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The Impact of Antiepileptic Therapy on Anesthetic Requirements and Postoperative Pain in Patients with Pharmacoresistant Focal Epilepsy: a Retrospective Study

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ABSTRACT

Background: Patients with pharmacoresistant focal epilepsy (PRE) are exposed to long-term antiepileptic drug (AED) therapy, which may influence anesthetic pharmacology and postoperative pain. However, evidence regarding the perioperative effects of chronic AED treatment remains scarce. The study aims to investigate the association of AED regimen, epilepsy duration, anesthetic consumption, and surgery with postoperative pain in patients with pharmacoresistant focal epilepsy.

Methods: The retrospective cohort study included 92 participants with pharmacoresistant focal epilepsy who underwent neurosurgical intervention between 2019 and 2024. Patients were stratified according to AED regimen into carbamazepine monotherapy (n=27), carbamazepine plus levetiracetam (n=44), and other AED combinations (n=21). Intraoperative anesthetic consumption, postoperative visual analogue scale (VAS) pain scores, analgesic requirements, and perioperative outcomes were compared. Predictors of postoperative pain were evaluated using multivariable logistic regression analysis.

Results: Patients receiving combination AED therapy had significantly longer epilepsy duration than those receiving carbamazepine monotherapy (median 16–18 vs. 8 years, p<0.01). Intraoperative consumption of fentanyl, propofol, sevoflurane, and neuromuscular blocking agents did not differ among treatment groups (all p>0.05). Postoperative pain occurred in 58.7% of patients, with similar VAS trajectories, analgesic requirements, and time to first analgesic request across groups. Surgery type was not associated with postoperative pain in either univariate or multivariable analyses. Multivariable logistic regression demonstrated no independent association between postoperative pain and AED regimen, intraoperative fentanyl dose, sex, or surgical procedure (all p>0.05). Sensitivity analysis limited to patients receiving carbamazepine or levetiracetam as the primary AED yielded consistent results, although levetiracetam showed a non-significant trend toward lower odds of postoperative pain compared with carbamazepine (adjusted OR 0.47, 95% CI 0.19–1.18).

Conclusions: Long-term AED regimen, epilepsy duration, and surgery type were not independently associated with anesthetic requirements or early postoperative pain in patients undergoing surgery for pharmacoresistant focal

epilepsy. Standardized perioperative anesthesia and postoperative analgesia appear to provide effective pain control regardless of chronic AED therapy. Larger prospective multicenter studies are needed to confirm these findings and evaluate the perioperative effects of individual AEDs.

Keywords: pharmacoresistant focal epilepsy; antiepileptic drugs; carbamazepine; levetiracetam; postoperative pain; anesthesia; epilepsy surgery; neurosurgery.

Introduction

Epilepsy is a neurological condition affecting approximately 70 million people worldwide, with a prevalence of 4 to 10 cases per 1,000 individuals [1,2]. Despite the ongoing development of various antiepileptic drugs (AEDs), drug resistance remains a significant issue. About one-third of epilepsy patients experience pharmacoresistant epilepsy (PRE) [3,4]. The most common type linked to treatment resistance is focal epilepsy, which represents over half of all cases [4]. Focal PRE refers to resistance to two or more AEDs and is often associated with a long disease duration. Some PRE patients are considered candidates for surgical intervention [5].

Over the last decade, epilepsy surgery has advanced with improvements in neuroimaging, high-resolution structural and functional mapping, intraoperative neuronavigation, and minimally invasive approaches. Nonetheless, challenges persist in personalized perioperative management, including optimizing anesthesia, maintaining hemodynamic stability, and managing antiepileptic drug interactions [1,6]. When a patient with pharmacoresistant epilepsy opts for surgery, care transitions from a neurologist-epileptologist to a multidisciplinary team that includes anesthesiologists and other specialists [6,7]. Long-term use of antiepileptic drugs in these patients alters neurological networks, affecting cognitive function and anesthetic response during surgery [6,8]. These changes might indicate that there is individual variability in how patients respond to anesthetic and analgesic drugs, including differences in pharmacokinetics and pharmacodynamics, the potential for drug interactions, and variations in postoperative pain levels [9].

The current range of antiepileptic drugs includes both microsomal enzyme inducers like carbamazepine and agents with minimal drug interactions such as levetiracetam. Cytochrome P 450 inducers accelerate the metabolism of opioids and certain anesthetic agents, thereby increasing intraoperative needs and reducing the effectiveness of analgesics and anesthetics [10]. Data on how antiepileptic drugs affect the minimum alveolar concentration (MAC) of inhalational anesthetics are limited [11]. Drugs with a low potential for interactions, such as levetiracetam, are associated with fewer early postoperative complications like seizures, agitation, and delayed recovery, especially in neurosurgical patients [12]. In non- neurosurgical populations, these drugs show a potential opioid- sparing effect; however, evidence in focal pharmacoresistant epilepsy remains limited [12]. Combination therapy may pose risks of adverse interactions and cumulative side effects such as dizziness, drowsiness, and ataxia, which can worsen during the perioperative period [13]. Postoperative pain syndrome (PPS) is also a key consideration during perioperative management of patients undergoing epilepsy or brain tumor surgery [14]. Neurosurgical procedures, especially craniotomies, are linked to significant postoperative pain, which if poorly controlled, can lead to complications like hypertension, tachycardia, increased intracranial pressure, agitation, sleep disturbances, delayed awakening, and longer ICU stays [14,15]. Inadequate pain management after craniotomy may increase stress responses, raise opioid use, and impair neurocognitive recovery [15] . Post- craniotomy pain peaks within the first 24–48 hours, with the worst intensity on the first postoperative day, correlating with higher analgesic use, delayed extubation, and longer recovery times [14]. Despite progress in epilepsy surgery and available anesthetic guidelines, research on how antiepileptic drugs, epilepsy duration, and their characteristics influence anesthetic and analgesic needs and postoperative pain levels is limited [7].

However, most existing research has concentrated on seizure prevention rather than personalized perioperative pain management. Many studies evaluate how effective various AED regimens, such as monotherapy versus combination therapy, are at stopping intraoperative and early postoperative seizures, especially in patients undergoing craniotomy or awake surgery [14,15,16]. Exploring this topic could help predict optimal anesthetic and opioid doses, personalize multimodal pain relief, decrease practice variability, and promote quicker recovery [17,18].

Considering all the above-mentioned, the aim of this study is to assess the impact of antiepileptic therapy characteristics and epilepsy duration on anesthetic consumption and the severity of postoperative pain in neurosurgical patients with focal pharmacoresistant epilepsy.

Methods

Study design and data selection

An investigation was conducted at the Medical Center Hospital of the President's Affairs Administration of the Republic of Kazakhstan using a retrospective cohort design. Between 2019 and 2024, a total of 130 patients who underwent surgical treatment for pharmacoresistant focal epilepsy were analyzed. After applying the inclusion and exclusion criteria, 38 patients were excluded for failing to meet the study criteria or for incomplete clinical data. The final analysis included 92 patients.

Inclusion criteria:

– age ≥18 years;

– confirmed diagnosis of pharmacoresistant focal epilepsy;

– undergone neurosurgical intervention with available medical documentation;

– completed perioperative period (up to 48 hours postoperatively).

Exclusion criteria:

– patients with generalized forms of epilepsy;

– patients with incomplete data on key variables;

– presence of severe comorbid somatic conditions (e.g., decompensated respiratory diseases, respiratory failure, end-stage cardiovascular failure, cancer, stage IV hepatic or renal failure);

– massive intraoperative bleeding; repeat surgeries.

As part of the retrospective analysis, anesthetic records of the patients included in the study were reviewed. Patients received general anesthesia according to the standardized protocol in the neurosurgery department.

Anesthesia induction started with intravenous propofol at 2 mg/kg and fentanyl at 2 μg/kg. Additional fentanyl was given every 20–30 minutes at 1–2 μg/kg, adjusted based on clinical signs of anesthesia depth. The adequacy of anesthesia was evaluated using clinical signs, hemodynamic responses (MAP, HR), and, when available, bispectral index (BIS) monitoring. Stable hemodynamics within ±20% of baseline and BIS values between 40 and 60 were considered indicative of sufficient anesthesia. Extra fentanyl doses were administered as needed to maintain these parameters.

Neuromuscular relaxation was achieved using rocuronium bromide at 0.6 mg/kg, followed by IV boluses of pipecuronium bromide at 0.01–0.02 mg/kg as needed. Anesthesia was maintained with sevoflurane at a MAC of 1.0–1.2%, with fresh gas flow set at 25–30% of the patient's minute ventilation, based on ideal body weight. The effectiveness of anesthesia was monitored using hemodynamic parameters, including invasive arterial blood pressure (IABP), heart rate (HR), and ECG. Perioperative respiratory status and oxygenation were assessed using arterial blood gases, capnography, and continuous pulse oximetry (SpO_2), with SpO_2 maintained≥93%. Intravenous fluids were administered per local protocol to support tissue perfusion. In cases of hemodynamic instability, defined as a mean arterial pressure (MAP) below 65 mmHg despite adequate fluid resuscitation, vasopressor support with titrated intravenous norepinephrine was initiated and continued until the target pressure was achieved.

Recorded Parameters for Analysis

Intraoperative parameters: total fentanyl dose (μg) administered during surgery; total volume of inhalational anesthetic (sevoflurane, mL); duration of anesthesia (minutes).

Postoperative pain assessment: visual analog scale (VAS) pain scores recorded at 2, 4, 6, 8, and 12 hours postoperatively; total dose of analgesics administered within the first 24 hours after surgery; and time to first request for analgesia. Postoperative analgesia comprised a three-step pain management protocol based on World Health Organization (WHO) guidelines [19]. The protocol was introduced into routine clinical practice alongside the standardized VAS scale. The analgesic protocol consisted of:

– Step 1: VAS 1–4 (mild pain) – nonsteroidal anti-inflammatory drugs (NSAIDs): intravenous Intrafen 800 mg or intramuscular ketoprofen 100 mg;

– Step 2: VAS 4–6 (moderate to severe pain) – synthetic opioids (tramadol 100 mg IM) and/or NSAIDs (Intrafen 800 mg IV or ketoprofen 100 mg IM);

– Step 3: VAS 7–10 (severe pain) – opioids (morphine 10 mg IM) and NSAIDs.

Additional recorded parameters: age, sex, body mass index (BMI); type of neurosurgical procedure; duration of epilepsy; duration of mechanical ventilation; Richmond Agitation-Sedation Scale (RASS) score [20]; length of stay in the intensive care unit (ICU); and presence of postoperative seizures.

Patients were stratified by antiepileptic drug therapy and divided into three groups: Group I, carbamazepine monotherapy; Group II, carbamazepine and levetiracetam combination; and Group III, other double antiepileptic drug combination.

Ethics

This study was conducted in accordance with an expanded protocol for the secondary analysis of de-identified medical data, with ethical approval from the Hospital's Local Bioethics Committee (Institutional Review Boards), as stated in Protocol No. 4, dated December 20, 2024, and in accordance with the Declaration of Helsinki.

Statistical analysis

Statistical analyses were conducted in SPSS Statistics (version 26) and JASP. Normality of variables was assessed with the Shapiro–Wilk test. For non-normally distributed data, the Mann–Whitney U test was used. Categorical variables were analyzed with the chi-squared test or Fisher's exact test, as appropriate. Multivariable logistic regression was performed to identify factors associated with postoperative pain. A p-value of <0.05 was considered statistically significant.

Results

Clinical and demographic characteristics of patients

92 patients who underwent surgery for pharmacoresistant focal epilepsy were included in the study. The mean age was 32.5 ± 8.0 years. Of the cohort, 54 (58.7%) were male, and 38 (41.3%) were female. The mean BMI value was 23.9 ± 4.7 kg/m². The distribution of surgical procedures was as follows: lobectomy – 75.0%, tumor resection – 10.9%, cortical dysplasia resection – 4.3%, cavernous hemangioma excision – 4.3%, arteriovenous malformation resection – 3.3%, ulegyria resection – 2.2%.

Carbamazepine was the most commonly used main antiepileptic medication (57/92, 62.0%) across all groups, followed by levetiracetam (32/92, 34.8%), valproic acid (2/92, 2.2%), and lamotrigine (1/92, 1.1%). Among patients on combination therapy (n = 65), additional antiepileptic medications were distributed as follows: carbamazepine (23/65, 35.4%), levetiracetam (21/65, 32.3%), lamotrigine (10/65, 15.4%), valproic acid (6/65, 9.2%), and topiramate (5/65, 7.7%). The results of patient stratification by antiepileptic therapy are presented in Table 1.

Table 1 Main AED and Additional AED by Treatment Group

Variable	Carbamazepine (n=27)	Carbamazepine + Levetiracetam (n=44)	Other AED (n=21)
Main AED			
Carbamazepine	27 (100.0%)	21 (47.7%)	9 (42.9%)
Levetiracetam	-	23 (52.3%)	9 (42.9%)
Valproic acid	-	-	2 (9.5%)
Lamotrigine	-	-	1 (4.8%)
Additional AED			
None	27 (100.0%)	-	-
Carbamazepine	-	23 (52.3%)	-
Levetiracetam	-	21 (47.7%)	-
Lamotrigine	-	-	10 (47.6%)
Valproic acid	-	-	6 (28.6%)
Topiramate	-	-	5 (23.8%)

Daily doses of the main antiepileptic medication varied across the groups as follows: 800 (400–800) mg/day in the carbamazepine group, 1000 (800–1625) mg/day in the carbamazepine + levetiracetam group, and 1000 (400–1500) mg/day in the other AED group. Pairwise group comparison with Bonferroni adjustment revealed a significantly lower antiepileptic medication dose in the carbamazepine group than in the carbamazepine + levetiracetam group ($p < 0.001$). There was no such difference between carbamazepine and other AED groups. Among patients on combination treatment, where an

Table 2 Comparison of the clinical and demographic characteristics among the groups

Parameter	Group I: Carbamazepine (N=27)	Group II: Carbamaze-pine + Levetiracetam (N=44)	Group III: Other AED Combinations (N=21)	p-value
Demographic and Clinical Data				
Age, years,	34.0 (27.5 – 38.5)	30.5 (26.0 – 35.25)	32 (29.0–38.0)	0.36[1-2] 0.43[2-3] 0.86[1-3]
Gender, n (%)				
Male	18 (66.7%)	24 (54.5%)	12 (57.1%)	0.594
Female	9 (33.3%)	20 (45.5%)	9 (42.9%)	
BMI (kg/m^2)	22.9 (22.11–28.46)	21.99 (19.11–26.08)	24.57 (20.2–28.2)	0.047[1-2*] 0.122-3 0.98[1-3]
Main Antiepileptic Drug (mg/day)	800 (400–800)	1000 (800–1625)	1000 (400–1500)	0.000021-2 0.1972-3 0.1121-3
Additional Antiepileptic Drug (mg/day)		1000 (400–1812.5)	350 (200–750)	0.00042-3
Epilepsy duration, years	8.0 (3.5–13.5)	16.0 (12.0–24.25)	18.0 (10.0–22.0)	0.0011-2 0.7412-3 0.0041-3
ASA	2 (2–2)	2 (2–2)	2 (2–2)	0.821-2 0.722-3 0.881-3
ASA I	0	1	0	
ASA II	26	41	2	
ASA III	1	2	1	
Anesthesia duration, min	555 (460–625)	555 (480–611.25)	555 (518.4–607.6)	0.821-2 0.512-3 0.651-3
Mechanical ventilation in ICU, min	65.0 (52.5–117.5)	100.0 (58.75–136.25)	60.0 (45.0–100.0)	0.271-2 0.0362-3* 0.261-3
RASS score	−2.0 (−3.0 to −2.0)	−2.5 (−3.0 to −2.0)	−2.0 (−3.0 to −2.0)	0.711-2 0.212-3 0.351-3
ICU stay, hours	15.0 (13.5–16.0)	14.0 (13.0–16.0)	14.0 (12.5–16.0)	0.771-2 0.522-3 0.361-3
Hospital stay, days	12.0 (8.0–14.5)	12.5 (10.0–16.0)	16.0 (14.0–19.0)	0.161-2 0.012-3 0.0021-3
Post-op seizures, incidence	1/27 (3.7%)	4/44(9.1%)	5/21 (23.8%)	0.6431-2** 0.1352-3** 0.0731-3**
Anesthetic Consumption Parameters				
Fentanyl dose, µg/kg	14.94 (12.90–18.18	15.56 (11.30–20.56	14.08 (11.24–17.54)	0.661-2 0.322-3 0.551-3
Propofol dose, mg,	132.0 (120.0–164.0)	131.0 (107.5–146.5)	142.0 (114.0–162.0)	0.121-2 0.422-3 0.521-3
Inhalational anesthetic, mL/hr	11.01 (8.45–14.09)	10.17 (8.19–12.49)	10.59 (8.81–14.29)	0.341-2 0.462-3 0.941-3
Pipecuronium, mg	12.0 (12.0–13.0) n=24	12.0 (8.0–16.0) n=35	10 (9.26–12.04) n=19	0.571-2 0.332-3 0.111-3
Rocuronium, mg	200 (175–200) n=3	150 (100–150) n=9	150 (100–200) n=2	0.221-2 1.002-3 1.001-3

*Me – median, (Q1–Q3) – interquartile range, p – Mann-Whitney test, *Bonferroni adjustment did not reveal a statistically significant difference. **Fisher's exact test. For gender distribution χ^2 ($\chi^2 = 1.04$, $p = 0.594$) was applied.*

additional antiepileptic medication is added, the dose of the additional antiepileptic medication was significantly higher in the carbamazepine + levetiracetam group, at 1000 (400–1812.5) mg/day, compared with the other AED group at 350 (200–750) mg/day ($p < 0.001$). Table 2 represents demographical, preoperative and intraoperative parameters across the groups.

The median age ranged from 30.5 to 34 years and did not differ significantly among the groups ($p > 0.05$). The gender distribution was also comparable across groups. The median BMI was higher in patients receiving combination therapy with other antiepileptic drugs than in those receiving carbamazepine monotherapy ($p = 0.047$), although after Bonferroni adjustment, the difference was not statistically significant ($p = 0.140$).

Disease duration varied significantly across groups ($p = 0.001$). The shortest duration of epilepsy was observed in patients receiving carbamazepine monotherapy, whereas those on combined therapy with levetiracetam and other AEDs had a significantly longer disease duration ($p = 0.011$), 8, 16, and 18 years, respectively. Mann–Whitney U tests with Bonferroni correction showed that the first group (carbamazepine group) had shorter disease duration than the second group (carbamazepine + levetiracetam; $p = 0.0021$) and the third group (other AED combinations; $p = 0.011$). The latter two groups did not differ in disease duration ($p > 0.05$). According to the ASA physical status classification system, most patients were classified as ASA class II ($p > 0.05$).

Anesthesia duration was comparable among the three groups, with values of 555 (460–625) min, 555 (480–611.25) min, and 555 (518.4–607.6) min, respectively ($p > 0.05$). Mechanical ventilation time tended to be higher in the carbamazepine + levetiracetam group, with the median 100.0 (60.0–135.0) min, in comparison to the carbamazepine group, with the median 65.0 (53.8–116.3) min, and the other AED group, with the median 60.0 (45.0–92.5) min. However, the difference among the groups did not reach statistical significance ($p=0.07$). Pairwise analysis demonstrated a statistically significant difference between the second and third groups ($p = 0.036$), but after Bonferroni correction, this significance was not maintained. The level of sedation, assessed using the RASS, was comparable across all groups ($p > 0.05$), suggesting similar arousal levels.

ICU stay did not differ significantly between the groups, with values of 15, 14, and 14 hours, respectively ($p > 0.05$). However, there was a difference in the overall hospital length of stay across the groups ($p = 0.0033$). The overall hospital stay of the first group comprised 12.0 (8.0–14.5) days, 12.5 (10.0–16.0) days for the second group, and 16.0 (14.0–19.0) days for the last group, illustrating the statistically significant discrepancy between the first two groups and the third one ($p = 0.006$ and $p = 0.026$, respectively). The incidence of postoperative seizures was relatively low across groups. Although the postoperative seizure rate was higher in the third group, 5/21 (23.8%), compared to the first group, 1/27 (3.7%), and the second group, 4/44 (9.1%), the difference did not reach statistical significance ($p = 0.074$).

