesorectum, alongside metastatic (M1b) involvement of pelvic lymph nodes and bones. Bone scintigraphy confirmed widespread osteoblastic metastases. Transperineal biopsy confirmed acinar adenocarcinoma with a Gleason score of 9 (4+5). Staging confirmed T4N1M1b disease.

The treatment began in July 2019 with the initiation of androgen deprivation therapy (ADT) using degarelix (Firmagon) alongside the chemotherapy and bisphosphonate treatment that included ten cycles of taxane (Docetaxel 140 mg) and zoledronic acid. Although there was an initial decline in PSA levels, this response was not maintained upon the clinical follow-up. By 2021, due to disease progression marked by a PSA rise from 56.72 to 123.90 ng/mL, the treatment was adjusted to include four courses of enzalutamide. This approach was followed by chemotherapy with cabazitaxel (also four courses), leading to unsatisfactory outcomes due to the PSA levels rising to 306.76 ng/mL in February 2022. The treatment was shifted to abiraterone (Zytiga) as PSA levels rose, reaching 281 ng/mL in July 2022. Palliative radiotherapy was added to the treatment in September 2022, delivering up to 60 Gy to the prostate and pelvis. Still, it failed to provide adequate disease control, as indicated by a post-treatment PSA of 1460 ng/mL. In 2023, the patient underwent two cycles of lutetium-177 Prostate-specific membrane antigen (PSMA) therapy abroad, but unfortunately, this led to rapid PSA progression exceeding 1530 ng/mL, accompanied by a clinical decline.

Genetic Testing and Clinical Outcomes

The patient confirmed a family history of early-onset breast cancer in his younger sister, who passed away in her 40s. The patient was recruited as part of a large study investigating genetic factors of prostate cancer in Kazakhstan [10] (Ministry of Science and Higher Education of the Republic of Kazakhstan 0123PK00358), and his peripheral blood was collected for genetic analysis. We used the ReliaPrep™ Blood gDNA Miniprep System for DNA extraction, library preparation, and enrichment. Next-generation DNA sequencing was performed using the TruSight Rapid Capture kit on the Illumina MiSeq platform. We filtered out variants with ≤10 reads. Reference genome mapping (hg19) using the Burrows-Wheeler Aligner was followed by variant calling with the Genome Analysis Toolkit. Genetic variants were annotated per Human Genome Variation Society (HGVS) nomenclature, and sequencing results were analyzed in VCF format using IGV viewer, Variant Studio, SnpEff, SnpSift, and OpenCravat. The final variant analysis returned in September 2024 (posthumously) identified a pathogenic BRCA2 heterozygous variant (NM _ 000059.4(BRCA2):c.7008-2A>T), aligning with his aggressive clinical course and the family history of cancer. Despite multilevel therapy composed of ADT, taxane chemotherapy, as well as novel anti-androgens and radiopharmaceuticals, the patient decline was rapid. The patient was lost to follow-up after the visit to the oncologist in late 2023. At that time, he was assessed at 80% by Karnofsky's performance scale. The patient passed away due to the disease progression in May 2024.

Discussion

This case report described the first patient from Kazakhstan with PC due to a pathogenic variant in the BRCA2 gene with poor response to conventional chemo- and radiotherapy. The BRCA2 (NM _ 000059.4(BRCA2):c.7008-2A>T) variant emphasizes the value of integrating genetic profiling into advanced prostate cancer management, even in resource-limited settings. The variant likely contributed to poor responses to ADT-directed therapies and taxanes and possibly explains the history of early onset breast cancer in this patient's sibling. While lutetium-177 PSMA provided a temporary benefit, rapid progression is consistent with the inadequate therapeutic efficacy due to the genetic defect in the BRCA2 DNA-repair pathway. Several clinical trials report the high value of PPRAi treatment in prostate cancer patients with disrupted genes like BRCA1 and BRCA2, demonstrating significantly prolonged survival and increased efficiency of chemotherapy and radiotherapy [11, 12]. This case illustrates the practical use of genetic testing to detect pathogenic variants of BRCA2 in Central Asia and the timely integration of genetic results into clinical decision-making.