Intraoperative consumption of the main anesthetics, analgesics, and neuromuscular blocking agents was comparable across the three groups. Induction dose of propofol was 132.0 (120.0–164.0) mg, 131.0 (107.5–146.5) mg, and 142.0 (114.0–162.0) mg in the carbamazepine, carbamazepine + levetiracetam, and other AED groups, respectively, demonstrating similar distribution ($p = 0.27$). Median values of inhalational anesthetic dose were 11.01 (8.45–14.09) mL/hour in the carbamazepine group, 10.17 (8.19–12.49) mL/hour in the carbamazepine + levetiracetam group, and 10.59 (8.81–14.29) mL/hour in the other AED group, illustrating also comparable consumption. Fentanyl doses adjusted for body weight showed no significant differences among the groups and comprised 14.94 (12.90–18.18), 15.56 (11.30–20.56), and 14.08 (11.24–17.54) mcg/kg, respectively. Due to prolonged surgery time, pipecuronium was preferred, and there was no difference in its consumption across groups ($p = 0.317$), although the median dose for the third group tended to be lower. Rocuronium was administered to a patient population with small sample sizes across three groups: n = 3, 9, and 2, respectively. The median dose of rocuronium in the carbamazepine group was the highest, at 200 (175–200) mg, while in the second and third groups it was 150 (100–150) mg and 150 (100–200) mg, respectively.

Postoperative pain assessment of patients

Postoperative pain was assessed using the VAS immediately after surgery, at 2, 4, 6, 8, and 12 hours postoperatively. The need for additional analgesia and the time to the first request for pain medication were also recorded. The results are presented in Table 3.

Postoperative pain was observed in more than half of the patient population across the groups, comprising 66.7%, 52.2%, and 66.7%, respectively. The differences in pain occurrence among the groups were not statistically significant ($p = 0.27$), indicating comparable pain severity in the early postoperative

Table 3 Assessment of postoperative pain syndrome across the treatment groups

Parameter	Group I: Carbamazepine, N=27	Group II: Carbamazepine + Levetiracetam, N=44	Group III: Other AED combinations, N=21	p-value
Patients with pain, n (%)	18 (66.7%)	22 (52.2%)	14 (66.7%)	0.27**
Postoperative pain (VAS)				
2 hours after surgery (points)	1.0 (0.0–2.5)	2.0 (1.0–3.0)	3.0 (2.0–4.0)	$0.098^{1\text{-}2}$ $0.18^{2\text{-}3}$ $0.028^{1\text{-}3*}$
4 hours after surgery (points)	3.0 (2.0–8.0)	3.0 (2.0–4.0)	3.0 (2.0–4.0)	$0.42^{1\text{-}2}$ $0.92^{2\text{-}3}$ $0.60^{1\text{-}3}$
6 hours after surgery (points)	2.0 (2.0–3.0)	3.0 (2.0–3.0)	3.0 (3.0–3.0)	$0.55^{1\text{-}2}$ $0.20^{2\text{-}3}$ $0.05^{1\text{-}3}$
8 hours after surgery (points)	3.0 (2.0–3.0)	3.0 (2.0–3.0)	3.0 (2.0–3.0)	$0.70^{1\text{-}2}$ $0.15^{2\text{-}3}$ $0.29^{1\text{-}3}$
12 hours after surgery (points)	2.0 (0.5–2.0)	1.0 (1.0–2.0)	2.0 (1.0–2.0)	$0.74^{1\text{-}2}$ $0.79^{2\text{-}3}$ $0.97^{1\text{-}3}$
Time of analgesic requirement after surgery (hours)	4.0 (4.0–4.0)	4.0 (2.25–5.75)	3.0 (2.0–4.75)	$0.99^{1\text{-}2}$ $0.24^{2\text{-}3}$ $0.17^{1\text{-}3}$
Frequency of additional analgesia after surgery with morphine, n (%)	8 (29.6%)	7 (15.9%)	7 (33.3%)	
Frequency of additional analgesia after surgery with tramadol, n (%)	4 (14.8%)	7 (15.9%)	4 (19.0%)	
Frequency of additional analgesia with NSAID after surgery, n (%)	6 (22.2%)	8 (18.2%)	3 (14.3%)	

*Me – median, (Q1–Q3) – interquartile range, p – Mann-Whitney test, ** Chi-square test.*

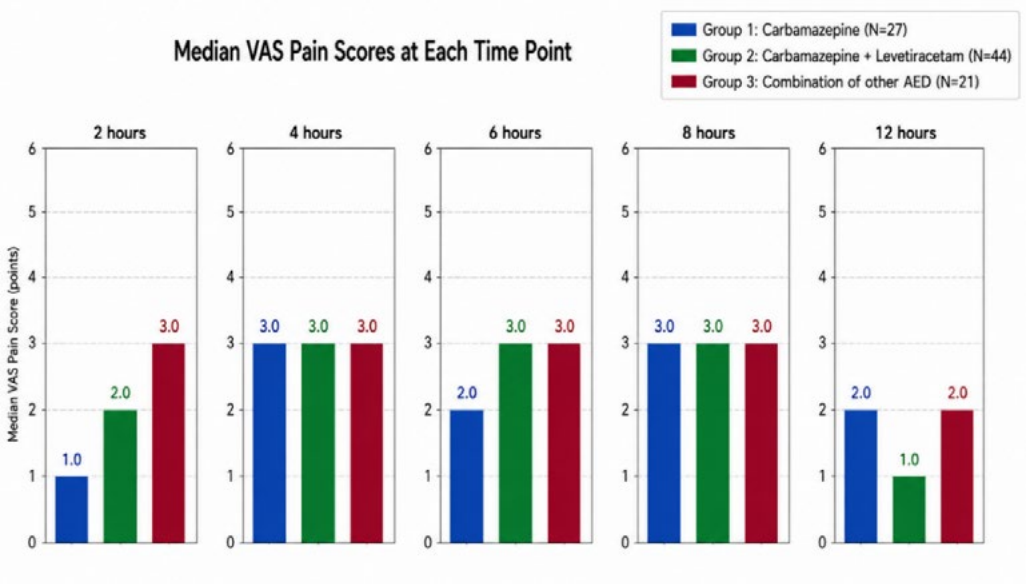

Figure 1 – Trend of median VAS over 12 hours in the postoperative period

period. When compared between the second and the third group, due to additional drug combination, patients in the third group had twice the odds of experiencing post-operative pain compared with those in the second group. However, the difference was not statistically significant (Fisher's exact test, p = 0.287). The data is illustrated in supplementary table S1.

Pain intensity measured by VAS at 2 hours postoperatively varied among the groups, being 1.0 (0.0–2.5), 2.0 (1.0–3.0), and 3.0 (2.0–4.0) in the carbamazepine, carbamazepine + levetiracetam, and other AED groups, respectively. The overall difference in pain intensity was borderline (p = 0.05). Pairwise comparison showed that the carbamazepine group had a lower pain intensity score than the other AED group (p=0.028); however, after Bonferroni adjustment, the discrepancy was not statistically significant (p=0.084).

At 4 hours, pain intensity among the groups was as follows: 3.0 (2.0–8.0), 3.0 (2.0–4.0), and 3.0 (2.0–4.0), indicating a similar distribution of pain intensity (p=0.715). At 6 hours postoperatively, median VAS scores were 2.0 (2.0–3.0), 3.0 (2.0–3.0), and 3.0 (3.0–3.0) in the carbamazepine, carbamazepine + levetiracetam, and other AED groups, respectively. Pairwise comparison showed lower pain intensity in the first group than in the third group (p = 0.05); however, after Bonferroni correction, the difference did not reach statistical significance (p = 0.075). At 8 hours postoperatively, the VAS pain score was 3.0 (2.0–3.0) in all groups (p = 0.322). While at 12 hours, the VAS pain score was comparable (p = 0.932) and tended to decline, comprising 2.0 (0.5–2.0), 1.0 (1.0–2.0), and 2.0 (1.0–2.0), respectively. Figure 1 demonstrates the VAS pain intensity trend over 12 postoperative hours.

The median number of hours to the first postoperative analgesic request was lower in the third group, 3.0 (2.0–4.75), than in the first, 4.0 (4.0–4.0), and the second, 4.0 (2.25–5.75) groups, although no statistically significant differences were noted (p = 0.35). Regarding additional analgesia, its frequency (p = 0.332) and the types of analgesia (morphine, tramadol, and NSAIDs; p = 0.68) were also comparable across the groups.

To identify predictors of postoperative pain, we performed a univariate analysis of preoperative and intraoperative factors. No significant associations were found with age, gender, BMI, epilepsy duration, main antiepileptic drug doses, anesthesia and surgery duration, anesthetic and analgesic doses, pipecuronium, ventilation time, and RASS parameter (Table S2, supplementary materials). There was no statistically significant difference in the proportion of patients with post-operative pain considering surgery types (Fisher test, p = 0.73, Table S3 supplementary material). To stabilize the statistical model, we combined rare procedures in the common category named “other procedures” which included cortical dysplasia resection (5/92), cavernous hemangioma excision (4/92), arteriovenous malformation resection (3/92), ulegyria resection (2/92). Post-operative pain occurred in 41/69 patients after lobectomy, 7/14 patients after other surgery, and 6/9 after tumor resection.

To identify more robust associations for predicting postoperative pain risk factors, multivariable logistic regression was performed on clinically relevant parameters. Considering 54 events of pain out of 92, we selected a few parameters for stable configuration of the model. Table 4 represents the results. The model included intraoperative fentanyl, antiepileptic drug regimen across three groups and type of the surgery. Carbamazepine monotherapy and lobectomy were used as references.

The model demonstrated no statistically significant independent predictors of post-operative pain. Nevertheless, compared with carbamazepine monotherapy, treatment with carbamazepine in combination with levetiracetam was associated with lower odds of post-operative pain (OR 0.46, 95% CI 0.16–1.31, p = 0.147). Patients receiving other AED medications had comparable odds of postoperative pain with carbamazepine monotherapy (OR 1.00, 95% CI 0.28–3.59, p = 0.998). Considering surgery, patients undergoing tumor resection had 42% higher odds of postoperative pain compared to the lobectomy surgery patients (OR 1.33, 95% CI 0.28–6.23,

Table 4 Multivariable regression analysis considering presence of post-op pain and intraoperative parameters

Predictor	Adjusted OR	95% CI	p-value
Fentanyl (mcg/kg)	1.04	0.96–1.13	0.316
Carbamazepine + Levetiracetam vs. Carbamazepine	0.46	0.16–1.31	0.147
Other AED vs. Carbamazepine	1.00	0.28–3.59	0.998
Other surgery vs. Lobectomy	0.74	0.21–2.58	0.631
Tumor resection vs. Lobectomy	1.33	0.28–6.23	0.717

Table 5 Multivariable regression analysis considering presence of post-op pain and other parameters

Variable	Adjusted OR	95% CI	p-value
Male vs Female	0.87	0.34–2.26	0.781
Fentanyl (per mcg/kg)	1.02	0.94–1.12	0.599
Levetiracetam vs Carbamazepine	0.47	0.19–1.18	0.109
Other surgery vs. Lobectomy	0.59	0.17–2.07	0.413
Tumor resection vs. Lobectomy	1.34	0.29–6.15	0.703

p = 0.717). On the contrary, patients undergoing cortical dysplasia resection, cavernous hemangioma excision, arteriovenous malformation resection, and ulegyria resection demonstrated 23% lower odds of post-operative pain than those undergoing lobectomy surgery (OR 0.74, 95% CI 0.21–2.58, p = 0.717).

Carbamazepine (n = 57) and levetiracetam (n = 32) were the most commonly used antiepileptic drugs as primary treatment options (89/92), whereas the sample sizes for valproic acid (n = 2) and lamotrigine (n = 1) were extremely small. We decided to investigate the incidence of postoperative pain among patients receiving two most common main antiepileptic medication. Thus, we performed the second multivariable logistic regression as a sensitivity analysis and in addition, included gender parameter to investigate the differences in pain perception, since difference in the postoperative analgesic requirements have been reported prior [21]. Table 5 demonstrates the results.

After adjustment for selected parameters, no independent predictor of postoperative pain was identified in the secondary analysis (p > 0.05 for all variables). Although non-significant, male patients showed lower odds of post-op pain than females (OR 0.87, 95% CI 0.34–2.26, p = 0.781), and patients receiving levetiracetam demonstrated lower odds of post-op pain compared to those receiving carbamazepine antiepileptic medication as a primary treatment (OR 0.47, 95% CI 0.19–1.18, p = 0.109). The odds of developing postoperative pain assuming a surgery type preserve similar trends as during primary analysis. The consistency of the findings between the primary and sensitivity analyses suggests that the exclusion of the three patients receiving other main AEDs did not significantly influence the study conclusions.

When patients were stratified by the primary antiepileptic drug. It was shown that participants receiving levetiracetam had a longer duration of epilepsy than those receiving carbamazepine: 18.0 (12.0–24.0) vs. 12.0 (6.0–18.0) years, p = 0.028. No other associations were observed.

Discussion

The retrospective cohort study evaluated how prolonged antiepileptic therapy interacts with general anesthesia agents and the potential effect of antiepileptic therapy on postoperative pain in patients with pharmacoresistant focal epilepsy. The findings show that patients receiving dual therapy have significantly longer epilepsy duration than those receiving carbamazepine monotherapy. Despite pharmacokinetic differences among the antiepileptic medications, intraoperative consumption of fentanyl, sevoflurane, propofol, and neuromuscular blocking agents is comparable across groups. Postoperative pain intensity follows a similar trajectory across groups. In multivariable analysis, duration of epilepsy and antiepileptic drug regimen do not emerge as independent predictors of postoperative pain.

In the present study, disease duration is longer in participants receiving dual therapy. This aligns with clinical reasoning: patients with longer epilepsy often require polypharmacotherapy because monotherapy becomes less effective and pharmacoresistance develops. In the study, among patients receiving antiepileptic drug treatment for more than 5 years, 59% report inefficiency of first-line antiepileptic therapy, and 70% report inefficiency of second-line antiepileptic medication. Inadequate selection of the first- and second-line treatments increases the risk of pharmacoresistance by 18- and 9-fold, respectively. Every additional year of epilepsy duration increases the risk of pharmacoresistance by 7% [22]. According to the recently conducted retrospective study, patients on polypharmacy have the onset of epilepsy at an older age and experience a higher frequency of seizures before the therapy compared to the monotherapy group. In contrast, patients on monotherapy tend to experience five years of remission and are more prone to discontinue the treatment compared to the polypharmacy group [23].

Contrary to expectations, the antiepileptic medication regimen is not significantly associated with differences in consumption of general anesthesia agents. Enzyme-inducing antiepileptic drugs, such as carbamazepine, enhance the function of cytochrome P450 enzymes [24], potentially increasing the requirements for general anesthesia agents. Exposure to long-term antiepileptic therapy may result in faster recovery after, possibly due to hepatic microsomal enzyme induction and decreased GABA receptor sensitivity [25,26]. Our findings, however, do not demonstrate a significant discrepancy in propofol or inhaled anesthetics consumption across the groups.

Previous study reports an increased intraoperative fentanyl consumption depending on the number of antiepileptic medications received by patients [27]. Conversely, in the present study, there is no statistically significant difference in fentanyl consumption between the participants receiving monotherapy or combined dual antiepileptic therapy. Extended exposure to anticonvulsant therapy may result in resistance to non-depolarizing muscle relaxants due to increased liver metabolism, acetylcholine receptors upregulation, and increased protein binding, reducing the availability of muscle relaxants [28]. In a study comparing the effect of cisatracurium among patients undergoing craniotomy, patients exposed to chronic antiepileptic medications demonstrate higher clearance and lower steady-state plasma concentration of cisatracurium than participants without a history of antiepileptic drug use [29]. In the current study, however, the statistically significant difference in pipecuronium consumption is not observed.

According to the current observations, the median post-op mechanical ventilation time in the ICU is 35% and 40% longer in the carbamazepine + levetiracetam than in the carbamazepine and other AED groups, respectively. In the second group, each patient was prescribed levetiracetam either as a primary or a secondary medication, unlike in other groups. According to a recent study, patients receiving levetiracetam have a longer interval to the next rocuronium maintenance dose than the control group, resulting in longer recovery [30]. This could be explained by potential competition for excretion via P-glycoprotein transmembrane drug efflux pump, since levetiracetam and rocuronium are transported by the same pump [30]. Delayed elimination of neuromuscular agents may result in prolonged postoperative mechanical ventilation in the ICU.

Postoperative pain is defined as acute nociceptive pain following surgical intervention because of tissue injury and

inflammatory mediator release [31]. In the present study postoperative pain is measured by VAS and ranges from 1 to 3 points, although indicating mild to moderate pain. Pain trajectories demonstrate similar trends, although patients receiving other AED medications tend to report higher pain scores at 2 and 6 hours, however, these differences lose significance after adjustment for multiple comparisons. Later during post-op period, the differences between groups decrease, also likely due to the standardized pain management protocol. In the current study the time of additional analgesic requirement is comparable across the groups, although the third group shows slightly shorter median time towards the additional analgesic requirement. This may suggest that the antiepileptic drug regimen does not significantly influence acute postoperative pain perception and is effectively managed by postoperative pain management protocols. According to a recent investigation including 42 studies, high-quality evidence demonstrates postoperative pain reduction by NSAIDs up to 24 hours in patients undergoing craniotomy [32]. In addition, pain perception may be altered in patients depending on the anatomical location of the epilepsy foci, as the recent study shows association between reduction in mechanical pain sensitivity and hippocampal damage in the patients with temporal lobe epilepsy [33]. This demonstrates that not only pharmacological interaction, but also anatomical structures involved may influence the pain perception in patients with chronic epilepsy.

A multivariable regression analysis finds no statistically significant associations between postoperative pain and intraoperative fentanyl, antiepileptic drug regimen, gender, or surgical procedure. Importantly, the similarity of the primary and secondary analyses strengthens the confidence that the observed findings are consistent after exclusion of patients receiving valproic acid or lamotrigine as a primary medication. The regression model shows a non-significant trend toward lower odds of postoperative pain among patients receiving levetiracetam as a primary antiepileptic drug in a dual combination treatment compared to carbamazepine-treated patients either as a mono or duo therapy. Investigations in the elective laparoscopic cholecystectomy report reduced postoperative pain scores as well as opioid consumption in patients receiving perioperative levetiracetam [34]. A clinical study conducted in rats demonstrates that levetiracetam possesses an antihyperalgesic effect, to a certain extent mediated by GABA, serotonin, opioid, and alpha-2-adrenergic receptors, in response to inflammatory stimuli [35]. Nevertheless, our findings did not reach statistical significance, and the observed trend should be regarded as hypothesis-generating rather than evidence of a true analgesic effect. Larger investigations are required to determine whether levetiracetam possesses clinically meaningful perioperative analgesic properties in patients with chronic epilepsy.

The type of neurosurgical procedure was not associated with the occurrence of postoperative pain in either the univariate or multivariable analyses. Although patients undergoing tumor resection demonstrated numerically higher odds of postoperative pain than those undergoing lobectomy in both analyses, the confidence interval is wide, and the association was not statistically significant. Likewise, the pooled group named other surgical procedures in both multivariable logistic regression models demonstrates lower odds of postoperative pain outcomes compared to lobectomy. It is possible that differences in surgical complexity and further tissue damage may vary among surgery types. These findings suggest that, within the scope of standardized perioperative anesthesia, surgery type may have only a limited influence on early postoperative pain. However, since the results do not reach statistical significance, larger sample size should be considered in the future. Likewise, no independent association between gender and postoperative pain is observed. While higher postoperative pain scores and greater fentanyl consumption have been reported among females undergoing laparoscopic cholecystectomy [21], the present study is not powered to detect modest gender-specific outcomes.

In the present study, epilepsy duration was not associated with postoperative pain intensity or analgesic requirements. In the study, comparing pain perception between participants with epilepsy and healthy individuals, sensitivity to pain was reduced in those with temporal lobe epilepsy and was associated with the extent of hippocampal damage [33]. The extent of hippocampal damage may indirectly reflect the frequency of epileptogenic firing and thus the duration of epilepsy. Nevertheless, evidence on the relationship between epilepsy duration and postoperative pain remains limited. Interestingly, our data shows that participants receiving levetiracetam as a first-line medication demonstrate longer duration of epilepsy compared to carbamazepine group. Overall, the second and third group representing combined therapy demonstrate longer disease course compared to carbamazepine monotherapy group, supporting the tendency of polypharmacy development with time [22]. Our findings suggest that disease chronicity alone may not be a major determinant of acute postoperative pain, instead, individual pain sensitivity [21], and perioperative analgesic strategies [32] may have greater influence on pain outcomes.

An additional notable finding was the longer hospital stay for patients on AED combinations other than carbamazepine plus levetiracetam. While the postoperative seizure rates were similar across groups, the higher seizure occurrence and more complex treatment regimens in this subgroup may contribute to the extended hospitalization. Consequently, the length of hospital stay likely reflects overall disease severity rather than solely perioperative pain or anesthetic influences. Further investigations in this direction are warranted.

The analysis indicates that none of the examined preoperative or intraoperative variables are associated with the occurrence of postoperative pain in patients with epilepsy. Recent study, however, demonstrates that preoperative high frequency heart rate variability as a mirror of the vagus nerve firing inversely and independently predicts the intensity of postoperative pain [36]. This suggests that postoperative pain following epilepsy surgery may be influenced more strongly by individual pain sensitivity, psychological characteristics, surgical site, and postoperative analgesic management. Current findings suggest that standardized anesthesia and postoperative analgesia provide effective pain control among the patients with pharmacoresistant focal epilepsy receiving continuous antiepileptic treatment. Third, anesthetic depth monitoring was not consistently available, which could impact the assessment of anesthetic requirements

Finally, due to retrospective design, the study may have been underpowered to detect moderate differences among the treatment groups. The sample size for valproic acid and lamotrigine as a primary medication was extremely small. Anesthetic depth monitoring was not consistently available, which could impact the assessment of anesthetic requirements. In addition, postoperative pain scale VAS is a subjective measure which may not always extrapolate a definitive pain status of a patient. In addition, the use of both pipecuronium and

rocuronium, according to individual anesthesiologist preference may compromise clear outcomes regarding the interaction of AED and neuromuscular blocking agents.

Conclusion

Long-term antiepileptic drug therapy, epilepsy duration, and surgical procedure were not identified as independent predictors of anesthetic consumption or early postoperative pain after epilepsy surgery. The comparable outcomes observed across treatment groups suggest that standardized perioperative anesthetic and analgesic protocols may minimize potential differences related to chronic antiepileptic therapy. Larger prospective multicenter studies are needed to confirm these observations and evaluate the influence of specific antiepileptic drugs on perioperative outcomes.

Supplementary materials

The Supplementary information includes:

• Supplementary Table S1. Post-operative pain between group II ang group III;

• Supplementary Table S2. Univariate Analysis of Factors Associated with Postoperative Pain;

• Supplementary Table S3: The presence of postoperative pain according to surgery type stratification.

This supplemental materials have been provided by the authors to give readers additional information about their work.

The file can be accessed using: https://www.editorialpark.com/download/article-supp/767/SUPPLEMENTARY-MATERIALS-JCMK-42946-2026-R3.docx.

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Effectiveness and Safety of Lanadelumab in the Treatment of Patients with Hereditary Angioedema in Routine Clinical Practice in the Russian Federation

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Abstract

Background: Hereditary angioedema (HAE) caused by C1 inhibitor deficiency is a rare condition marked by recurrent swelling of the skin and mucous membranes. This study evaluated the effectiveness and safety of lanadelumab in routine clinical practice in Russia (IISR-2021-200085).

Methods: A prospective cohort of 30 individuals with HAE was observed. Most patients were women (87%), with a mean age of 34 years; Seventeen percent of participants were adolescents. Prior to initiating lanadelumab, 16 patients (53%) had been receiving long-term prophylaxis, which was discontinued once lanadelumab was started. Treatment outcomes were assessed by comparing attack frequency, medication use for acute events, disease control scores, and quality of life before and after lanadelumab therapy.

Results: Before treatment, patients reported an average of 8.6 attacks per month. After 6 months of lanadelumab treatment, the mean attack frequency was reduced to 0.5 per month ($p < 0.001$). Use of acute therapies decreased from 4.73 to 0.43 doses per month ($p < 0.001$). Disease control improved substantially, and quality-of-life scores improved from 58.2 to 25.3 points ($p < 0.001$). No significant lanadelumab-related adverse events were observed.

Conclusion: In routine clinical practice, lanadelumab proved highly effective in reducing attack rates, enhancing disease control, and improving quality of life in patients with hereditary angioedema. The therapy was well tolerated, with no serious safety concerns identified. These findings reinforce lanadelumab as a reliable long-term prophylactic option for hereditary angioedema management.

Keywords: hereditary angioedema, HAE, angioedema, C1-inhibitor, bradykinin, lanadelumab.

Introduction

Hereditary angioedema (HAE) due to C1-esterase inhibitor (C1-INH) deficiency is characterized by recurrent, unpredictable episodes of localized swelling of the skin and mucous membranes, episodes of severe abdominal pain (abdominal attacks). The disease follows an autosomal dominant inheritance pattern and is most commonly linked to mutations in the SERPING1 gene, leading either to reduced levels of C1-INH enzyme (type I HAE) or to impaired C1-INH activity (type II HAE) [1–3].

HAE attacks vary widely in severity and frequency, significantly impairing patients' physical, psychological, and social functioning—even during symptom-free intervals. Laryngeal edema can be life-threatening, while abdominal attacks are intensely painful and often result in unnecessary surgical or diagnostic procedures. Unlike mast cell–mediated angioedema, HAE attacks do not respond to antihistamines or corticosteroids.

Long-term prophylaxis (LTP), short-term prophylaxis (STP), and acute attack management form the basis of HAE treatment. Earlier options such as danazol, progestins, tranexamic acid, and fresh frozen plasma were repurposed from other indications but demonstrated partial effectiveness in HAE. The advent of targeted therapies, including icatibant and plasma-derived or recombinant C1-INH (administered subcutaneously or intravenously), has significantly altered the prognosis and quality of life for patients. However, individuals with frequent attacks continue to require effective and well-tolerated long-term preventive therapy. Existing prophylactic agents have limitations: attenuated androgens are associated with significant adverse effects, intravenous C1-INH requires frequent administration (off-label for LTP, licensed in Russia for treatment of acute HAE attacks), and antifibrinolytics and progestins are effective only in selected cases [4]. This underscores the need for more effective and safer options for prophylaxis.

Lanadelumab is a fully human monoclonal antibody (IgG1/κ) that inhibits the activity of plasma kallikrein (IgG1/κ-light chain). It is administered subcutaneously at a recommended starting dose of 300 mg every two weeks, with the option to extend the interval to every four weeks in well-controlled patients (no HAE attacks) [5–7]. Lanadelumab combines high efficacy, a favorable safety profile, and convenient administration, representing a major advance in the management of HAE.

International WAO/EAACI guidelines position lanadelumab as a first-line prophylactic treatment for HAE [8]. In Russia, expert consensus and updated federal clinical guidelines also endorse its use in patients with high disease activity, frequent attacks, or inadequate response or contraindications to other preventive therapies [9,10].

Its efficacy and safety have been confirmed in the multicenter randomized double-blind, placebo-controlled phase III clinical trial HELP (Hereditary Angioedema Long-term Prophylaxis), which enrolled 125 patients [11], and subsequently in the open-label HELP OLE extension study [7]. Additional evidence has emerged from non-interventional studies such as EMPOWER/ENABLE, which evaluated lanadelumab prophylaxis in adolescents [12].

Nonetheless, given that clinical trial populations are highly selected, the short period of use of lanadelumab, the data from clinical trials may not reflect the variability of routine practice. Real-world data are therefore crucial to confirm the drug's effectiveness across diverse patient groups. Although several small cohort studies have been conducted internationally [13–16], due to the disease's rarity, all studies were conducted on limited patient samples and no published data have addressed the use of lanadelumab in the Russian population. Key aspects of lanadelumab administration in real-world practice remain to be defined:

1. transitioning patients from previous LTP regimens to lanadelumab.
2. The role of STP in patients achieving complete symptom control on lanadelumab.
3. Strategies for extending injection intervals once remission is achieved.
4. Defining the maximum safe dosing interval.
5. Duration of disease control after treatment discontinuation.
6. The feasibility of dose reduction in patients with sustained remission.

Given these gaps, we initiated a two-year, non-interventional, retrospective/prospective study to assess the long-term effectiveness and safety of lanadelumab in routine clinical practice in Russia. This article reports interim study results.

Aim of the study: The objective of this study was to evaluate the long-term effectiveness and safety of lanadelumab as prophylactic therapy in patients with hereditary angioedema (HAE) caused by C1-esterase inhibitor (C1-INH) deficiency under routine clinical practice conditions in the Russian Federation.

Methods

Study Design

This study was designed as a non-interventional, retro-prospective study involving patients with hereditary angioedema (HAE) caused by C1-esterase inhibitor (C1-INH) deficiency who started lanadelumab therapy in accordance with current usage

guidelines. A total of 30 patients were enrolled, with planned follow-up over a 24-month period. The present article reports interim results based on data collected at multiple time points.

Eligibility Criteria

Inclusion criteria comprised patients aged ≥12 years with a confirmed diagnosis of HAE caused by C1-INH deficiency [3]. Written informed consent was obtained from all participants or their legal representatives. The decision to prescribe lanadelumab had to be made independently of study enrollment, and lanadelumab had to be prescribed in accordance with the drug's instructions for use. The data on disease activity during the preceding six months were required. For retrospective inclusion, patients were additionally required to have complete medical records documenting angioedema attacks, adverse events, and interventions from the initiation of lanadelumab.

Exclusion criteria included absence of informed consent, contraindications to lanadelumab as defined in the product labeling, pregnancy or lactation, and concurrent participation in other interventional clinical trials.

Terms and Conditions

The study was conducted across multiple expert centers in the Russian Federation, including institutions within the ACARE international network: the NRC "Institute of Immunology" (FMBA of Russia, Moscow) and the Allergy and Immunology Center of Moscow City Clinical Hospital No. 52. Additional participating sites included the Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, V.I. Razumovsky Saratov State Medical University, "Vologda Regional Clinical Hospital" and Murmansk

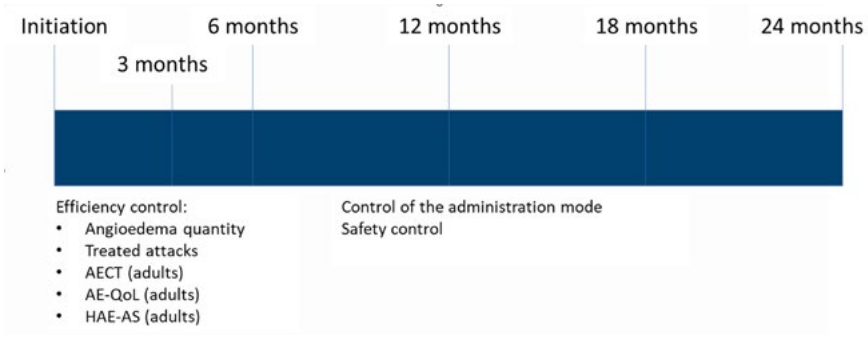

Figure 1 – Study Design

Arctic University, Murmansk ,V.A. Baranov Republic Hospital of Petrozavodsk, Regional Center of Allergology and Immunology of Smolensk, Clinical hospital № 1 of Sverdlovsk region.

Data Sources

Retrospective data were obtained from medical records, while prospective data were collected during scheduled follow-up visits in accordance with the study protocol. All information was recorded in standardized individual registration cards (IRC). Retrospective PRO (patient-reported outcome) data were extracted from routinely maintained patient diaries/PROM records.

Study Duration

The study started in December 2021 and is ongoing. Each patient is to be followed for a total of 24 months. Assessments are conducted at baseline and subsequently at 3, 6, 12, and 24 months, unless participation is discontinued earlier (Figure 1).

Medical Intervention

Patients included in this non-interventional study had been prescribed lanadelumab as part of standard clinical care. The first 16 patients that were enrolled retrospectively in December 2021 were already receiving lanadelumab therapy, with comprehensive medical records available for each patient, which enabled the integration of retrospective data with prospective observations. 14 additional patients were prospectively included between December 2021 and October 2022. All patients continued prospective follow-up after enrollment.

Effectiveness and safety were assessed using PRO tools filled out at control points. Evaluated variables included attack frequency, anatomical location and severity of angioedema episodes (mild: temporary or minor discomfort; moderate: partial activity limitation; severe: significant activity limitation requiring assistance), use of AE management medications, adverse events, and other medical interventions.

The following validated PRO instruments were employed (Table 1):

1) AE-QoL (Angioedema quality of life Questionnaire) [17];
2) AAS28 (Angioedema Activity Score 28) [18];
3) HAE-AS (HAE Activity Score) [19];
4) AECT (Angioedema Control test) [20].

The Primary Outcome of the Study

The primary endpoint was change in disease activity before and during lanadelumab therapy. Additional objectives included evaluation of an algorithm for extending dosing intervals, analysis of medical interventions and adverse events during treatment, and assessment of patient self-administration.

Table 1 Comparative analysis of instruments for assessing hereditary angioedema severity [20, 21]

	AAS28	HAE-AS	AECT	AE-QoL
Title	Angioedema activity score 28	HAE activity score	Angioedema control test	Angioedema quality of life
Evaluation period	4 weeks	6 months	4 weeks / 3 months	4 weeks
Completion method	Prospective	Retrospective	Retrospective	Retrospective
Parameter Assessed	Frequency and duration of angioedema episodes	Frequency and duration of angioedema episodes	Disease control	Impact on quality of life

Subgroup Analysis

No predefined subgroup analyses were conducted.

Outcome Documentation

Medical records covering 6 months prior to lanadelumab initiation and the treatment period of 24 months were analyzed. All relevant data were transferred to IRCs (individual registration cards) and stored electronically (Microsoft Excel).

Ethical Review

The study protocol was reviewed and approved by the local ethics committee of the NRC "Institute of Immunology" FMBA of Russia, Protocol No. 12, dated June 12, 2021.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (USA), RStudio (USA), and Microsoft Excel (USA). Data are presented as descriptive summaries, tables, and graphical representations. Continuous variables with non-normal distributions are presented as median (Q1; Q3), while categorical variables are presented as counts and percentages.

At the initial stage of statistical analysis, group comparisons were performed using the Friedman test (Friedman ANOVA) and Kendall's coefficient of concordance (W). Statistical significance was defined as $p < 0.05$. In cases where statistical significance was identified, pairwise comparisons were subsequently conducted using the Wilcoxon signed-rank test, with p-values adjusted according to the Benjamini–Hochberg method to control for multiple comparisons.

List of abbreviations

HAE — hereditary angioedema

HAE-PLG — HAE with a mutation in the plasminogen gene

HAE-FXII — HAE with a mutation in the coagulation factor 12 (FXII) gene

AE — angioedema
LTP – long-term prophylaxis
STP – short-term prophylaxis
CRF – case report form
PRO – Patient-reported outcome
AE-QoL – Angioedema quality of life
AAS28 – Angioedema activity score 28
HAE-AS – HAE activity score
AECT – Angioedema control test

Results

A total of 30 patients were enrolled in the study, comprising 26 (87%) female and 4 (13%) male individuals. The mean age at initiation of lanadelumab therapy was 33,96 years (range: 13–70 years; median: 32.5 years). 25 patients (83%) were adults, while the remaining 5 participants (17%) were adolescents between 12 and 17 years of age. With respect to HAE subtype, 28 individuals (93%) had type I HAE, and 2 patients (7%) were diagnosed with type II HAE. The mean body mass index (BMI) across the cohort was 26.12 kg/m². Supplementary Table S1 contains a detailed characterization of the study cohort.

At the time of interim analysis, 26 patients had completed their scheduled follow-up. Data from some visits were unavailable in cases where the injection interval exceeded 40 days. Four patients discontinued early: one due to planning of the pregnancy, two due to confirmed pregnancies, and one patient discontinued therapy at his own request after achieving complete disease control.

Main Results of the Study

Before initiating lanadelumab, all patients had previously received various forms of long-term prophylactic therapy (Supplementary Table S2). 16 (53%) patients were on LTP regimens at the time of enrollment, which included either tranexamic acid, danazol, progestins, or intravenous C1-INH. Of these, 15 patients discontinued prior prophylaxis upon starting lanadelumab. One patient (patient 29) had been receiving two concurrent prophylactic therapies prior to initiating lanadelumab: desogestrel 0.075 mg daily and tranexamic acid 3000 mg daily. Following the start of lanadelumab, tranexamic acid was discontinued, while desogestrel was continued at 0.075 mg daily as prescribed by her gynecologist for contraceptive purposes."

Among the 16 patients receiving LTP, 8 terminated their prior therapy on the day lanadelumab was initiated. Seven of these individuals remained attack-free, while one had an angioedema episode on day 4, which required treatment with icatibant. The remaining seven patients discontinued their prophylaxis gradually.

In total, 28 of 30 patients (93%) had previously received at least one line of prophylactic therapy. Tranexamic acid was the most frequently used agent but was reported as ineffective in 27 cases. Among these, effectiveness could not be adequately assessed in three patients due to short treatment duration or subtherapeutic dosing secondary to adverse events. Six patients demonstrated a partial response, while 18 had no therapeutic benefit.

Data of the LTP before initiation of lanadelumab treatment are presented in Supplementary Table S2. In total, 28 of 30 patients (93%) had previously received at least one line of long-term prophylactic therapy. Tranexamic acid was the most frequently used agent but was reported as ineffective in 27 cases. Among these, effectiveness could not be adequately assessed in three patients due to short treatment duration or subtherapeutic dosing secondary to adverse events. 6 patients demonstrated a partial response, while 18 had no therapeutic benefit.

Ten patients were previously prescribed danazol. It was partially effective in 4 patients, and 6 patients showed no improvement at all with danazol. Two of the 6 were taking danazol for a short period of time because of side effects, so it was not possible to assess the benefit of the treatment. Ten patients previously received progestins, 4 out of 10 patients had an incomplete response, while in 6 patients the treatment was ineffective. Six patients were treated with intravenous C1 esterase inhibitor, 5 of whom had a suboptimal response and 1 patient did not respond to therapy. Two patients did not receive any of the long-term prophylaxis before lanadelumab therapy (patients 17 and 23).

Ten patients had been treated with danazol. 4 patients reported partial benefit, while 6 patients experienced no clinical improvement. Two of the 6 discontinued danazol early because of adverse effects, precluding assessment of effectiveness.

Ten patients had previously been treated with progestins; 4 out of 10 patients achieved an incomplete response, and in 6 patients the treatment demonstrated no clinical benefit.

Six patients had previously been prescribed intravenous C1-INH as prophylaxis. 5 of these patients showed suboptimal responses, while 1 patient demonstrated no response.

Two patients (patients 17 and 23) had not received any prior LTP before starting lanadelumab.

Patient Self-Injection Ability

At the initiation of lanadelumab therapy, 22 patients (73%), including three adolescents, were trained in self-injection and demonstrated no difficulties with the procedure. During follow-up, an additional six patients successfully transitioned to self-administration. In total, 28 of 30 patients (93%) were able to administer the medication independently. Two patients (patients 2 and 16) were unable to perform injections at home and continued to receive lanadelumab in a clinical setting.

Maintenance of Drug and Regimen Adherence

Among the eight patients receiving lanadelumab at healthcare facilities, three patients experienced deviations from the prescribed regimen owing to factors unrelated to the treatment itself: one patient missed an injection because of a comorbid gout flare, another because of limited clinic availability during public holidays, and a third due to work-related commitments. These patients were not supplied with lanadelumab for home administration, as regional regulations required in-clinic dosing.

All participants initially received lanadelumab 300 mg every 14 days, in line with approved recommendations. In patients who achieved disease control (defined as no HAE attacks for at least three months), dosing intervals were extended. As no standardized protocol exists for interval extension, an algorithm was applied in clinical practice: after three months of remission, the injection interval was extended to 21 days; if remission persisted for a further three months, the interval was extended to 28 days. In selected cases, dosing intervals were prolonged up to four weeks.

Current Regimen Adherence

At the six-month follow-up, injection intervals were analyzed for 29 patients (patient 30 was excluded due to non-adherence to the prescribed regimen). 14 patients (48%) continued the standard 14-day regimen, 8 (27.6%) were on a 21-day interval, and 6 (21.1%) had extended dosing interval to 28–30 days. Notably, one patient successfully extended the interval to 56 days while maintaining complete disease control; this individual subsequently withdrew from the study at their own request due to sustained remission.

Attack Frequency and Treatment Need

The effectiveness of lanadelumab therapy was assessed using the following parameters: the frequency of AE or abdominal attack before and during the treatment period, the need for acute attack treatment drugs, disease activity parameters and quality of life assessed using questionnaires on patient-reported outcome (PRO).

The median monthly attack frequency per patient decreased markedly following initiation of lanadelumab therapy. At baseline, patients experienced a median of 7.0 [5.66–12.0] attacks per month. After 6 months of treatment, this value declined to 2.0 [0.0–5.0], with sustained low attack rates observed at 12, 18, and 24 months (1.0 [0.0–2.0], 1.0 [0.0–2.0], and 1.0 [0.0–4.0], respectively).

A statistically significant difference in median monthly attack frequency across the evaluated time points was identified (Friedman test, p = 0.0004; Kendall's W = 0.317), indicating a moderate effect size.

Pairwise comparisons demonstrated significant reductions in attack frequency between baseline and all subsequent time points, including 6 months (p = 0.0004), 12 months (p = 0.0001), 18 months (p = 0.0001), and 24 months (p = 0.00012).

Reduction in sample size at later time points reflected patient withdrawal: four discontinued therapy, and one was excluded due to irregular drug supply (Figure 2).