The NM _ 000059.4(BRCA2):c.7008-2A>T (often described as IVS13-2A>T or rs81002823) is an intronic pathogenic variant resulting from an A to T substitution of two nucleotides upstream from the exon 14 in the BRCA2 gene. ClinVar database characterizes this variant as pathogenic and associated with clinical phenotypes like breast-ovarian familial cancer in women as well as hereditary cancer-predisposing syndrome (Accession VCV000052246.30) [13]. This variant was shown to be linked to young-onset breast cancer in Caucasian and African women [14], as well as breast and concurrent breast with ovarian cancer in Italian patients with evidence of disrupted mRNA product [15, 16]. This variant is rare based on population cohorts from the Genome Aggregation Database (gnomAD) (Table 1, see the next page).

The aberrant splicing, resulting in either an abnormal protein or a transcript reported in the previous studies, was confirmed through in-silico analysis. Specifically, SpliceAI [17] predicts a loss of the exon 14 acceptor splice site (Δ score: 0.97) two base pairs upstream and a gain of a splicing acceptor site (Δ score: 0.25) twelve base pairs downstream of the variant. This is further confirmed by the Pangolin model [18], which shows Splice Loss Δ of 0.89 and Splice Gain Δ of 0.65 (https://

Table 1 Genetic Ancestry Group Frequencies of NM _ 000059.4(BRCA2):c.7008-2A>T allele in gnomAD v4.1

Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
European (non-Finnish)	1	1123670	0	8.90E-07
African/African American	0	73620	0	0
Admixed American	0	59936	0	0
Ashkenazi Jewish	0	29256	0	0
East Asian	0	44490	0	0
European (Finnish)	0	64024	0	0
Middle Eastern	0	5956	0	0
Amish	0	912	0	0
South Asian	0	89782	0	0
Remaining	0	60504	0	0
XX	1	777676	0	1.29E-06
XY	0	774474	0	0
Total	1	1552150	0	6.44E-07

spliceailookup.broadinstitute.org/) at the same respective sites (Figure 1). These disruptions are expected to lead to premature splicing events, likely triggering nonsense-mediated decay and low levels of functional BRCA2 protein in affected cells. Our bioinformatic predictions match the pathogenic mechanisms described in the medical literature. However, our limitation is a lack of experimental validation through RNA sequencing and protein quantification to validate the variant's effects in vivo.

The genetic landscape of PC varies significantly across different populations, with numerous studies identifying key mutations in genes like BRCA2, HOXB13, CHEK2, and ATM that contribute to hereditary prostate cancer with proven economic value and increase in quality-adjusted life-years when first-degree relatives undergo genetic testing in addition to the patients [8, 19, 20]. These variants are well-documented in Western and East Asian populations, including Europe, North America, and Japan, where genetic testing and research on prostate cancer are more widespread [21, 22]. However, no large-scale genetic studies describe high-risk pathogenic variants in populations from Kazakhstan or other Central Asian countries. To date, there have been few reports of pathogenic BRCA2 variants associated with breast cancer from Central Asia [23, 24]. This case from Kazakhstan is the first evidence of the pathogenic BRCA2 c.7008-2A>T variant likely contributing to the metastatic castration-resistant PC in this population. We want to demonstrate the importance of genetic studies of PC in Central Asia to understand better the region-specific genetic variants contributing to the disease pathogenesis and to guide personalized treatment and risk assessment in patients and their families.

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Informed Consent: We received written informed consent from the patient to participate in the study. A copy of the written informed consent is available upon request from the corresponding author.

Data availability statement: The corresponding author can provide the data supporting the study's conclusions upon request. Due to ethical and privacy constraints, the data are not publicly accessible.