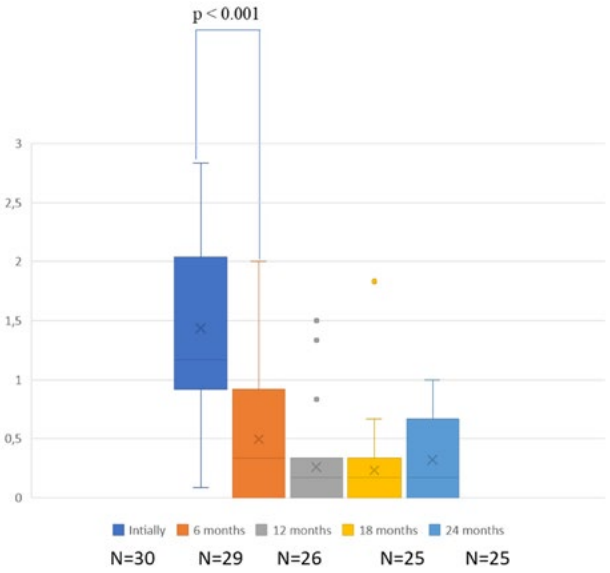

Figure 2 – Median monthly number of attacks per patient prior to and during lanadelumab therapy

A reduction in attack frequency was observed across most anatomical subtypes. Pairwise comparisons between baseline and 6 months demonstrated statistically significant decreases in peripheral attacks (p = 0.0003), abdominal attacks (p = 0.004), and facial attacks (p = 0.009). No statistically significant changes were observed for upper airway attacks (p = 0.286).

When stratified by severity, significant reductions were observed in moderate (p = 0.004) and severe attacks (p = 0.00001), whereas changes in mild attacks did not reach statistical significance (p = 0.586).

By the end of the two-year observation period, 72% of patients reported no severe attacks, 88% reported no upper airway involvement, and 80% reported no abdominal attacks, indicating a marked improvement in overall disease control.

A statistically significant reduction in the use of on-demand therapy was observed as early as 3 months after initiation of lanadelumab (p = 0.0001). This reduction remained stable throughout subsequent follow-up visits, indicating sustained disease control and decreased treatment burden. (Figure 3).

No significant differences were observed in the use of on-demand therapy at subsequent control points (6, 12, 18, and 24

Table 2 Median monthly HAE attack frequency

Time frame	Monthly frequency of HAE attack types	Median	Q1 (first quartile)	Q3 (third quartile)
Before lanadelumab treatment (30 patients)	Peripheral angioedema	4	2	5
	Abdominal attacks	3	2	5
	Face	0	0	1
	Upper airways	0	0	0,5
	mild	0	0	2
	moderate	3	1	5
	severe	4	2	5
	total	7	5,66	12
After 6 months of lanadelumab treatment (29 patients)	Peripheral angioedema	0	0	1
	Abdominal attacks	1	0	2
	Face	0	0	0
	Upper airways	0	0	0
	mild	0	0	2
	moderate	1	0	2
	severe	0	0	0
	total	2	0	5
After 12 months of lanadelumab treatment (26 patients)	Peripheral angioedema	0	0	1
	Abdominal attacks	0	0	1
	Face	0	0	0
	Upper airways	0	0	0
	mild	0	0	0
	moderate	0	0	1
	severe	0	0	0
	total	1	0	2
After 18 months of lanadelumab treatment (25 patients)	Peripheral angioedema	0	0	1
	Abdominal attacks	0	0	1
	Face	0	0	0
	Upper airways	0	0	0
	mild	0	0	0
	moderate	0	0	1,5
	severe	0	0	0,5
	total	1	0	2
After 24 months of lanadelumab treatment (25 patients)	Peripheral angioedema	0	0	1
	Abdominal attacks	0,5	0	1,5
	Face	0	0	0
	Upper airways	0	0	0
	mild	0	0	1
	moderate	0	0	1,5
	severe	0	0	1
	total	1	0	4

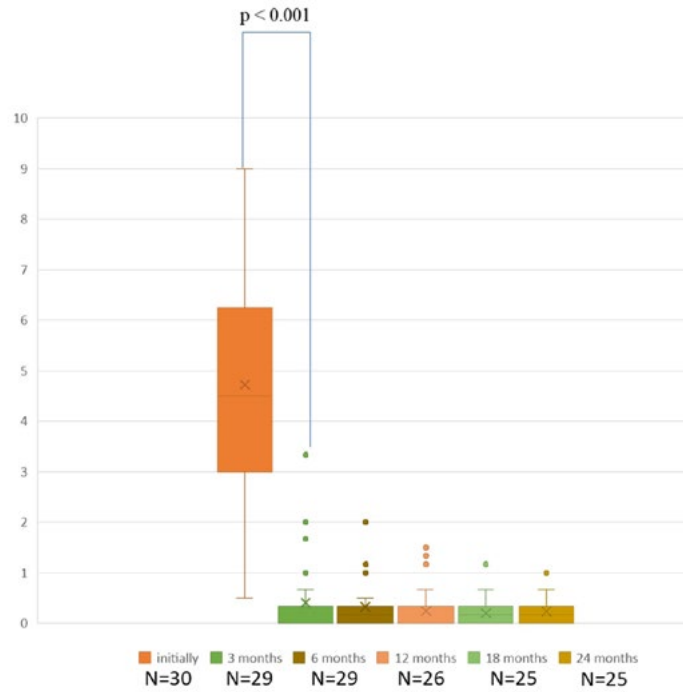

Figure 3 – Monthly use of on-demand medication per patient

months) compared with the third month, indicating sustained reduction in treatment need over time.

Patient-Reported Outcome (PRO) Values

The Angioedema Control Test (AECT) scores demonstrated a statistically significant improvement following initiation of lanadelumab therapy. Median AECT score increased from 5 (5;7) at baseline to 14 (13;16) after 3 months ($p < 0.00001$).

This improvement was sustained throughout the follow-up period, with median values of 15 (12;16) at 6 months, 16 (14;16) at 12 months, 15 (12;16) at 18 months, and 15 (12;16) at 24 months (Figure 4).

A statistically significant difference in AECT scores across all time points was observed (Friedman test, $p = 0.00001$; Kendall's $W = 0.51$), indicating a strong effect size. Pairwise comparisons confirmed significant differences between baseline and all subsequent visits (all $p < 0.00001$).

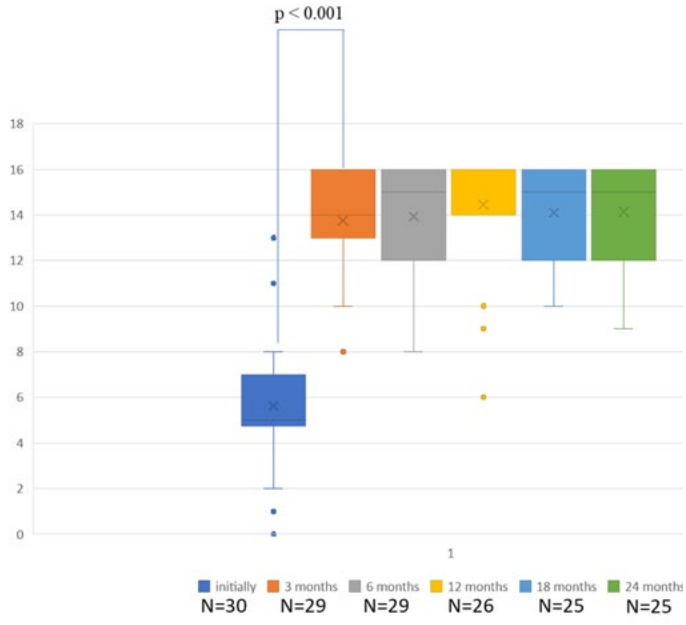

Figure 4 – AECT-based assessment of disease control prior to and during lanadelumab treatment

Improvement in Quality of Life

The Angioedema Quality of Life Questionnaire (AE-QoL) scores showed a marked improvement after initiation of lanadelumab therapy. Median AE-QoL score decreased from 60 (53;66) at baseline to 21 (9;40) after 3 months ($p = 0.0004$), indicating a substantial reduction in disease burden.

This improvement was maintained over time, with median values of 24.5 (11;38) at 6 months, 17 (9;25) at 12 months, 22 (7;29) at 18 months, and 22 (9;28) at 24 months.

Overall, AE-QoL scores differed significantly across time points (Friedman test, $p = 0.00001$; Kendall's $W = 0.51$), with significant pairwise differences between baseline and all subsequent visits (Figure 5).

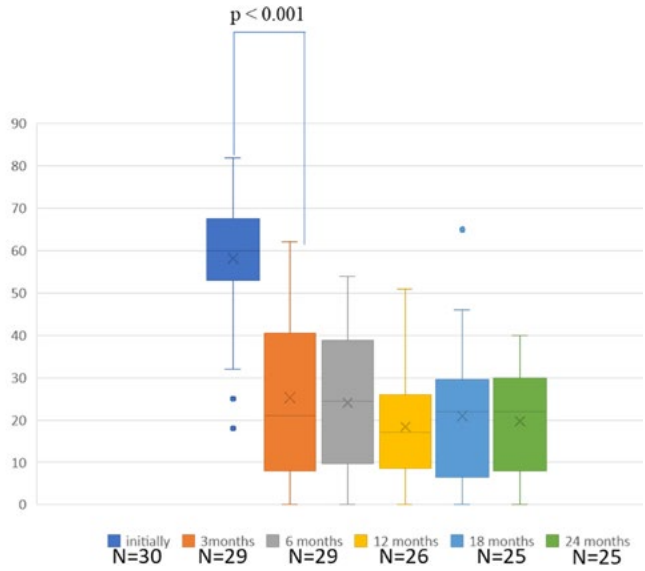

Figure 5 – AE-QoL scores prior to and during lanadelumab therapy

Notably, lower scores indicate less impairment, with reductions reflecting a clinically meaningful improvement in health-related quality of life.

The Hereditary Angioedema Activity Score (HAE-AS) decreased significantly following initiation of lanadelumab therapy. Median values declined from 15.5 (13;18) at baseline to 4 (1;5) at 6 months ($p = 0.0003$).

Further reductions were observed at 12 months (2 (0;4)), 18 months (2 (1;5)), and 24 months (4 (1;5)), indicating sustained suppression of disease activity over time.

HAE-AS scores differed significantly across time points (Friedman test, $p = 0.00001$; Kendall's $W = 0.55$), with significant differences between baseline and all subsequent follow-up visits (Figure 6, see the next page).

Impact of Medical, Dental, and Cosmetic Interventions

During the observation period, 18 patients (60%) underwent a total of 43 medical, dental, or cosmetic interventions that carried the potential to trigger HAE attacks. Of these, 36 procedures were performed without short-term prophylaxis (STP). Only three interventions (two performed with STP and one without) were followed by angioedema episodes, all occurring after minimally invasive procedures (conization of the cervix and removal of a uvular papilloma). Details are presented in Table 3.

Dental procedures were the most common (n = 28), followed by esophagogastroduodenoscopy (n = 5), cosmetic procedures (n = 5), minimally invasive gynecological or ENT interventions (n = 2), and surgical operations (n = 3) (Table 3). The majority were carried out safely, suggesting that

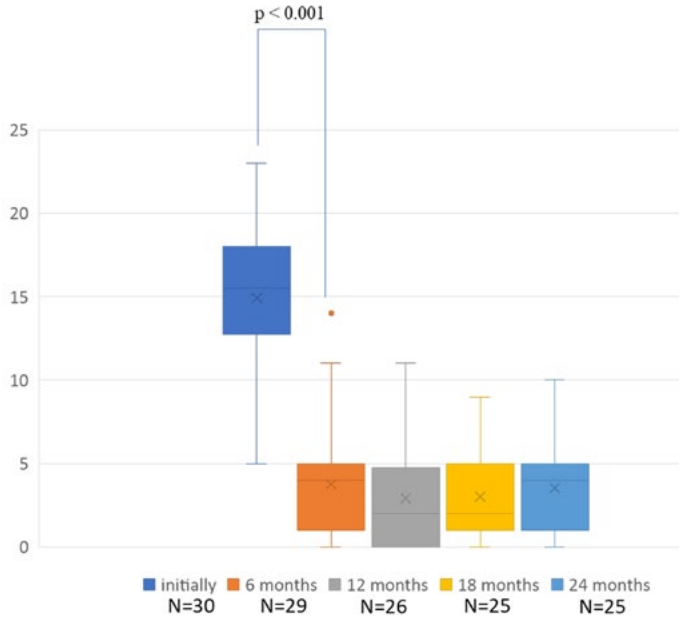

Figure 6 – HAE-AS score prior to and during lanadelumab therapy.

Table 3 Medical, dental, and cosmetic procedures performed during the 24-month period following initiation of lanadelumab therapy

Type of procedure	Number of procedures	Prior to short-term prophylaxis therapy (C1-inhibitor 1000 IU IV)	Angioedema
Dental procedures	28	2	0
Esophagogastroduodenoscopy	5	2	0
Cosmetic procedures	5	0	0
Minimally invasive procedures (cervical conization and excision of uvular papilloma)	2	0	2
Surgical procedures	3	3	1

Table 4 Reported adverse events

Adverse events after lanadelumab administration	Patient	Severity	Association with the drug	Outcome	Note
Small papular rash appearing the day following the injection	07	Mild	Probably related	Continues and reappears	-
Hyperemia at the injection site	15	Mild	Probably related	Reappears	-
Uterine bleeding	22	Severe (hospitalization)	Not related	Hospitalization	Probably due to tranexamic acid discontinuation
Headache on the day of injection	02	Mild	Probably related	Reappears	-

lanadelumab therapy may reduce the need for routine STP in certain patients with stable disease control, although access to on-demand medication remains essential.

Adverse Events

During the observation period, four adverse events were documented. Three were considered likely related to lanadelumab administration, while one was unrelated. All treatment-related adverse events were classified as mild and corresponded to reactions described in the instructions for use. A single serious adverse event was recorded in Patient 22, who experienced uterine bleeding requiring hospitalization. This event was not attributed to lanadelumab and was considered most likely secondary to discontinuation of tranexamic acid. Importantly, no treatment-related serious adverse events occurred during the study, and none of the reported adverse events led to therapy discontinuation (Table 4).

Discussion

The study demonstrated the effectiveness of lanadelumab as a long-term prophylactic therapy in patients with HAE caused by C1-esterase inhibitor deficiency. A substantial reduction in attack frequency, a marked decrease in the need for on-demand medication, and significant improvements in patients' quality of life were observed. Moreover, the favorable safety profile of lanadelumab was confirmed by the absence of treatment-related serious adverse events.

The efficacy and safety of lanadelumab have previously been established in a double-blind, randomized clinical trial, which identified 300 mg administered subcutaneously every 14 days as the optimal dosing regimen [5]. Real-world experience with lanadelumab has since been documented in several countries, including Canada, Germany, France, and the United Kingdom [13,15,22–23]. However, data from prolonged observation periods under routine clinical practice conditions remain limited. The present study addresses this gap by providing long-term real-world evidence from a Russian cohort undergoing lanadelumab prophylaxis.

The predominance of female patients (87%) in our cohort is noteworthy and consistent with demographic patterns observed in other real-world studies, where women represented 75% of participants in Canada, 72.6% in the United Kingdom, 68.8% in France, and 55.6% in Germany [8,15,23]. This imbalance may reflect both increased occurrence of HAE among females and the limitations associated with attenuated androgens, which are more frequently discontinued or avoided in women due to adverse effects [8]. However, danazol is an effective drug for long-term prophylaxis, generally better tolerated by male patients [24].

The majority of patients enrolled in this study (97%) were diagnosed with type I HAE. This predominance is influenced by natural distribution of type I and II in population of HAE patients.

The sustained reduction in attack frequency and improvement in disease control observed in our cohort, as well as in Canadian, British, French, and German real-world cohorts, highlights an important conclusion: lack of effectiveness of previous prophylactic therapies does not predict the ineffectiveness of lanadelumab.

Transitioning patients from prior long-term prophylactic regimens to lanadelumab remains a clinical challenge for which standardized guidance is currently lacking. In our cohort, one patient experienced uterine bleeding after discontinuing

tranexamic acid, illustrating the potential complexities associated with withdrawing prior therapies – particularly antifibrinolytics. Additional data are therefore needed to inform safe and effective strategies for tapering or discontinuing tranexamic acid. A similar discontinuation problem exists for attenuated androgens, prompting the initiation of the SHAERPA study, which aims to generate evidence-based recommendations for the discontinuation of danazol and related agents. Importantly, subgroup analyses by Fain et al. [15] indicate that the type or effectiveness of previous prophylactic therapy does not influence the subsequent therapeutic response to lanadelumab.

Lanadelumab is intended for self-administration according to established guidelines. In our study, 28 of 30 patients were successfully trained to self-inject the medication. All individuals who undertook self-administration performed the procedure correctly on the first attempt and continued to do so reliably thereafter. Among patients administering the drug at home, only one deviation from the prescribed dosing schedule occurred (a missed injection day), excluding cases related to drug-supply issues. By contrast, three deviations were recorded among patients receiving injections exclusively in medical facilities. These findings underscore the practical advantages of self-administration. Nevertheless, the adoption of flexible, patient-directed dosing regimens requires ongoing clinical monitoring to assess disease activity and quality of life, ensuring timely adjustment of treatment plans. Administration in healthcare settings, while necessary for some patients, may increase the likelihood of regimen interruptions due to factors such as appointment availability and institutional workflows, which may adversely impact therapeutic consistency.

A group of researchers from France analyzed patients' attitudes toward training in self-administration and toward ease of administration in a cohort of patients. In a French cohort, the majority of patients (71%, n = 54) reported that training in self-administration was very easy, with a mean difficulty score of 8.8 on a 10-point scale (1 = very difficult; 10 = very easy). Satisfaction with the ease of lanadelumab administration remained consistently high throughout follow-up, with mean scores of 8.1 at one month and 8.7 at six months (1 = not at all satisfied; 10 = very satisfied) [15].

The approved dosing regimen for lanadelumab recommends administration of 300 mg every 14 days, with the option to extend the interval to 28 days in patients achieving stable disease control (according to the official usage guidelines). Real-world evidence has demonstrated even greater flexibility, with some studies reporting successful interval extensions to 30 days and, in selected cases, up to 90 days [15]. Despite these observations, no standardized protocol for extending dosing intervals has yet been established. Buttgereit et al. proposed a pragmatic approach in which the first three injections are administered at 14-day intervals, followed by incremental 3-day extensions contingent upon sustained remission. This method allowed for individualized adjustment of the dosing interval for each patient. A study by Inmaculada Martinez-Saguer accessed the number of patients in the cohort able to extend the injection intervals for more than 17 days. Among 106 patients treated for no less than four months, 33 patients (31 %) administered lanadelumab with extended injection intervals (more than 17 days between injections). The average period of lanadelumab therapy before an attempt to extend the injection interval was 110 +/- 91 days. The proportion of patients with extended interval regimens increased to 48 % (among 44 patients receiving therapy for 12 months) after 12 months of lanadelumab therapy (Table 1). The average period of lanadelumab therapy before an attempt to extend the injection interval was 144 +/- 95 days. However, In Maculada Martinez-Saguer give no details on their approach to injection interval extension. [25] A group of researchers from Great Britain also analyzed the injection intervals in their cohort of patients. In a cohort of 60 patients, half (n = 30) were able to extend the dosing interval to at least three weeks. Among these, 27 patients transitioned to a 28-day regimen, and one patient successfully extended the interval to five weeks. The authors note that it took an average of 15.2 ± 12.1 weeks to extend the interval, but give no details on their approach to interval extension [13].

In another study, all patients were initially prescribed the standard lanadelumab regimen of 300 mg every 14 days. Dose adjustment was planned for a control visit 6 months. However, in two patients, the intervals were extended after 3 months due to severe pain at the injection sites. After 6 months of therapy, 10 of 39 remaining participants continued therapy with extended administration regimen: 3 patients received injections every 3 weeks and 6 patients – every 4 weeks. The extension of injection intervals was driven by good disease control, painful injections or by patient request. In contrast, in one instance the injection interval was reduced to 1 week, but the authors did not disclose the details of this clinical decision in the article [15].

In our study, interval extension was initiated after the first six injections. This strategy was based on the pharmacokinetic profile of lanadelumab, as steady-state concentrations are typically achieved by approximately day 70, aside from cases involving deviations from the recommended dosing schedule. A three-month period was also considered adequate for evaluating both therapeutic effectiveness and potential adverse effects. Thus, dosing intervals were extended by seven days in patients who achieved complete remission, followed by an additional seven-day extension if remission persisted, allowing for a final interval of 28 to 30 days. Considering the cost of therapy, the possibility of optimizing lanadelumab administration regimen by extending the injection intervals without diminishing the therapy effectiveness is of great pharmacoeconomic importance. We have developed a scheme for optimizing lanadelumab administration regimen based on our own observations and on literature data (Figure 7).