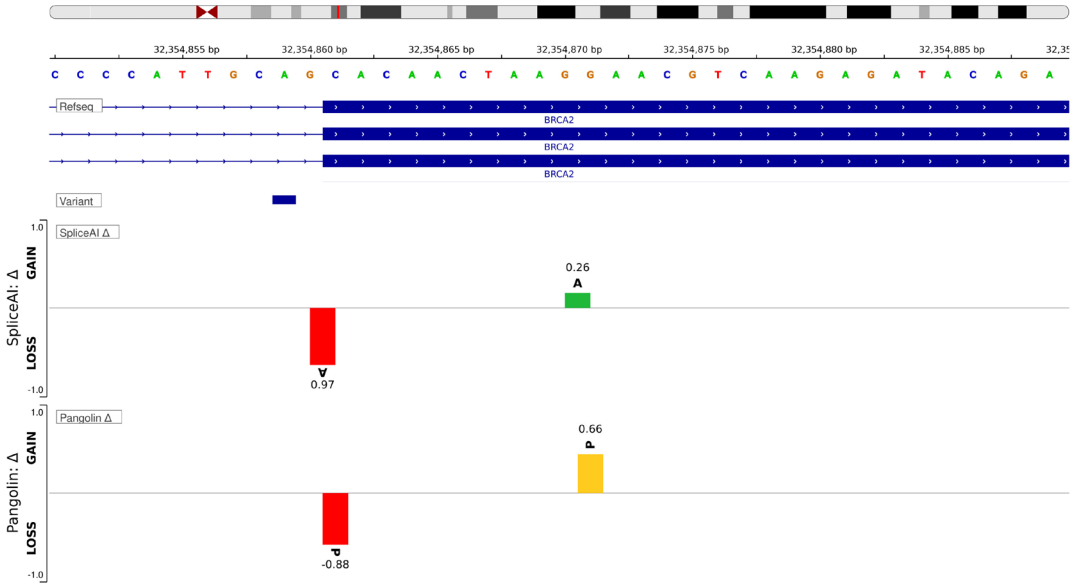

Figure 1 – SpliceAI visualization showing predicted splicing alterations for the BRCA2 variant NM _ 000059.4(BRCA2):c.7008-2A>T

The plot represents intron 13 and exon 14, splicing events with impact scores, and a custom legend indicating the positions of acceptor gain and loss predicted by the SpliceAI and Pangolin models.

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Delayed Open Chest Closure in Prosthetic Aortic Root Endocarditis: a Case Report

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Abstract

Surgical therapy for aortic valve endocarditis can be complicated by paravalvular abscess formation, which is associated with high morbidity and mortality. We report a case of complicated infective endocarditis treated using a delayed sternalclosure (DSC) strategy. DSC after cardiac surgery may be an effective option in managing complicated aortic root endocarditis. On admission, a 60-year-old male presented with symptoms of heart failure and a high-grade fever of unknown origin. He had previously undergone aortic valve reimplantation (David procedure) 10 years earlier for aortic regurgitation and root dilation. Transesophageal echocardiography and contrast-enhanced computed tomography confirmed a para-aortic infiltrate and vegetation on the free margin of the right coronary cusp. The patient underwent explantation of the infected Valsalva prosthesis with thorough debridement of the surrounding infected native aortic root tissues. Due to the extensive spread of infection, DSC with mediastinal drainage was performed. Mediastinal re-exploration and irrigation with Betadine solution were conducted for meticulous washing of all infected areas. The patient's postoperative course was uneventful, with preserved valve function and no recurrence of abscess at 3-year follow-up. DSC can be considered a therapeutic option in advanced cases of infective endocarditis.

Keywords: aortic root, heart valve prosthesis, infective endocarditis, abscess, delayed closure, sternum

Introduction

Prosthetic aortic root endocarditis is a rare but devastating complication, associated with high morbidity and mortality due to its aggressive nature and the frequent involvement of perivalvular structures [1]. The disease is often complicated by paravalvular abscess formation, leading to conduction disturbances, valvular dysfunction, and systemic embolization [2, 3]. Surgical intervention remains the cornerstone of management, particularly in cases with annular destruction or prosthesis dehiscence, but operative risk is significantly increased in patients with active infection and friable tissue [4]. In such scenarios, achieving complete source control through radical debridement can be technically challenging and may require non-standard strategies.

Delayed sternal closure (DSC) is an established approach in cardiac surgery, typically applied in patients with hemodynamic instability, coagulopathy, or myocardial edema [5]. While extensively studied in congenital heart surgery and transplantation, its use in infective endocarditis is less frequently reported. Recent literature has shown that DSC may reduce the risk of postoperative mediastinitis and facilitate repeated surgical access for infection control in patients undergoing complex debridement procedures [6, 7]. Moreover, the choice of prosthetic material in such cases is crucial. Stentless bioprostheses, such as the Freestyle valve, have demonstrated favorable outcomes

in destructive aortic root infections due to their anatomical conformity and resistance to reinfection compared to homografts [8].

This case report presents a patient with prosthetic aortic root endocarditis and a paravalvular abscess who underwent successful treatment through radical debridement, stentless valve implantation, and delayed open chest closure. To our knowledge, this is one of the few reported cases where DSC was strategically employed to facilitate staged debridement and improve postoperative outcomes in this setting.

Case presentation

Upon admission, a 60-year-old male patient presented with clinical manifestations of heart failure, including dyspnea, along with a high fever of 39.9°C of unknown origin that had persisted for several days. Additionally, he exhibited signs of neurological impairment, characterized by speech difficulties and disorientation, which prompted his visit to the emergency department. Patient had undergone aortic valve (AV) reimplantation operation (David surgery) 10 years before.

Lab studies showed elevated inflammatory markers: White blood cell count: 14.2×109/L, C-reactive protein: 298 mmol/L, Procalcitonin: 19.5 mcg/L, D-dimer: 17.26 mg/L.

Transesophageal echocardiography in the projection of the non-coronary cusp showed an infiltrate about 5 x 3 cm where no blood flow is observed in the cavity (Figure 1). Additionally, the infiltrate extended into the interatrial septum, with the anterior portion thickened to 1.04 cm.

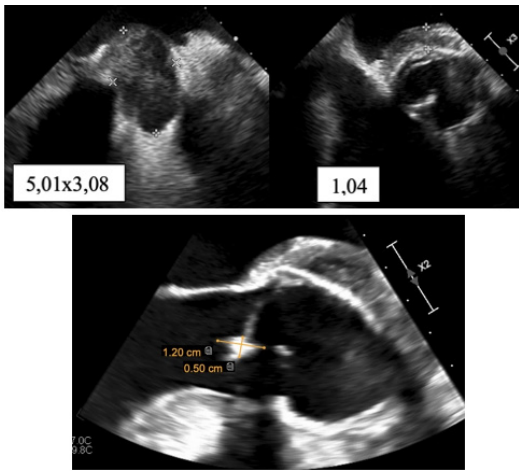

Figure 1 – Echocardiographic features of aortic root abscess with vegetation on the right coronary cusp

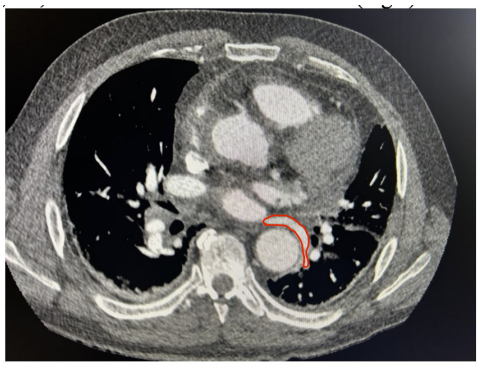

Figure 2 – CT features of aortic root abscess. Red outline depicts the paravalvular abscess cavity surrounding the aortic root

SpliceAI visualization showing predicted splicing alterations for the BRCA2 variant NM _ 000059.4(BRCA2):c.7008-2A>T

In the projection of the right coronary cusp, small additional formations were observed, one of which was elongated and protruded into the left ventricular outflow tract. The right coronary cusp exhibited thickened edges and reduced mobility. Aortic valve insufficiency was characterized by two regurgitant jets, graded as moderate to severe. The vena contracta measured 5 mm, with the jet directed toward the anterior mitral valve (MV) leaflet. MV insufficiency was assessed as mild to moderate.