A primary aim of our study was to evaluate the effectiveness of lanadelumab as a long-term prophylactic therapy in routine clinical practice in the Russian Federation. The median monthly attack frequency per patient decreased from 7.0 (5.66; 12.0) attacks at baseline to 2.0 (0.0; 5.0) after 6 months of therapy. These findings are consistent with the results of the randomized

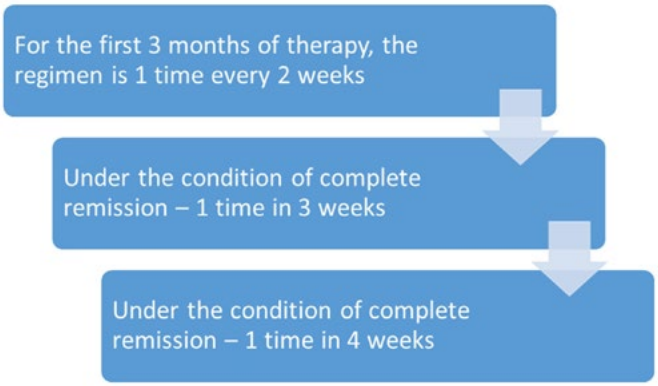

Figure 7 – Standardized protocol for extending lanadelumab dosing intervals

HELP (Hereditary Angioedema Long-term Prophylaxis) trial, which demonstrated a marked reduction in attack frequency among patients receiving lanadelumab prophylaxis [5]. Direct numerical comparison should be interpreted with caution because the present study reports median values, whereas the HELP trial reported mean attack rates. Furthermore, our cohort had a higher baseline disease burden and was evaluated under real-world clinical practice conditions, including individualized extension of dosing intervals in selected patients. Despite these differences, both studies demonstrated substantial and sustained reductions in HAE attack frequency during lanadelumab treatment.

A marked reduction was also observed in attacks of greater clinical severity. The median frequency of severe attacks decreased from 4 (2; 5) attacks per month at baseline to 0 (0; 0) after 6 months of therapy, while the median frequency of moderate attacks decreased from 3 (1; 5) to 1 (0; 2) attacks per month. These findings are directly comparable with those of the HELP trial, as both studies applied the same criteria for attack severity classification. In the HELP study, the combined rate of moderate and severe attacks after 6 months of treatment averaged 0.20 attacks per month in patients receiving lanadelumab every four weeks and 0.32 attacks per month in those receiving lanadelumab every two weeks [5].

Similarly, real-world data from France demonstrated a low attack burden during lanadelumab prophylaxis, with a mean monthly attack frequency of 0.16 attacks per patient among individuals receiving the standard 300 mg every-14-days regimen [15].

The need for on-demand treatment decreased significantly as early as the third month of therapy ($p = 0.0001$) and remained low throughout follow-up. This finding is consistent with the results of the HELP trial, in which the mean frequency of attacks requiring on-demand treatment was 0.26 attacks per month among patients receiving lanadelumab every 14 days and 0.53 attacks per month among those receiving lanadelumab every 28 days [5].

Lanadelumab's proven to be highly effective already at the 3 months visit. Heightened disease control remained consistent throughout the following months of therapy. This finding is crucial for real-world clinical practice, when the patient does not have strict selection criteria for comorbidities, concomitant medication and other characteristics that may be the exclusion criteria in the clinical trials.

These findings suggest meaningful long-term advantages despite the high cost of lanadelumab, including a reduction in expenditures associated with on-demand medications, missed workdays, medical consultations, and hospitalizations. It is also conceivable that the improved disease control achieved with lanadelumab may reduce the cost of short-term prophylaxis and, importantly, enhance the safety of urgent invasive procedures that could otherwise pose life-threatening risks in the absence of adequate prophylactic measures. Nevertheless, these potential economic and clinical benefits require further investigation and more comprehensive validation.

Our study demonstrated a statistically significant improvement in disease control three months after the initiation of lanadelumab therapy, as reflected by the AECT scores (Fig. 4). Notably, this enhanced level of control was maintained between the third and sixth months, indicating that lanadelumab not only achieves rapid stabilization of disease activity but also sustains it over time. Across all assessment points, the proportion of patients achieving high levels of disease control ranged from 89% to 100% according to AECT results.

HAE significantly burdens patients' lives. Highly effective drugs like danazol have been in used for this condition; however, they do not improve patient's overall quality of life given the development of adverse events. Many patients stop LTP in routine life because the burden of the medication intake may disturb even greater than the disease itself. Therefore, upon evaluating the effectiveness of therapy in real-world practice, we paid great attention to assessing quality of life. In our study lanadelumab therapy resulted in a significant enhancement in quality of life after only 3 months, the result comparable to the HELP study and in other cohort studies [5,7,22].

One of the most important practical issues regarding lanadelumab therapy is short-term prophylaxis before invasive interventions. According to the HELP and HELP OLE studies, the number of invasive procedures resulting in HAE attacks was comparable in patients who used short-term prophylaxis and those who did not. In our study, 36 interventions were performed without STP with HAE attack developing only in two instances, while 7 interventions were performed with STP with one following HAE attack. The data obtained is insufficient to advise against STP. However, prolonged observation period may change the approaches to STP in patients receiving lanadelumab with complete control over the disease symptoms and a stable access to acute attack management drugs in sufficient quantities. Regardless, the access to acute attack management drugs is mandatory [6,7].

Our findings confirm that lanadelumab maintains a favorable safety profile, consistent with previously published data [5,7]. All observed adverse events corresponded to those described in the product information and were classified as mild. Importantly, none of these events interfered with patients' ability to perform self-administration.

This study represents the first real-world investigation of lanadelumab use in routine clinical practice in the Russian Federation. The present report summarizes findings from a 24-month observation of 30 patients, demonstrating both the effectiveness and safety of lanadelumab in a real-world setting. In addition to confirming its clinical benefits, our results provide valuable insights into practical aspects of therapy, including strategies for extending dosing intervals, the safety of medical and dental interventions performed during treatment, and the feasibility of home-based self-administration.

Conclusion

Current HAE management guidelines identify the primary therapeutic goals as complete prevention of attacks, sustained disease control, and preservation of patients' quality of life [8]. Lanadelumab—approved globally in 2018 and in Russia in 2021—has demonstrated, through its convenient subcutaneous administration and favorable safety profile, a strong capacity to meet these objectives. Our findings further support its role as an effective and well-tolerated long-term prophylactic option in routine clinical practice.

Study Limitations

As HAE is a rare (orphan) disease, a formal sample size calculation was not possible to perform due to the limited number of available patients. Consequently, all individuals with HAE caused by C1-inhibitor deficiency who met the inclusion criteria were enrolled. While this approach enabled the inclusion of the entire accessible patient population, it inherently restricts the representativeness of the sample and limits the generalizability

of the findings to broader HAE populations beyond the study cohort.

Supplementary materials

The Supplementary information includes:

• Supplementary Table S1. Data about the patients enrolled in the study;

• Supplementary Table S2. Analysis of baseline and prior long-term prophylactic therapies before initiation of lanadelumab.

This supplemental materials have been provided by the authors to give readers additional information about their work.

The file can be accessed using: https://www.editorialpark.com/download/article-supp/768/Supplementary-materials.docx.

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Placenta-Derived Mesenchymal Stem Cells in Chronic Kidney Disease via NADPH Oxidase

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Abstract

Introduction: Chronic kidney disease (CKD) is associated with gradual deterioration of renal structure and function, accompanied by persistent fibrosis and oxidative imbalance. Excessive generation of reactive oxygen species (ROS), particularly through NADPH oxidase pathways, contributes to inflammatory and profibrotic signaling during disease progression. This study investigated whether human placenta-derived mesenchymal stem cells (MSCs) could modulate oxidative stress and fibrotic responses in an experimental CKD model.

Methods: A subtotal nephrectomy model was used to establish CKD in rats. Following disease induction, placenta-derived MSCs were administered intravenously. Renal tissues were evaluated for markers associated with oxidative injury, including ROS, hydrogen peroxide (H_2O_2), and NADPH oxidase-4 (NOX4). Fibrosis-related molecules, including fibronectin, collagen IV, and α-smooth muscle actin (α-SMA), were also assessed and compared among study groups.

Results: MSC administration was associated with reductions in oxidative stress markers, including ROS, H_2O_2, and NOX4 expression. A trend toward reduced expression of fibrosis-related markers was also observed following MSC treatment; however, reductions in protein expression did not reach statistical significance for all comparisons.

Conclusion: Placenta-derived MSC treatment was associated with modulation of oxidative stress– and fibrosis-related pathways in experimental CKD. Although reductions in several molecular markers were observed following treatment, additional studies with larger sample sizes and longer follow-up periods are needed to confirm the therapeutic potential of MSCs and to further clarify their effects on renal fibrosis and disease progression.

Keywords: mesenchymal stem cells; chronic kidney disease; NADPH oxidase 4; oxidative stress; renal fibrosis.

Introduction

Mesenchymal stem cells (MSCs) have become an important focus of regenerative medicine because of their capacity to participate in tissue repair and restoration of cellular balance. These cells exhibit self-renewal properties and are capable of differentiating into multiple mesenchymal lineages under appropriate microenvironmental conditions. In addition to differentiation, MSCs exert biological effects through paracrine signaling, releasing growth factors, cytokines, and extracellular vesicles that contribute to regulation of inflammation, apoptosis, and tissue remodeling [1–3].

Among the available MSC sources, placental tissues have attracted considerable interest because they are easily obtainable, associated with minimal ethical concerns, and characterized by relatively low immunogenicity. Placenta-derived MSCs demonstrate high proliferative potential and may provide a rich source of progenitor cells suitable for regenerative applications. Experimental evidence suggests that these cells can influence tissue repair through immunomodulatory and antifibrotic actions. Previous investigations in renal injury models have further demonstrated that MSC-based interventions may reduce inflammation, improve renal morphology, and attenuate fibrotic progression in chronic kidney disease [4–9].

Chronic kidney disease (CKD) is a progressive disorder associated with gradual deterioration of renal structure and function. Persistent injury within the kidney promotes extracellular matrix accumulation, tubular

remodeling, and irreversible tissue damage. Fibrosis represents a major pathological process in CKD and contributes substantially to nephron loss and declining renal performance. As disease severity increases, structural alterations within renal tissue become increasingly resistant to endogenous repair mechanisms [10–11].

Oxidative stress is widely recognized as an important contributor to CKD progression. Excessive generation of ROS disrupts intracellular signaling pathways and enhances inflammatory activation. ROS accumulation may also accelerate cellular injury by promoting lipid peroxidation, mitochondrial dysfunction, and extracellular matrix deposition. Among enzymatic systems involved in ROS production, NADPH oxidases have been identified as major regulators of redox signaling within renal tissue [12–13].

NOX4 is the predominant NADPH oxidase isoform expressed in the kidney and has been linked to multiple forms of renal injury. Increased NOX4 activity has been associated with enhanced oxidative burden, activation of profibrotic pathways, and progression of tubulointerstitial damage. Experimental observations indicate that NOX4 contributes to extracellular matrix accumulation and myofibroblast activation, partly through interactions with transforming growth factor-beta–related signaling mechanisms [14–20]. Suppression of NOX4 activity has been reported to reduce oxidative injury and alleviate fibrotic remodeling in nephropathy models [21].

Given the close relationship between oxidative stress, fibrosis, and CKD progression, therapeutic strategies targeting these mechanisms may offer meaningful clinical benefit. The present study was designed to investigate the effects of human placenta-derived mesenchymal stem cells on oxidative stress markers and fibrosis-related molecular pathways in a nephrectomy-induced CKD rat model, with particular emphasis on NOX4 expression and renal fibrogenesis.

Methods

Study Design and Experimental Animals

An experimental chronic kidney disease (CKD) model was established using 42 male Wistar rats weighing 250–300 g. Animals were obtained from the institutional Experimental Animal Research Unit and housed under standardized laboratory conditions (20–22°C, 12-h light/12-h dark cycle) with free access to standard pellet chow and tap water throughout the study.

All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals and complied with internationally accepted principles for animal research. The study protocol was approved by the Institutional Experimental Animal Ethics Committee (Approval No. 74; December 3, 2018).

The animals were assigned to six experimental groups (n = 7 per group): (1) Sham-operated control (SHAM), (2) nephrectomy (NEPH), (3) nephrectomy followed for 15 days (NEPH+15), (4) nephrectomy followed for 30 days (NEPH+30), (5) nephrectomy followed by MSC treatment and evaluated after 15 days (MSC+15), and (6) nephrectomy followed by MSC treatment and evaluated after 30 days (MSC+30).

Isolation and Culture of Human Placenta-Derived Mesenchymal Stem Cells

Human term placental tissues were obtained from the Department of Obstetrics and Gynecology of a university hospital following written informed consent. The amniotic membrane was carefully separated from the chorionic membrane under sterile conditions and repeatedly washed with physiological saline containing penicillin/streptomycin and amphotericin B to minimize microbial contamination.

The amniotic membrane was cut into approximately 3 × 3 cm fragments and incubated with dispase to facilitate epithelial cell removal. Tissue digestion was subsequently performed using collagenase and DNase in RPMI 1640 medium supplemented with fetal bovine serum (FBS) and L-glutamine. The resulting cell suspension was filtered through a 100-μm cell strainer and centrifuged to obtain the cellular pellet.

The recovered cells were cultured in mesenchymal stem cell growth medium and maintained at 37°C in a humidified atmosphere containing 5% CO_2. Culture medium was replaced at regular intervals, and adherent cells were expanded through serial passaging until sufficient cell numbers were obtained for transplantation.

Flow Cytometric Characterization

The immunophenotypic characteristics of the isolated cells were evaluated by flow cytometry. Cells exhibited positive expression of the mesenchymal stem cell markers CD44, CD73, CD90, and CD105, whereas hematopoietic markers (CD34, CD45, CD14, CD19, and HLA-DR) were not expressed. These findings confirmed the mesenchymal phenotype of the isolated cells and were consistent with the minimal criteria established by the International Society for Cellular Therapy (ISCT) for mesenchymal stem cells.

Induction of Chronic Kidney Disease and Experimental Groups

Chronic kidney disease was induced using a 5/6 subtotal nephrectomy model. Animals were assigned to six experimental groups as described above. Human placenta-derived mesenchymal stem cells (1×10^6 cells) suspended in 500 μL Dulbecco's Modified Eagle Medium (DMEM) were administered via tail vein injection to the treatment groups.

Because human-derived MSCs were transplanted into rats, cyclosporine A was administered subcutaneously at a dose of 1 mg/day beginning one day before cell transplantation and continued for seven days thereafter to minimize xenogeneic immune rejection.

Twenty-four-hour urine samples were collected using metabolic cages prior to sacrifice. At the end of each experimental period, animals were anesthetized with ketamine hydrochloride and 2% xylazine. Blood samples were collected from the abdominal artery, after which the animals were euthanized. Kidney tissues were harvested, immediately stored at −80°C for subsequent biochemical and molecular analyses, and serum samples were separated by centrifugation and stored at −20°C until analysis.

Western Blot Analysis

Renal tissue samples were homogenized in lysis buffer supplemented with protease inhibitor cocktail. Protein concentrations were determined prior to electrophoresis, and equal amounts of protein were separated by SDS–PAGE and transferred onto nitrocellulose membranes. Membranes were blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBS-T) and incubated overnight at 4°C with primary antibodies against NOX4, fibronectin, collagen IV, and α-smooth muscle actin (α-SMA). Following incubation with the appropriate horseradish peroxidase-conjugated secondary antibodies, protein bands were visualized using enhanced chemiluminescence. Densitometric analysis was

performed using Alpha DigiDoc 1000 software (Alpha Innotech Corporation, San Leandro, CA, USA), and protein expression levels were normalized to β-actin.

Quantitative Real-Time PCR Analysis

Total RNA was isolated from renal tissue using TRIzol reagent, and complementary DNA (cDNA) was synthesized from the extracted RNA. Quantitative real-time PCR was performed using SYBR Green chemistry on a QuantStudio™ 3 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Gene expression levels of NOX4, fibronectin, collagen IV, and α-SMA were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and relative expression was calculated using the 2-ΔΔCt method. Melting curve analysis was performed to verify amplification specificity.

Assessment of Oxidative Stress Parameters

Hydrogen peroxide (H_2O_2) levels in renal tissue homogenates were determined using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Thermo Fisher Scientific, Cat. No. A22188) according to the manufacturer's instructions. Reactive oxygen species (ROS) production was evaluated using the OxiSelect™ In Vitro ROS/RNS Assay Kit (Cell Biolabs, Inc., San Diego, CA, USA). Fluorescence measurements were obtained using a microplate reader, and values were normalized to tissue protein concentration.

Urinary Albumin-to-Creatinine Ratio

Urinary albumin-to-creatinine ratio (ACR) was determined using the Albumin-to-Creatinine Ratio Assay Kit (BioVision, Cat. No. K551-100) according to the manufacturer's instructions. Albumin and creatinine concentrations were measured separately, and the final ACR was expressed as mg/g creatinine.

Outcome Measures

The primary outcome measures were oxidative stress–related parameters, including NOX4 expression, reactive oxygen species (ROS), and hydrogen peroxide (H_2O_2) levels. Secondary outcome measures included fibrosis-related markers (fibronectin, collagen IV, and α-smooth muscle actin [α-SMA]) and urinary albumin-to-creatinine ratio (ACR).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Data are presented as mean ± SEM. Comparisons among experimental

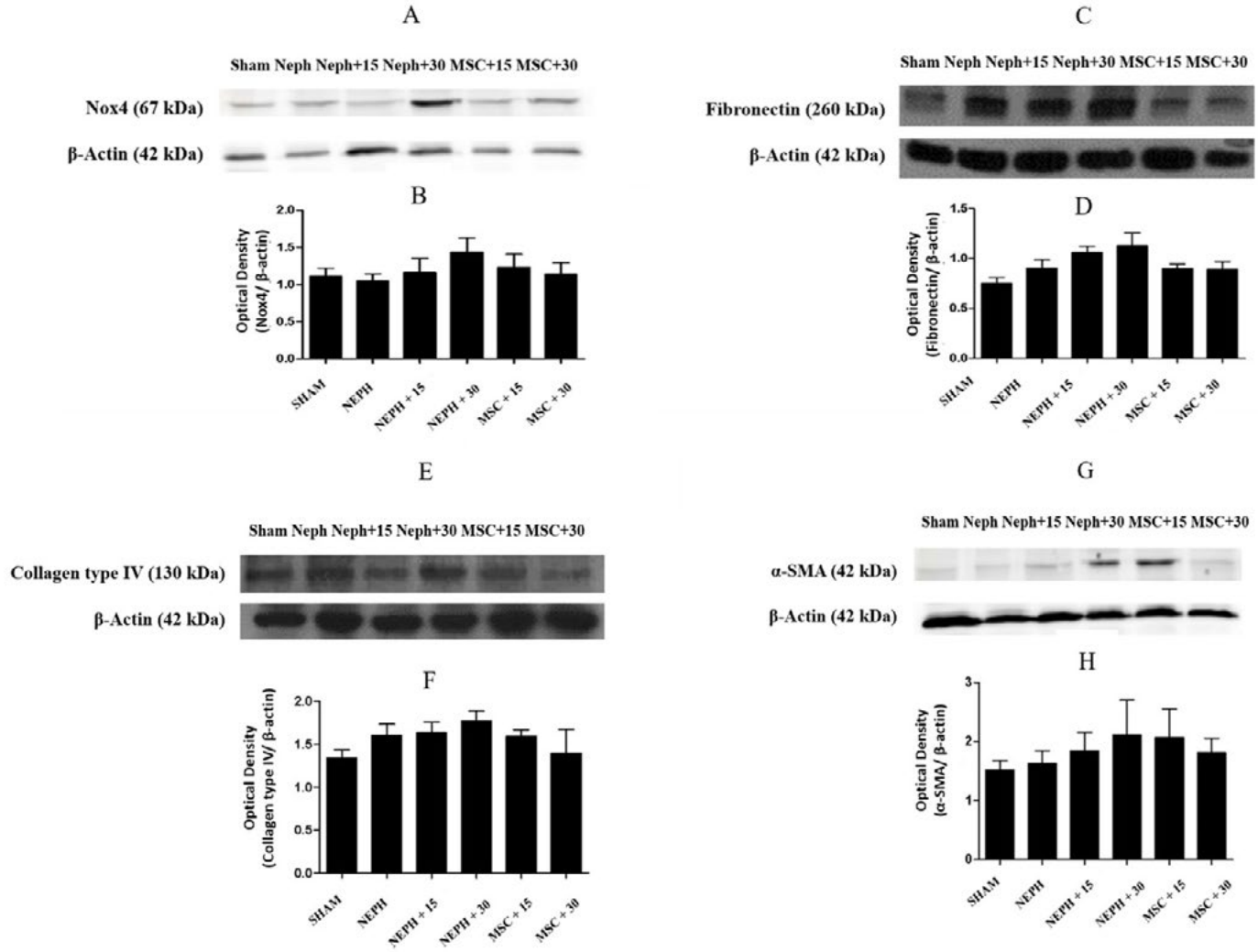

Figure 1 – Western blot analysis of oxidative stress– and fibrosis-related proteins in renal tissues from the experimental groups. Representative western blot images showing protein expression of (A) NOX4, (C) fibronectin, (E) collagen IV, and (G) α-SMA. Corresponding densitometric analyses normalized to β-actin are presented in (B), (D), (F), and (H), respectively.

Data are presented as mean ± SEM (n = 7 per group). Statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. Abbreviations: NOX4, NADPH oxidase 4; α-SMA, alpha-smooth muscle actin.

groups were performed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. Western blot densitometric analyses were performed using Alpha DigiDoc 1000 software (Alpha Innotech Corporation, San Leandro, CA, USA). A two-sided p value < 0.05 was considered statistically significant.

Results

Isolation and Characterization of Placenta-Derived Mesenchymal Stem Cells

Isolated human placenta-derived mesenchymal stem cells exhibited the characteristic spindle-shaped, fibroblast-like morphology and maintained stable growth throughout serial passaging. Flow cytometric analysis confirmed the mesenchymal immunophenotype of the cultured cells, demonstrating positive expression of CD44, CD73, CD90, and CD105, while hematopoietic markers (CD34, CD45, CD14, CD19, and HLA-DR) were not expressed. These findings confirmed that the isolated cells fulfilled the phenotypic characteristics of mesenchymal stem cells.

Protein Expression Analysis by Western Blot

Western blot analysis demonstrated increased expression of NOX4, fibronectin, collagen IV, and α-SMA in nephrectomy groups compared with the SHAM group, with the greatest increases generally observed in animals evaluated at the 30-day time point. Although MSC-treated groups showed lower protein expression levels than the corresponding nephrectomy groups, these reductions did not reach statistical significance for all comparisons. Representative western blot images and corresponding densitometric analyses are presented in Figure 1.

Gene Expression Analysis

Quantitative real-time PCR analysis demonstrated significant alterations in the expression of oxidative stress- and fibrosis-related genes among the experimental groups (Figure 2).

NOX4: NOX4 mRNA expression was significantly increased following nephrectomy, with the highest expression observed in the NEPH+30 group compared with the SHAM group. MSC treatment was associated with lower NOX4 mRNA expression at both 15- and 30-day evaluation time points. Significant differences were identified for selected pairwise

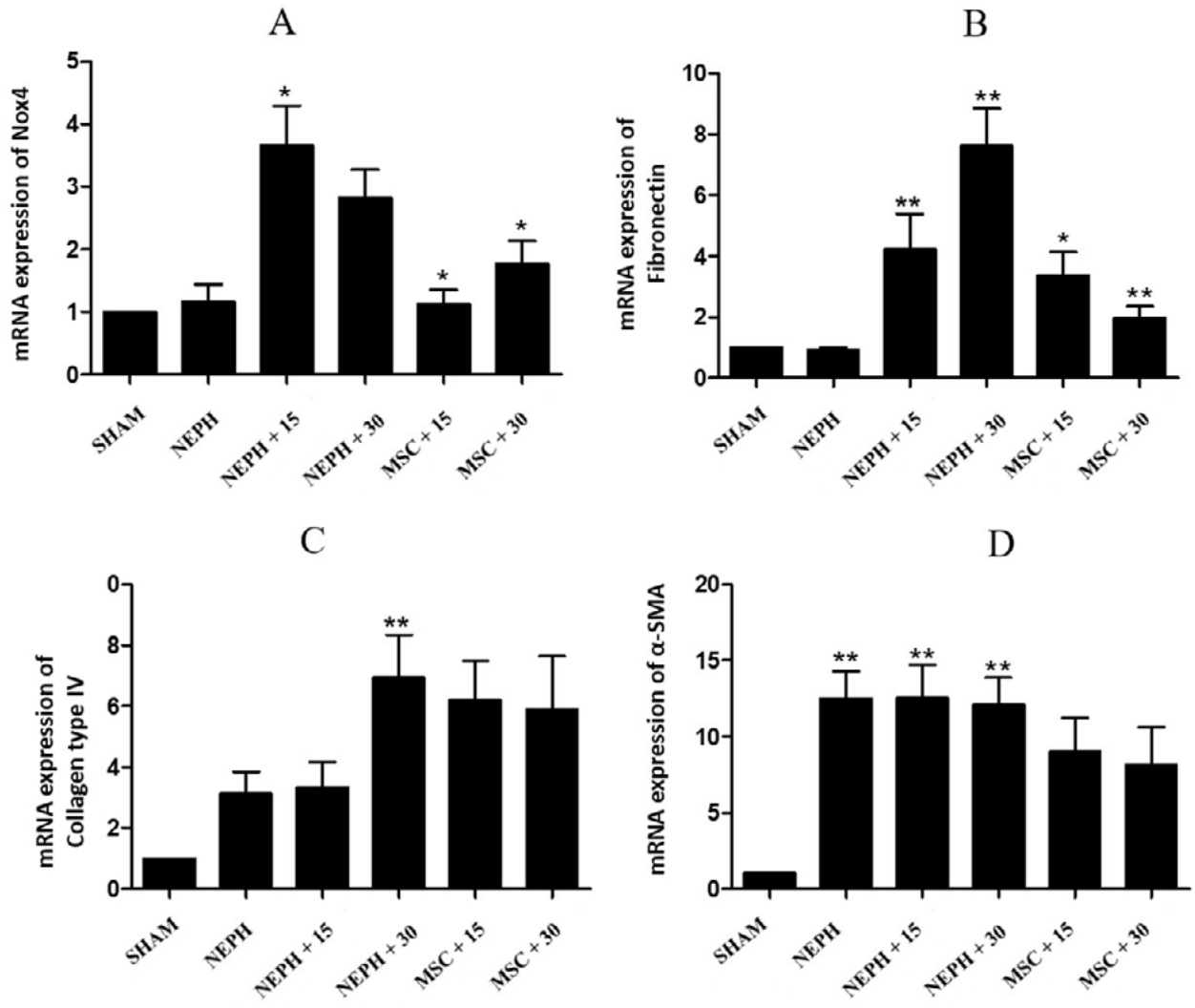

Figure 2 – Relative mRNA expression levels of oxidative stress– and fibrosis-related genes determined by quantitative real-time PCR. (A) Relative NOX4 mRNA expression, (B) fibronectin mRNA expression, (C) collagen IV mRNA expression, and (D) α-SMA mRNA expression.

*Gene expression levels were normalized to GAPDH and calculated using the 2^−ΔΔCt method. Data are presented as mean ± SEM (n = 7 per group). Statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. Asterisks indicate statistically significant differences compared with the SHAM group (*p < 0.05, **p < 0.001). Abbreviations: NOX4, NADPH oxidase 4; α-SMA, alpha-smooth muscle actin.*

comparisons, whereas differences between MSC-treated and the corresponding nephrectomy groups were not statistically significant in all comparisons (Figure 2A).

Fibronectin: Fibronectin mRNA expression was elevated in nephrectomized animals relative to SHAM controls. MSC-treated groups exhibited lower fibronectin expression than the corresponding nephrectomy groups, with the greatest reduction observed in the MSC+30 group. However, statistically significant differences were observed only in selected pairwise comparisons (Figure 2B).

Collagen IV: Collagen IV mRNA expression increased following nephrectomy, particularly in the NEPH+30 group. Although MSC treatment was associated with lower collagen IV expression, statistically significant differences were limited to specific pairwise comparisons (Figure 2C).

α-SMA: α-SMA mRNA expression was significantly increased in nephrectomy groups compared with the SHAM group. Lower α-SMA expression was observed following MSC treatment at both evaluation time points; however, these reductions did not reach statistical significance for all pairwise comparisons (Figure 2D).

Oxidative Stress Parameters

Renal H_2O_2 levels were significantly increased in nephrectomized animals compared with the SHAM group. MSC treatment was associated with lower H_2O_2 levels, with a statistically significant reduction observed in the MSC+30 group relative to the SHAM group (Figure 3A).

ROS production showed a similar overall pattern. Nephrectomy was associated with increased oxidative stress, whereas MSC-treated animals exhibited lower ROS levels than the corresponding nephrectomy groups. However, these differences did not reach statistical significance in all pairwise comparisons (Figure 3B).

Urinary Albumin-to-Creatinine Ratio

Urinary ACR was significantly increased in nephrectomized animals compared with the SHAM group, indicating impaired renal function. MSC-treated groups demonstrated lower ACR values than the corresponding nephrectomy groups at both evaluation time points. A statistically significant reduction in urinary ACR was observed following MSC treatment, suggesting partial preservation of renal function (Figure 3C).

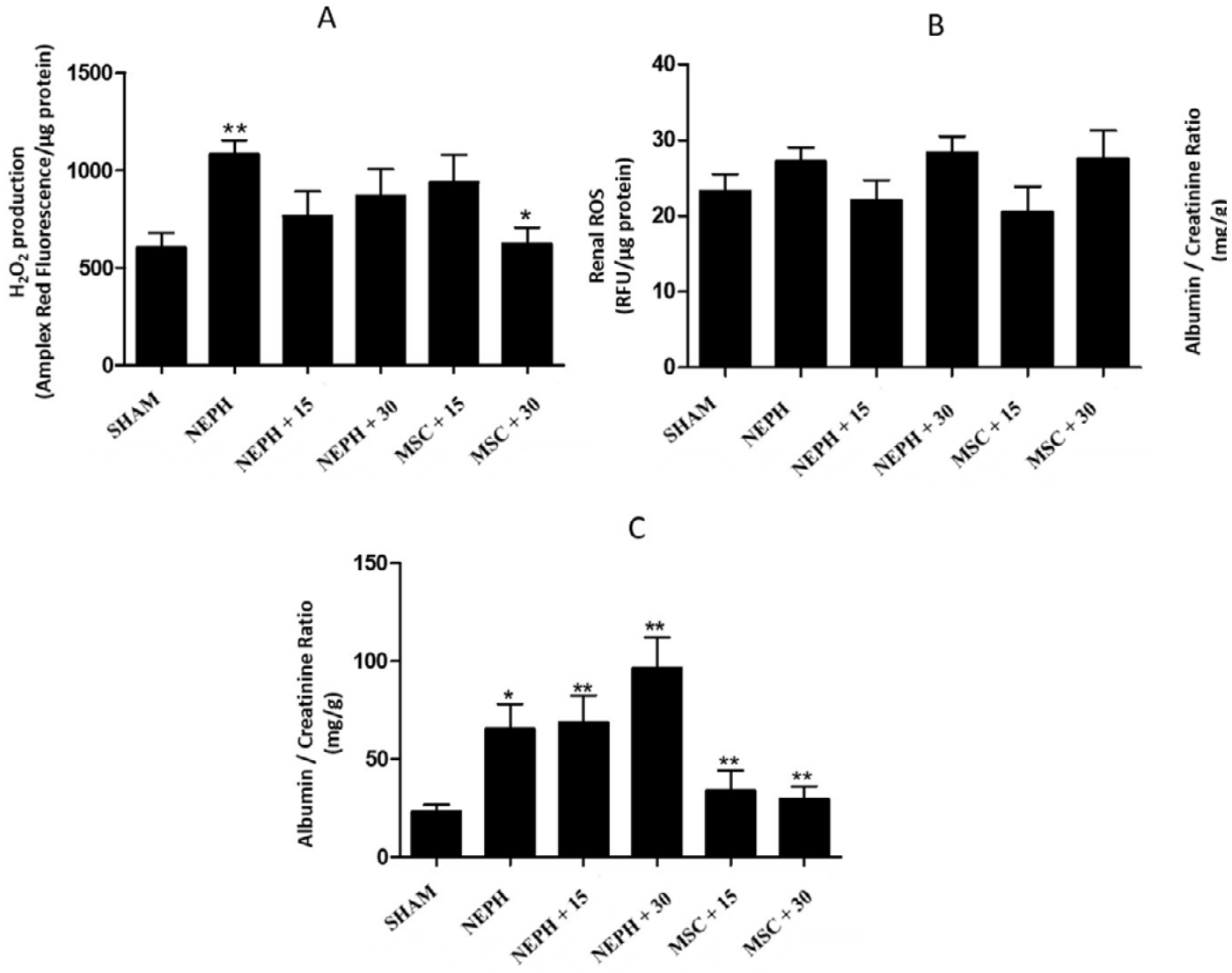

Figure 3 – Oxidative stress parameters and urinary albumin-to-creatinine ratio in the experimental groups. (A) Renal hydrogen peroxide (H_2O_2) levels determined using the Amplex Red assay. (B) Renal reactive oxygen species (ROS) levels. (C) Urinary albumin-to-creatinine ratio (ACR). Data are presented as mean ± SEM (n = 7 per group).

*Statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. Asterisks indicate statistically significant differences compared with the SHAM group (*p < 0.05, **p < 0.001). Abbreviations: NOX4, NADPH oxidase 4; α-SMA, alpha-smooth muscle actin.*

Discussion

The present study investigated the effects of human placenta-derived mesenchymal stem cells on oxidative stress and fibrosis in a nephrectomy-induced chronic kidney disease model. The findings demonstrated that MSC administration was associated with reduced oxidative stress, reflected by lower ROS and H_2O_2 levels together with modulation of NOX4 expression. In addition, MSC treatment was associated with trends toward reduced expression of fibrosis-related markers, although corresponding protein-level changes did not reach statistical significance in all comparisons. Furthermore, lower urinary albumin-to-creatinine ratios observed in MSC-treated animals suggested partial preservation of renal function.

Chronic kidney disease is characterized by progressive structural and functional deterioration of renal tissue. Persistent oxidative stress, inflammatory signaling, and extracellular matrix accumulation contribute to ongoing renal injury and fibrotic remodeling. These mechanisms interact throughout disease progression, ultimately leading to nephron loss and irreversible decline in kidney function [10–13].

Mesenchymal stem cells have been increasingly investigated as regenerative candidates because of their immunomodulatory and reparative properties. Although their differentiation potential remains important, accumulating evidence indicates that many therapeutic effects of MSCs are mediated through paracrine mechanisms rather than direct tissue replacement. Secreted cytokines, growth factors, and extracellular vesicles contribute to suppression of inflammation, regulation of fibrosis, and promotion of endogenous repair pathways [1–3].

Placenta-derived MSCs represent an attractive source for experimental and translational applications because placental tissue is abundant, readily accessible, and associated with fewer ethical concerns than many alternative stem cell sources. In addition, placenta-derived MSCs exhibit low immunogenicity and high proliferative capacity, supporting their potential use in regenerative strategies targeting chronic organ injury [4–6].

One of the principal findings of the present study was the modulation of NOX4 expression following MSC treatment. NOX4 is highly expressed in renal tissue and plays a central role in reactive oxygen species generation. Increased NOX4 activity has been associated with oxidative injury, mitochondrial dysfunction, and activation of profibrotic signaling pathways in chronic kidney disease [12–15]. In the present study, nephrectomy was associated with increased NOX4 expression, whereas MSC treatment was accompanied by lower NOX4 expression, supporting a potential role for MSCs in the regulation of renal redox homeostasis.

Excessive ROS generation promotes inflammatory activation and extracellular matrix accumulation within damaged renal tissue. Consistent with previous reports, nephrectomy-induced CKD was associated with elevated ROS and H_2O_2 levels. MSC treatment was associated with lower oxidative stress parameters, suggesting that MSCs may contribute to restoration of redox balance within injured renal tissue [22–24].

Fibrosis-related pathways were also influenced by MSC treatment. Elevated expression of fibronectin, collagen IV, and α-SMA in nephrectomy groups reflected extracellular matrix accumulation and myofibroblast activation characteristic of progressive renal fibrosis. Although lower expression of these markers was observed following MSC administration, corresponding protein-level changes did not reach statistical significance in all comparisons. This apparent discrepancy between gene and protein expression may reflect differences in post-transcriptional regulation, protein turnover, translational efficiency, and the temporal dynamics of mRNA and protein expression. Therefore, the present findings should be interpreted as evidence of partial modulation of profibrotic pathways rather than definitive suppression of renal fibrosis.

The interaction between NOX4 activity and transforming growth factor-β (TGF-β)-associated signaling has been highlighted in previous experimental studies. Increased NOX4 signaling amplifies fibrotic responses by promoting extracellular matrix protein synthesis and myofibroblast activation [16–20]. Furthermore, NOX4-dependent redox signaling contributes to extracellular matrix deposition and progressive renal scarring [24–26]. The lower NOX4 expression observed following MSC treatment therefore supports the hypothesis that MSCs may interfere with oxidative stress-related profibrotic signaling during CKD progression.

In addition to the molecular findings, lower urinary albumin-to-creatinine ratios were observed in MSC-treated animals. Albuminuria is a well-established marker of glomerular injury and an important predictor of CKD progression. The reduction in urinary ACR observed following MSC treatment suggests partial preservation of renal function and supports the biological relevance of the molecular findings.

Several limitations of the present study should be acknowledged. First, the relatively small sample size may have limited the statistical power to detect significant differences for some outcome measures. Second, only selected oxidative stress and fibrosis-related markers were evaluated. Future studies incorporating inflammatory mediators, mitochondrial function, and long-term tracking of transplanted cells would provide a more comprehensive understanding of the underlying mechanisms. In addition, only a single MSC dose and a relatively short observation period were investigated.

Because human placenta-derived MSCs were transplanted into rats, low-dose cyclosporine A was administered to minimize xenogeneic immune rejection. However, a nephrectomy plus cyclosporine A control group was not included; therefore, the independent contribution of cyclosporine A to the observed findings cannot be completely excluded. Furthermore, detailed maternal and pregnancy-related donor characteristics of the placental tissues, including maternal age, gestational age, and pregnancy-related clinical information, were not available. Since donor characteristics may influence the biological properties of placenta-derived MSCs, future studies should include more comprehensive donor characterization.

Despite these limitations, the present findings suggest that placenta-derived MSCs may modulate oxidative stress–related pathways in experimental CKD and may partially influence fibrosis-associated molecular responses. Although reductions in fibrosis-related protein expression did not reach statistical significance in all comparisons, the overall molecular findings, together with the reduction in urinary albumin-to-creatinine ratio, support further investigation of MSC-based therapies in chronic kidney disease. Additional experimental and translational studies involving larger cohorts and longer follow-up periods are warranted to further clarify the therapeutic potential and clinical applicability of this approach.

Conclusion

The findings of the present study suggest that human placenta-derived mesenchymal stem cells may modulate oxidative stress– and fibrosis-related pathways in experimental chronic kidney disease. MSC treatment was associated with

lower oxidative stress parameters, modulation of NOX4 expression, and trends toward reduced expression of fibrosis-related markers. Although reductions in fibrosis-related protein expression did not reach statistical significance in all comparisons, the overall findings suggest that MSC therapy may partially influence molecular pathways involved in CKD progression.

The observed reduction in urinary albumin-to-creatinine ratio further supports a potential beneficial effect of MSC treatment on renal injury in nephrectomy-induced CKD. Nevertheless, additional studies involving larger experimental cohorts, longer follow-up periods, broader mechanistic investigations, and well-controlled experimental designs are required to confirm these findings and to further clarify the therapeutic potential and translational applicability of MSC-based interventions in chronic kidney disease.

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Identification of an RFC4, CKS2, MCM5 Genes Based Prognostic Signature in Cervical Cancer Using Systems Biology and Machine Learning

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ABSTRACT

Introduction: Among women, cervical cancer continues to be a leading source of morbidity and death, yet there are few accurate prognostic markers. The study was performed to create a good prognostic molecular signature for cervical cancer by utilizing systems-level transcrip-tome analysis and machine learning methodologies together.

Methods: To find genes that were differentially expressed in TCGA-CESC (n=306 tumors,13 normals) and GEO GSE39001(39 tumors, 4 normals), GSEA was used to analyse gene expres-sion datasets, then WGCNA was done to find disease-related modules. The most crucial genes were then identified through analysis of protein-protein interactions. LASSO and Random For-est machine learning approaches were used to obtain a minimum prognostic gene set, external-ly validated via Kaplan-Meier survival analysis, time-dependent ROC, and Cox regression in TCGA-CESC. Immune infiltration correlations were determined, and possible therapeutic drugs were found by using 3D protein modelling with molecular docking.

Results: A variety of genes with differential expression were able to successfully differentiate cervical cancer samples from healthy cells (5,097 DEGs: 2,691 up, 2,406 down). The pathways linked to immunological responses, DNA replication, and cell cycle regulation were the most abundant in tumor-associated co-expression modules (r>0.7 correlation with cancer). RFC4, CKS2, and MCM5 were found to be important hub genes by combining DEG, WGCNA, and PPI studies (top-5 ranked across 4 centrality metrics).

Conclusion: In the TCGA-CESC cohort, the three-gene prognostic model served as an inde-pendent prognostic indicator and effectively classified patients into high- and low-risk catego-ries. Docking experiments showed substantial binding affinities, especially for RFC4, suggest-ing its potential relevance as a therapeutic target in cervical cancer, and gene expression levels were closely associated with immune cell infiltration.