Contrast-enhanced computed tomography (CT) revealed severe tricuspid AV regurgitation with mobile vegetation on the free margin of the right coronary cusp, along with an aortic root abscess measuring ~3.1 × 2.5 cm. (Figure 2).

Moreover, a brain CT scan revealed subacute or old ischemia in the left superior cerebellar artery and left posterior cerebral artery territories. Specialized stroke treatment was not administered due to the time elapsed since symptom onset.

Results from three initial sets of blood cultures obtained at admission confirmed the growth of Staphylococcus aureus at a concentration of 10^5 CFU/mL. Empirical antibiotic therapy was initiated, consisting of piperacillin–tazobactam 4.5 g four times daily, vancomycin 1 g twice daily (administered for 6 days), oxacillin 2 g six times daily (administered for 26 days), and gentamicin 280 mg once daily (administered for 7 days). The therapeutic response varied across the administered agents.

The patient underwent explantation of the infected Valsalva prosthesis with thorough debridement of surrounding native infected aortic root tissues, infiltrated tissue of right atrium, pulmonary artery and right ventricle outflow tract, left atrium roof, AV groove between right atrium and right ventricle, following right coronary artery.

For the restoration of aortic root anatomy, the stentless bioprosthesis Freestyle N27 (Medtronic Inc., Minneapolis, MN, USA) was implanted in full root fashion with subsequent reconstruction of the left ventricular (LV) outflow tract using continuous 4-0 Prolene sutures. Special care was taken to ensure a smooth transition between the bioprosthesis and native tissue to preserve laminar flow dynamics.

The patient was weaned off cardiopulmonary bypass without difficulty; however, due to the extensive spread of the infiltrative process to the surrounding heart areas, we decided to leave the chest open for later debridement.

The patient's chest wound was covered with sterile wound dressings and a soft iodine-impregnated membrane, with mediastinal drainage in place until definitive sternal closure. For mediastinal re-exploration and irrigation with povidone-iodine (Betadine) solution, the patient was transferred to the operating theater on two occasions to perform meticulous washing and debridement of all infected areas.

Sternal closure performed five days later, and the patient was extubated on the 7th postoperative day. The patient's postoperative course was uneventful. Intravenous administration of broadspectrum antibiotics was initiated and maintained for 6 weeks after the surgery.

Follow-up visits at five and seven months after the operation showed good AV function and no abscess in the pericardium. At the latest follow-up 3 yeas post-surgery, the patient remained asymptomatic with normal echocardiographic parameters and no signs of reinfection or prosthesis dysfunction. It is recommended to continue the same treatment. Infective endocarditis prophylaxis with amoxicillin before dental procedures and mitigation of purulent skin infection were advised.

Discussion

Mediastinitis and endocarditis account for approximately 3% of cases in one-year follow-up after cardiac surgery [4]. Moreover, osteomyelitis is one of the major non-cardiac complication of infective endocarditis [9]. Intracardiac abscess is another significant complication of infective endocarditis and associated in about 15% to 30% of cases [1, 2]. Paravalvular abscess often indicate severe valvular dysfunction and is linked to higher rates of morbidity and mortality [10]. Standard surgical techniques often require modifications to effectively manage the infection spread associated with this condition. Here we present the case of personalised surgical management of infective endocarditis with paravalvular abscess.

To the best of our knowledge, this is the first time when DSC was utilised to address meticulous debridement infected surrounding tissue and repeated irrigation [11]. In cardiac surgery routine DSC involves temporarily leaving the chest open after surgery, because of hemodynamic instability, bleeding/coagulopathy, cardiac oedema, and arrhythmias. Moreover, in our case DSC also helps to prevent postoperative complications such as mediastinitis. Therefore, managing prosthetic infective aortic root endocarditis often necessitates complex surgical interventions.

In cases of prosthetic aortic root endocarditis with extensive annular destruction, several surgical strategies are available, each with specific advantages and limitations. Homograft aortic root replacement has traditionally been considered the gold standard due to its infection resistance and favorable hemodynamic profile, but its availability is limited and long-term durability remains a concern [12]. Xenopericardial patch reconstruction and aortic root replacement with stentless bioprostheses, such as the Freestyle valve, have emerged as viable alternatives, offering easier handling, off-the-shelf availability, and comparable infection control in high-risk patients [8, 13]. In select centers, mechanical composite grafts are still used, particularly in younger patients, although they carry a higher risk of reinfection and require lifelong anticoagulation [14]. In our case, the use of a stentless Freestyle bioprosthesis allowed for extensive root reconstruction and optimal integration with surrounding debrided tissue. The addition of delayed sternal closure provided an important opportunity for staged debridement and infection control, highlighting the importance of tailoring the surgical strategy to the anatomical and infectious burden. Ultimately, the choice between homograft, xenograft, or prosthetic valve must be guided by patient factors, tissue destruction, and surgical expertise [6].

Treatment of endocarditis relies on observational studies and expert opinions [3]. DSC can be a valuable tool in the perioperative management of patients who cannot tolerate immediate chest closure, allowing for stabilization and reducing the risk of complications [11]. In our study, DSC was used for targeted removal of affected tissue, as demonstrated in cases by Kondov et al. and Gott et al., where DSC effectively managed complications like diffuse bleeding and delayed sternal closure [13, 15]. These approaches highlight DSC's utility in addressing complex postoperative challenges.

DSC is an established strategy in both adult and pediatric cardiac surgery, typically employed in the setting of hemodynamic instability, coagulopathy, or myocardial edema. However, its role in managing complex infective endocarditis with paravalvular abscess remains less commonly described. Our case demonstrates the utility of DSC not only as a temporizing measure, but as an integral part of a staged surgical debridement protocol aimed at infection control. Recent studies have emphasized the benefit of DSC in reducing mediastinal infections and facilitating repeated access to the surgical field for irrigation and inspection, particularly in high-risk patients with prosthetic valve endocarditis or extensive abscesses [5, 8]. Additionally, stentless bioprostheses, such as the Freestyle valve used in our patient, have shown favorable outcomes in destructive aortic root infections due to their adaptability and lower reinfection rates compared to homografts [16]. These modern approaches support the rationale for adopting personalized surgical strategies, especially in cases where standard one-stage procedures might be insufficient. As suggested by recent literature, individualized, flexible interventions—such as DSC combined with aggressive debridement—may offer improved long-term outcomes in this challenging cohort [7, 17].

Table 1 Timeline

Date	Intervention	
01.2013	Aortic valve reimplantation (David procedure)	
01.2021	Day 1 - Stentless bioprosthesis Freestyle implantation Day 2, Day 4 - Washing and debridement of all infected areas Day 5 - Sternal closure Day 7 - Extubation 6 weeks of antibiotic therapy	
07.2023	1st follow-up visit	• good AV prosthesis function • no abscess
09.2023	2nd follow-up visit	
01.2024	3-year follow-up visit	

Conclusion

This case demonstrates the successful use of delayed open chest closure as a strategic adjunct to complex surgical debridement in prosthetic aortic root endocarditis. Our tailored approach resulted in infection control, preserved cardiac function, and favorable long-term outcomes.

Author Contributions: Conceptualization, R. B.; methodology, S. A. and P. J.; validation, R. B. and E. R.; formal analysis, A. M. and Y. O.; investigation, S. A. and D. K.; resources, R. B.; data curation, Y. O.; writing – original draft preparation, S. A.; writing – review and editing, Y. O and D. K; visualization, E. R.; supervision, R. B. All authors have read and agreed to the published version of the manuscript.

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Informed Consent: Ethical approval was not sought for the present study because no identifiable images or information were used.

Data availability statement: All data generated or analyzed during this study are included in this published article

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