Keywords: Cervical cancer, Gene expression profiling, Prognostic signature, TCGA-CESC, Therapeutic compounds.

Introduction

Among women worldwide, cervical cancer continues to rank among the top causes of cancer-related morbidity and mortality [1–3]. Although screening programs and prophylactic HPV vaccination have evolved, cervical cancer is still a serious public health

concern because of late diagnosis, tumour heterogeneity, and a lack of therapeutic options for advanced-stage disease. Despite being a recognised etiological factor, persistent infection with high-risk HPV genotypes, notably HPV16 and HPV18, is unable to produce malignant transformation on its own [4,5]. The cervical carcinogenesis is a multistep process involving complex interactions between viral oncogenes and host cellular pathways, ultimately leading to deregulated gene expression, genomic instability, and malignant progression. At molecular level, cervical cancer is characterized by profound alterations in transcriptional programs governing cell cycle regulation, DNA replication, apoptosis, immune evasion, and metabolic reprogramming [6]. Viral oncoproteins E6 and E7 target p53 and retinoblastoma (RB) proteins, respectively, disrupting tumour suppressor pathways and causing chromosomal instability and unchecked cell growth. Identifying robust molecular signatures and regulatory networks underlying cervical cancer is therefore critical for improving early diagnosis, prognostic stratification, and therapeutic targeting [7,8].

High-throughput transcriptomic profiling has emerged as a powerful approach for dissecting cancer-associated molecular alterations at a genome-wide scale [9,10]. Openly available microarray and RNA sequencing datasets have enabled the systematic investigation of differentially expressed genes (DEGs) between tumour and normal tissues, providing valuable information on the biology of cervical cancer. Higher-order interactions among genes working in coordinated networks may be missed by conventional DEG-based analysis, which frequently concentrate on individual genes. Given the complex and multifactorial nature of cancer, systems-level approaches are required to uncover biologically meaningful gene modules and pathways that collectively drive disease phenotypes [11,12]. Principal Component Analysis (PCA) is the widely used tool of unsupervised dimensionality reduction technique for assessing global transcriptional variation across different biological conditions [13]. Weighted Gene Co-expression Network Analysis (WGCNA) is a systems biology technique that has been created to discover modules—groups of highly co-expressed genes—and link them to phenotypic or clinical characteristics[14,15]. Gene Set Enrichment Analysis (GSEA) provides complementary insights by evaluating whether predefined gene sets—representing biological pathways or functional categories—are significantly enriched between phenotypic states [16]. Integration of KEGG canonical pathways and Gene Ontology (GO) biological processes through GSEA facilitates a deeper understanding of the molecular programs activated or suppressed in cancer. Dysregulation of these pathways contributes to uncontrolled proliferation, genomic instability, and resistance to therapy. Conversely, pathways associated with endocytosis, neurodegeneration-related signaling, and cellular homeostasis may reflect context-dependent transcriptional states or compensatory mechanisms [17,18]. However, the coordinated regulation of these pathways at the network level and their relationship to disease status require further clarification.

By integrating unsupervised clustering, network-based analysis, and pathway enrichment approaches, this study aims to provide a comprehensive systems-level view of the transcriptional architecture underlying cervical cancer [19,20]. The identification of key gene modules, hub genes, and dysregulated pathways not only enhances our understanding of cervical cancer biology but also offers a rational framework for biomarker discovery and therapeutic target prioritization [21,22]. Unlike other single-gene approaches, this study innovatively combines WGCNA, machine learning techniques (LASSO & Random Forest), and PPI network analysis from both GEO and TCGA-CESC datasets to build and verify a minimal three-gene prognostic signature for cervical cancer. Collectively, our findings contribute to the growing body of evidence supporting network-driven strategies for elucidating complex cancer mechanisms and advancing precision oncology. In parallel, machine learning advances have provided the powerful tools needed to extract clinically meaningful signatures from high-dimensional omics data. Feature selection can be carried out, along with handling complex non-linear relationships, using algorithms such as LASSO, Random Forest that construct parsimonious prognostic models with improved interpretability [23–25]. When applied to cancer transcriptomes, these methods are able to reduce thousands of genes into a small set of robust predictors that stratify patients by risk and suggest potential therapeutic targets. A combined bioinformatics and machine-learning framework that could identify cervical cancer hub genes and construct a minimal prognostic molecular signature was applied in this study.

Methods

Datasets analyzed: GSE39001 (n=43: 39 tumors, 4 normals) microarray dataset and TCGA-CESC (n=319: 306 tumors, 13 normals) RNA-seq cohort with matched survival data.

Data Acquisition

GSE39001, the TCGA-CESC cohort, and the Gene Expression Omnibus (GEO) database were used to derive gene-expression profiles of cervical cancer and normal cervical tissues [26]. TCGA-CESC RNA-seq data was accessed via TCGAbiolinks R package (version 2.24.2) from GDC portal on March 15, 2025, utilizing Firehose legacy v2015 standardized processing (n=306 tumors, 13 normal).GSE39001 was the discovery set, while TCGA-CESC RNA-seq data with matched clinicopathological and survival information were used for external validation [27]. Imbalance addressed via PCA quality control and TCGA-CESC (n=306) independent validation. Raw data were downloaded from GEO query and TCGA bioLinks, log2-transformed or converted to TPM accordingly; samples without complete expression or clinical data were excluded from further analyses.

Principal component analysis

To assess sample quality and global variance structure, the normalised expression matrices were subjected to principal component analysis (PCA). Using the GSE39001 dataset, the plots were created in R to show the grouping of tumour and normal samples and identify any possible outliers that were eliminated prior to the DEG and network analysis that followed.

DEG analysis

The limma package in R was utilised to conduct a differential gene expression study between cervical cancer and normal cervical tissues [28]. The Gene Expression Omnibus (GEO) databases made the microarray dataset GSE39001 freely accessible. Quantile normalisation was applied after background correction to raw expression data in order to reduce technical variability and guarantee sample comparability. The appropriate platform annotation file was used to match probe identifiers to corresponding gene symbols. The average expression value was then computed when numerous probes corresponded to the same gene. Each gene's normalised expression data was fitted to linear models created from a

design matrix that modelled the experimental settings. Using empirical Bayes moderation on the standard errors of the estimated log2 fold changes improved statistical dependability. Table 2 shows the dataset and the parameters that were deemed substantially differentially expressed genes (DEGs) according to a Benjamini–Hochberg false discovery rate (FDR)–adjusted p-value < 0.05 and an absolute log2 fold change (|log2FC|) higher than the predetermined threshold. In order to show the overall distribution of fold changes and statistical significance, the resulting DEGs were visualised using volcano plots. Based on the most significant DEGs, heatmaps were created to show sample grouping and expression patterns.

Weighted gene co-expression network analysis

Using the GSE39001 dataset, a scale-free gene co-expression network was created using the Weighted Gene Co-expression Network Analysis (WGCNA) program. Genes were pre-filtered by variance (top 50% most variable across samples) and absolute expression (FPKM > 1 in ≥80% samples) to reduce noise and improve network robustness. High expression variability across samples was a criterion for choosing genes in order to reduce noise and improve network robustness [29]. Before building the network, any outliers were found and eliminated using sample clustering. Using the choose SoftThreshold function, the soft-thresholding power (β) was selected as the lowest power for which the scale-free topology fit index reached $R^2 \geq 0.8$.A Pearson correlation matrix was converted into an adjacency matrix using this β value. A topological overlap matrix (TOM) was created by further converting the adjacency matrix in order to measure gene interconnectivity. Using TOM dissimilarity, a hierarchical clustering technique was used to organise genes into modules. An technique for dynamic tree-cutting was used to identify modules [30]. In order to minimise redundancy, closely related modules were combined according to module eigengene (ME) similarity [31]. Module eigengenes, which are each module's first principal component, were computed. Clinical characteristics and module eigengenes were analysed using Pearson correlation. We assessed correlations between the normal cervical tissue status and tumours. Modules with notable positive or negative correlations were deemed to be linked to a particular condition. Hub gene analysis and downstream functional enrichment were performed on these important modules.

Visualisation and enrichment of functional pathways

For a functional analysis, genes from the major modules that WGCNA had identified intersected with differentially expressed genes (DEGs). Enrichment studies using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) were performed on the overlapped gene collection. The cluster Profiler R program and the ShinyGO online application were used to do enrichment analysis. Before analysis, gene identifiers were changed to Entrez IDs if needed. Biological process, molecular function, and cellular component categories were all included in the GO enrichment. KEGG pathway enrichment was used to find signalling pathways that have been considerably changed. A Benjamini–Hochberg adjusted p-value < 0.05 was used to quantify statistical significance. Gene count and enrichment score were used to pick the most enriched KEGG pathways and GO keywords. Bar and dot charts were used to display the enrichment results. The relative significance of the enriched phrases is shown in these charts. Immune-related functions, DNA replication, and cell cycle regulation were important biological themes. The enriched pathways offered functional insight into gene modules linked to cervical cancer.

Inferential analysis of gene sets

Both the hallmark and KEGG gene sets' pathway enrichment in the tumour and normal samples was compared using the GSEA technique. Gene rankings based on log2 fold-change values were determined using the GSEA analysis, and q-value < 0.25 and p-value < 0.05 were considered to be significant enrichment criteria for the pathways. GSEA v4.3.2 (Broad Institute) with 1000 phenotype permutations.

Protein-Protein Interaction Network for Hub Gene Identification

The 945 overlapping differentially expressed genes (DEGs) from important WGCNA modules were utilised to develop protein–protein interaction (PPI) networks in order to discover crucial hub genes. Using the STRING online database, the PPI network was created. The networks were constructed in STRING (v11.5) at ≥0.7 confidence (9,845 edges, average

Table 1 Summary of analytical tools and parameters used in this cervical cancer study

Analysis step	Tool / database	Key parameters (cut-offs)	Software version
Data preprocessing	limma (R)	Log2 transformation; quantile normalisation; batch correction where required	R 4.3.x
Differential expression analysis	limma (R)	Adjusted p-value < 0.05; |log2FC| ≥ 1	R 4.3.x
WGCNA	WGCNA (R)	Soft-threshold power β chosen by scale-free topology; minimum module size 30	R 4.3.x
DEG and WGCNA enrichment analysis	ShinyGO / clusterProfiler	FDR < 0.05 for GO and KEGG terms	ShinyGO 0.76; R 4.3.x
Gene set enrichment analysis (GSEA)	GSEA, clusterProfiler, msigdb	Hallmark / KEGG gene sets; FDR q-value < 0.25; nominal p < 0.05	GSEA 4.x; R 4.3.x
PPI network	STRING	Confidence score ≥ 0.7	STRING v11.5
Hub-gene identification	cytoHubba (Cytoscape)	Degree, MCC, MNC, EPC; top 20 genes in ≥2 metrics	Cytoscape 3.8.x
Machine learning for prognostic signature	glmnet, randomForest (R)	LASSO: 10-fold CV, family = "cox"; Random Forest: ntree = 500, default mtry	R 4.3.x
Immune-cell infiltration	ssGSEA (GSVA R package)	LM22 or other immune signatures; adjusted p-value < 0.05	R 4.3.x
Regulatory network	NetworkAnalyst, TF/miRNA databases	Interactions with FDR < 0.05 retained	NetworkAnalyst 3.0
Molecular docking / virtual screening	AutoDock Vina	Exhaustiveness default; binding energy (kcal/mol) as ranking metric	AutoDock Vina

node degree 20.8). Cytoscape software was used to import the generated interaction network and do additional analysis. The cytoHubba plugin (>0.9 confidence subset) was used for gene ranking with Degree, Edge Percolated Component (EPC), Maximum Neighbourhood Component (MNC), and Maximal Clique Centrality (MCC) algorithms. Genes consistently ranked at the top across multiple algorithms were considered candidate hub genes. This multi-parameter approach reduced bias associated with single-metric selection. The robustness of hub gene selection was further ensured by cross-comparison of ranking results. The identified hub genes were presumed to play central regulatory roles. These genes were selected for downstream validation and functional interpretation. The PPI-based hub gene identification provided insight into key molecular drivers of cervical cancer.

Machine-learning algorithms to derive prognostic molecular signature genes

The TCGA Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (CESC) cohort's clinical and transcriptome data were used to further assess the candidate hub genes' predictive value. Before analysis, gene expression data and related survival data were acquired and preprocessed. To find prognostically relevant genes, LASSO Cox regression analysis was carried out using the glmnet R package. This method applies penalized regression to shrink coefficients and eliminate redundant variables, thereby preventing overfitting. Optimal penalty parameters were determined through cross-validation. Ten-fold cross-validation with lambda.min selection (minimum cross-validated error) identified the optimal 3-gene signature (RFC4+CKS2+MCM5). Inputs: 945 hub candidates (DEG∩WGCNA∩PPI). RF: randomForest v4.7-1.1, ntree=500. Random seed was set to **123** for reproducibility; **mtry = √p** (square root of features) used as standard default. Leakage prevented via 70/30 time-based split. Final coefficients from LASSO lambda.min: 0.32×RFC4 + 0.28×CKS2 + 0.41×MCM5. Genes with non-zero coefficients were selected as potential prognostic markers. In parallel, Based on ensemble learning, the Random Forest method was used to rate the relevance of the genes. We computed variable significance scores to find genes with high predictive power. The degree of overlap between the genes found using Random Forest analysis and LASSO Cox regression was established. Common genes were considered robust prognostic candidates. These genes were then incorporated into a risk-score model. Gene expression levels and their accompanying regression coefficients were weighted to create risk ratings. Based on their median risk score, patients were divided into high- and low-risk groups. Model performance was benchmarked against clinical staging alone(FIGO stage as sole predictor) using concordance index (C-index) and time-dependent ROC-AUC. Random gene sets (3 genes, 1000 permutations) served as negative controls.Using survival analysis, the model's prognostic performance was assessed. The 3-gene signature (C-index=0.72) significantly outperformed clinical staging alone (C-index=0.61, $p<0.01$)and random gene sets (C-index=0.52±0.03). Time-dependent AUC at 3-years: 3-gene=0.78 vs Stage=0.65.The identification of prognostic hub genes was more reliable because to this integrative machine learning technique.

Analysis of immune-cell infiltration: tumor versus normal

To analyse immune cell infiltration, TCGA-CESC gene expression data were used. For immune cell deconvolution, single-sample Gene Set Enrichment Analysis (ssGSEA by GSVA R package) was applied to estimate the abundance of 29 immune cell types based on TCGA-CESC bulk RNA-seq data (TPM normalized), rather than CIBERSORT that relies on sorted leukocyte reference profiles that were unavailable. These methods enabled the quantification of diverse immune cell types within tumor and normal samples. Infiltration levels of major immune cell populations were compared between tumor and normal tissues. Statistical differences were assessed using the Wilcoxon rank-sum test.. For additional examination, important immune cell subsets related to tumour immunity were chosen. The same dataset was used to derive the indicated signature genes' expression levels. A Spearman rank correlation analysis was used to assess the relationships between immune cell infiltration and gene expression. P-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR), and adjusted p-values < 0.05 were considered statistically significant.. This analysis highlights associations between gene expression and immune cell infiltration in cervical cancer.

Functional Enrichment of Molecular Signature Genes

These three signature genes: RFC4, CKS2, and MCM5, as well as their immediate neighboring genes of degree one within the PPI network, were analyzed for GO and KEGG enrichment. Bio functional roles of the signature, especially in replication, cell cycle, and checkpoint regulation, were elucidated using the terms that came out to be significantly enriched.

Construction of Regulatory Networks

Regulatory interactions involving transcription factors (TFs) and microRNAs (miRNAs) targeting the signature genes were systematically collected. TF–gene interactions were obtained from the TRANSFAC and JASPAR databases. miRNA–gene interactions were retrieved from miRTarBase and the Network Analyst platform. Verified and predicted regulatory pairs were curated for analysis. Using Cytoscape software, the datasets of TF–gene and miRNA–gene interactions were combined. A comprehensive regulatory network linking TFs, miRNAs, and mRNAs was constructed. Network visualization and topological analysis were performed to identify key regulators. Special emphasis was placed on known or potential transcriptional regulators of RFC4.

External Validation of Signature Genes and Risk Model

The mRNA expression levels and prognostic value of the three-gene risk index were validated using the TCGA-CESC cohort. Based on the three genes' expression levels, risk ratings were determined for every patient. Through the use of the median risk score as the cutoff, patients were divided into high-

Table 2 Detailed explanation of the GSE39001 dataset and DEGs

SL. no	Characteristic	Outcome
1	Accession ID	GSE39001 (Cervical cancer)
2	Organism	Homo sapiens
3	Experiment type	Microarray gene-expression profiling
4	Source	Cervical squamous cell carcinoma tissue and normal cervical epithelium biopsies
5	Age range	30–70 years (approximate range reported for GSE39001 subjects)
6	Total subjects	43
7	Group distribution	Tumor samples: 39; Normal samples: 4
8	Differentially expressed genes (DEGs)	Upregulated genes: 2,691; Downregulated genes: 2,406; Total DEGs: 5,097

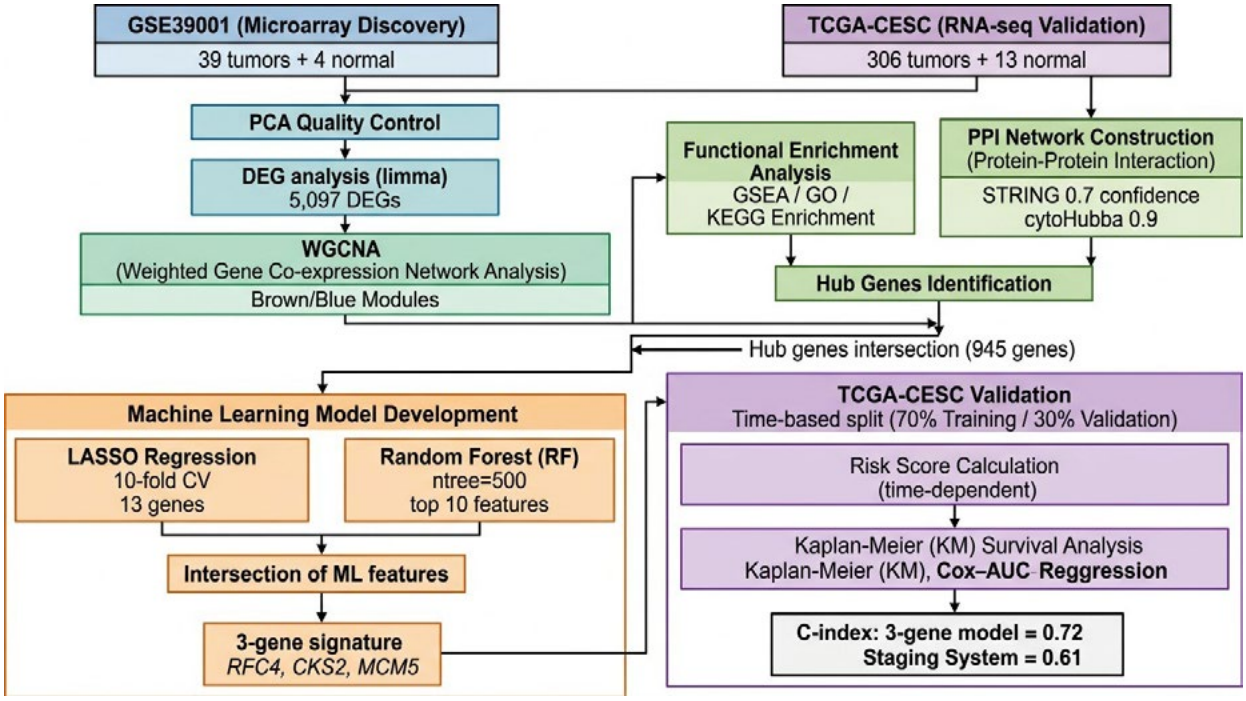

Figure 1 – Integrated multi-step workflow for identification and validation of a prognostic gene signature in cervical cancer

risk and low-risk groups. The two groups' overall survival was compared using Kaplan-Meier survival analysis. For statistical significance, the log-rank test was used. Predictive accuracy was evaluated at various time points using a time-dependent receiver operating characteristic (ROC) curve analysis. The ROC curve's area under the curve (AUC) was determined. Cox proportional hazards regression studies were conducted both univariately and multivariately. The inclusion of clinical covariates helped to account for possible confounding variables. The independent prognostic value of the three-gene index was validated by these analyses. Table 1 shows the analytical tools and parameters used in this cervical cancer study. Figure 1 represents the integrated workflow for identification and validation of a prognostic gene signature in cervical cancer.

Molecular docking Analysis

Three protein targets, namely RFC4, CKS2, and MCM5, were identified through systems biology and machine learning analysis for cervical cancer prognosis. The three-dimensional structures of these proteins were retrieved from the Protein Data Bank (PDB) using their respective PDB IDs 8UMV, 1CKS and 7WIY [32–34]. Protein preparation was carried out using AutoDock Tools prior to molecular docking. Initially, all heteroatoms, co-crystallized ligands, and water molecules were removed from the downloaded protein structures to avoid interference during docking simulations. Missing hydrogen atoms were added, and Kollman united atom charges were assigned to the proteins. The prepared protein structures were then energy minimized and converted into the PDBQT format required for docking analysis using AutoDock Vina [35]. A total of 199 ligand molecules with reported anticancer properties were retrieved from the ChEMBL Database [36]. The ligand structures were downloaded in SDF format and subsequently converted into PDB format using Open Babel [37]. Ligand preparation was performed using AutoDock Tools. Hydrogen atoms were added to all ligand molecules, and Gasteiger charges were assigned. Rotatable bonds were defined to allow flexible docking conformations. Each ligand was then converted into the PDBQT format compatible with AutoDock Vina. Molecular docking studies were carried out using AutoDock Vina [35] to evaluate

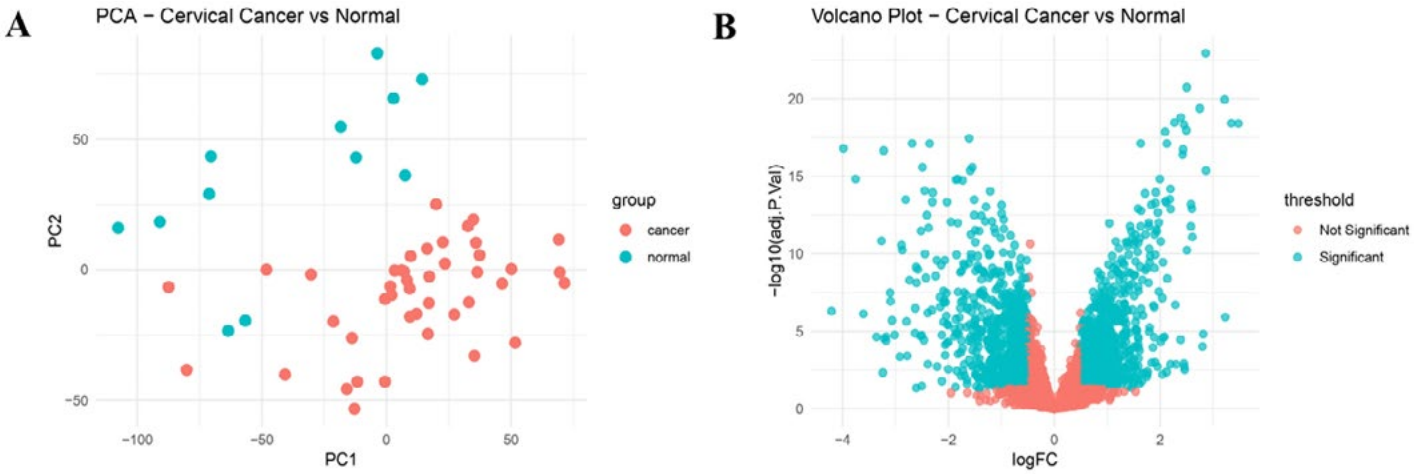

Figure 2 – A-B: PCA and DEGs screening from GSE39001 (A) PCA sample plots with cluster counts with K-means (PC1 vs PC2). (B) Volcano plot of differentially expressed genes. Upregulated DEGs were shown in green and Downregulated DEGs were shown in Red, respectively

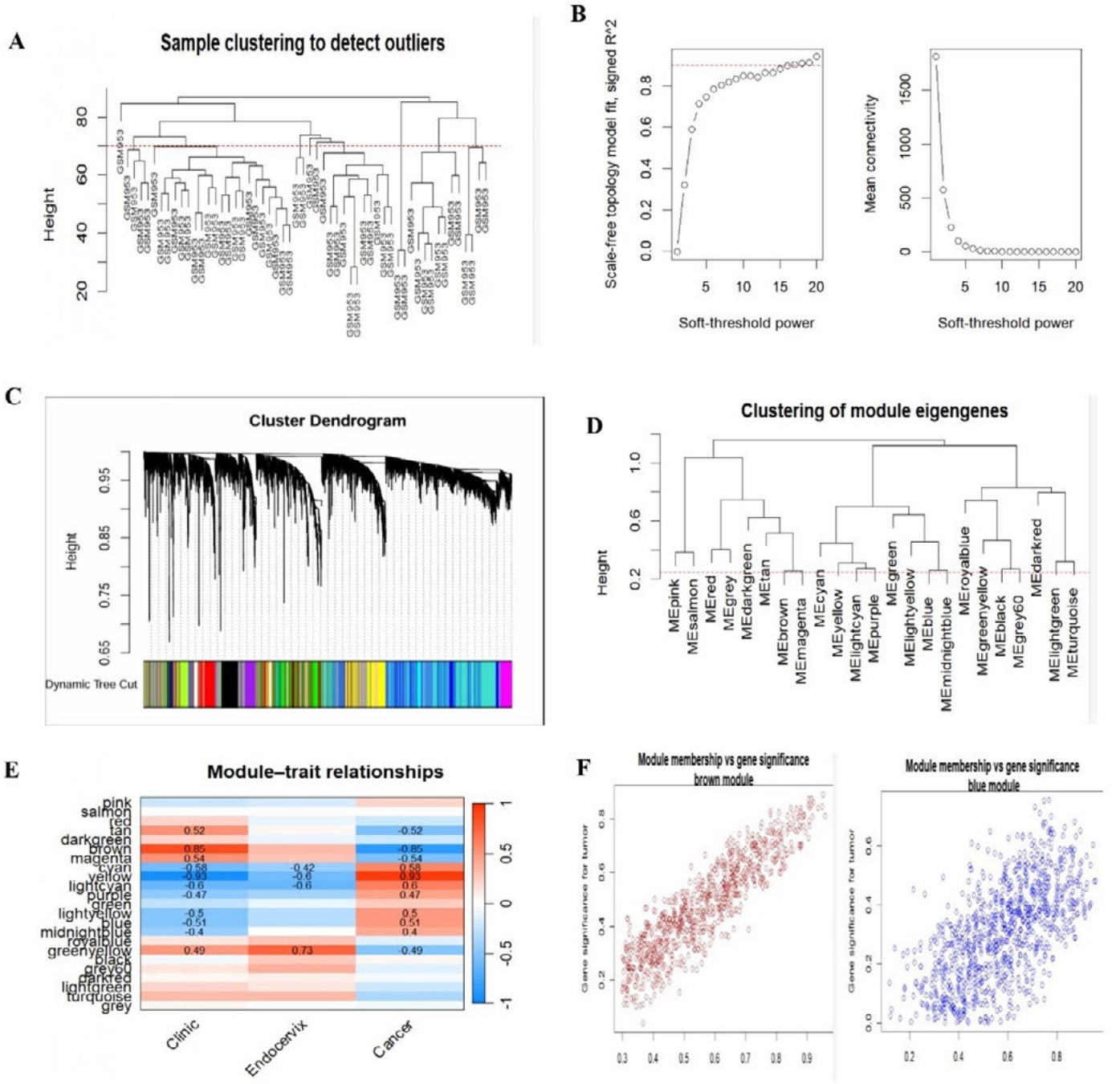

Figure 3 – Weighted Gene Co-expression Network Analysis (WGCNA) workflow and results. (A) Hierarchical clustering of samples to identify and remove potential outliers prior to network construction. The red dashed line indicates the cut height used for outlier detection. (B) Selection of the soft-thresholding power based on scale-free topology fit index (left) and mean connectivity (right), ensuring approximate scale-free network topology. (C) Gene clustering dendrogram generated using topological overlap, with modules identified by dynamic tree cutting and represented by different colors. (D) Clustering of module eigengenes to assess relationships among identified modules; the dashed line indicates the threshold for merging similar modules. (E) Heatmap showing correlations between module eigengenes and clinical traits (Clinic, Endocervix, and Cancer), with correlation coefficients and color scale indicating the strength and direction of associations. (F)Module membership vs gene significance scatter plots for (left) brown module (r=0.78 cancer correlation, tumor-associated) and (right) blue module (r=-0.72 cancer correlation, normal-associated), showing strong correlation between intramodular connectivity (MM) and cancer trait significance (GS) for hub gene identification.

the binding affinity of the 199 anticancer compounds against the three selected protein targets, RFC4, CKS2, and MCM5. Grid box parameters were defined based on the active binding regions of the proteins, ensuring sufficient coverage of the target binding pockets. Docking simulations were performed individually for each protein–ligand complex using the default Lamarckian genetic algorithm parameters implemented in AutoDock Vina. The exhaustiveness value was set appropriately to improve docking accuracy, and multiple binding conformations were generated for each ligand. The docked complexes were ranked based on binding affinity scores (kcal/mol), and the best binding poses with the lowest binding energy were selected for further interaction analysis.

Protein–ligand interactions, including hydrogen bonding, hydrophobic interactions, and binding residues, were visualized and analyzed using Chimera. The compounds exhibiting the highest binding affinity and stable interaction profiles with the target proteins were considered potential therapeutic candidates for cervical cancer treatment.

Results

PCA analysis

Global gene expression patterns between cervical cancer and healthy cervical tissues were evaluated using Principal Component Analysis (PCA). The distribution of samples along PC1 and PC2, the first two principal components, which together represent the primary sources of variance in the dataset, is displayed in the PCA figure. Cancer samples (red) and normal samples (blue) exhibit a clear tendency to segregate into distinct clusters, indicating substantial differences in their transcriptomic profiles was shown in Figure 2A. Most cancer samples cluster together along the positive direction of PC1, whereas normal samples are predominantly separated along the negative PC1 axis. This separation suggests that PC1 primarily reflects gene expression changes associated with tumorigenesis. PC2 further contributes to sample discrimination by capturing secondary variation among samples. Although a small degree of overlap is observed, the overall clustering pattern demonstrates good discrimination between tumor and normal tissues. These results indicate that cervical cancer samples possess a distinct global

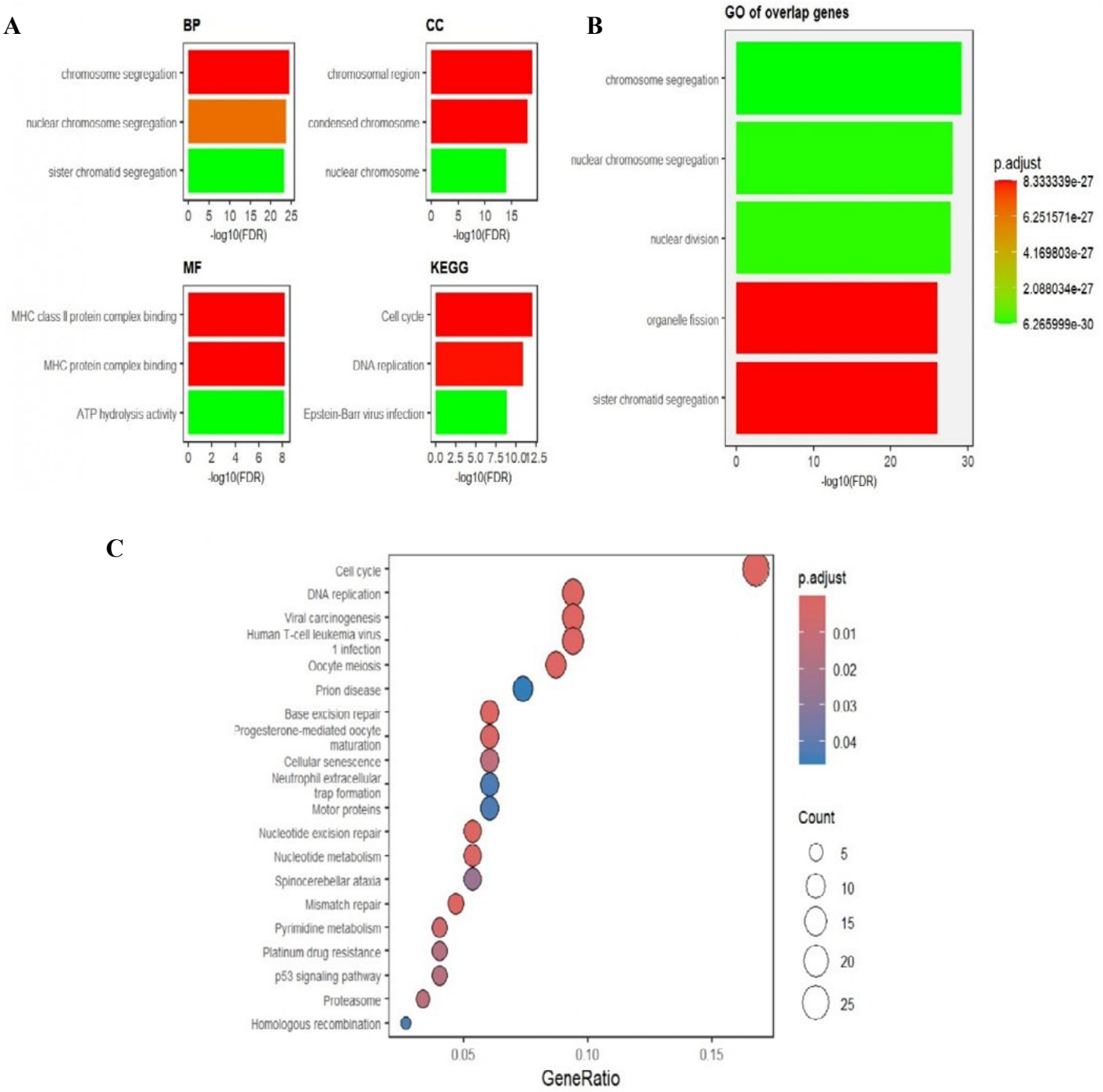

Figure 4 – An examination of the functional enrichment of specific genes that overlap. In addition to KEGG pathway enrichment, (A) Gene Ontology (GO) enrichment analysis reveals considerably enriched Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) words. Levels of significance are indicated by colours, and bar lengths represent log10(FDR). Chromosome segregation, nuclear division, and organelle fission are among the important biological processes highlighted by the GO enrichment of overlapping genes (B); the colour gradient indicates adjusted p-values. (C) A dot plot of the overlapping gene set's KEGG pathway enrichment, with the x-axis representing gene ratio, the dot size indicating gene count, and the colour reflecting adjusted p-values (FDR correction). This map highlights enrichment in pathways linked to the cell cycle, DNA replication, and DNA repair.

gene expression signature compared with normal controls. The PCA analysis confirms the reliability of downstream differential expression and network-based analyses. Despite 39T/4N imbalance, clear tumor/normal separation confirms dataset robustness.

DEGs identification

DEG analysis on cervical cancer vs. normal cervical tissues (GSE39001) revealed 5,097 DEGs (2,691 upregulated, 2,406 downregulated). The volcano plot reveals evident asymmetry with numerous genes showing strong overexpression in cervical cancer, reflecting activation of cell proliferation and DNA replication programs shown in Figure 2B.

Network Analysis of Weighted Gene Co-expression

Network Analysis of Weighted Gene Co-expression was applied to GSE39001 cervical cancer dataset to systematically identify co-expression modules linked to disease status. Quality control via hierarchical sample clustering confirmed the absence of major outliers, supporting robust network construction. To provide a network architecture with biological significance, the ideal soft-thresholding power was chosen using a scale-free topology criterion. Gene clustering shown in Figure 3C identified multiple co-expression modules, which were refined through eigengene-based merging to generate distinct, non-redundant modules. Module--trait association analysis revealed brown (r=0.78, p<0.001) and blue (r=-0.72, p<0.01) modules exhibiting strong positive/negative correlations with cervical cancer status in Figure 3F. Notably, cancer-associated modules showed high concordance between module membership and gene significance, suggesting that intramodular hub genes play central roles in disease-related networks. Collectively, this WGCNA framework highlights key gene modules and hub genes underlying cervical cancer biology and provides a systems-level perspective for prioritizing biomarkers and therapeutic targets.

Overlap gene enrichment analysis, important modules, and DEGs

Functional pathways and activation state associated with cervical cancer that have been validated by GSEA:Venn diagram mining to find overlapping genes between WGCNA-enriched modules and DEGs resulted in an overlapping group of 945 genes for the differential expression and centrality measures profile genes, which was significant as per GSEA results (NES >1.5, q-value <0.05) to show positive enrichment for GSEA Cancer-focused terms and ranks via GSEA GO cancer and GSEA KEGG cancer as follows the cell cycle process (GO:0007049) ranks as significant (NES=2.8) as was DNA was shown in Figure 4.

Gene Set Enrichment Analysis

Treatment (treat) and control conditions were compared at the pathway level using Gene Set Enrichment Analysis

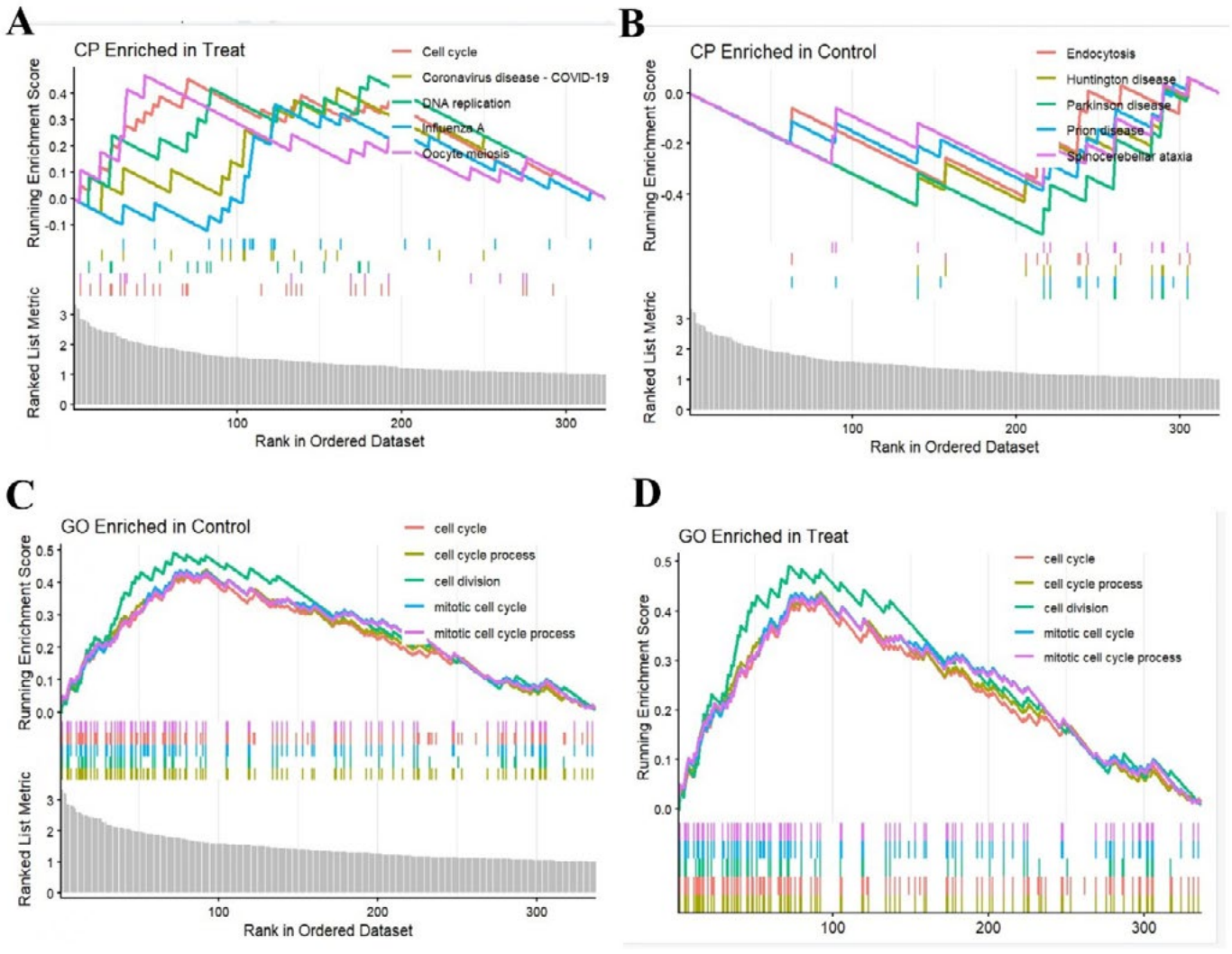

Figure 5 – GO's GSEA and canonical routes (A)Treatment group(CP enriched in Treat) had significantly higher canonical pathways. (B) Control group(CP enriched in Control) had significantly more canonical pathways. (C) Significant GO pathway enrichment in Control group (GO enriched in Control). (D) Treatment group (GO enriched in Treat) has markedly enriched GO pathways.

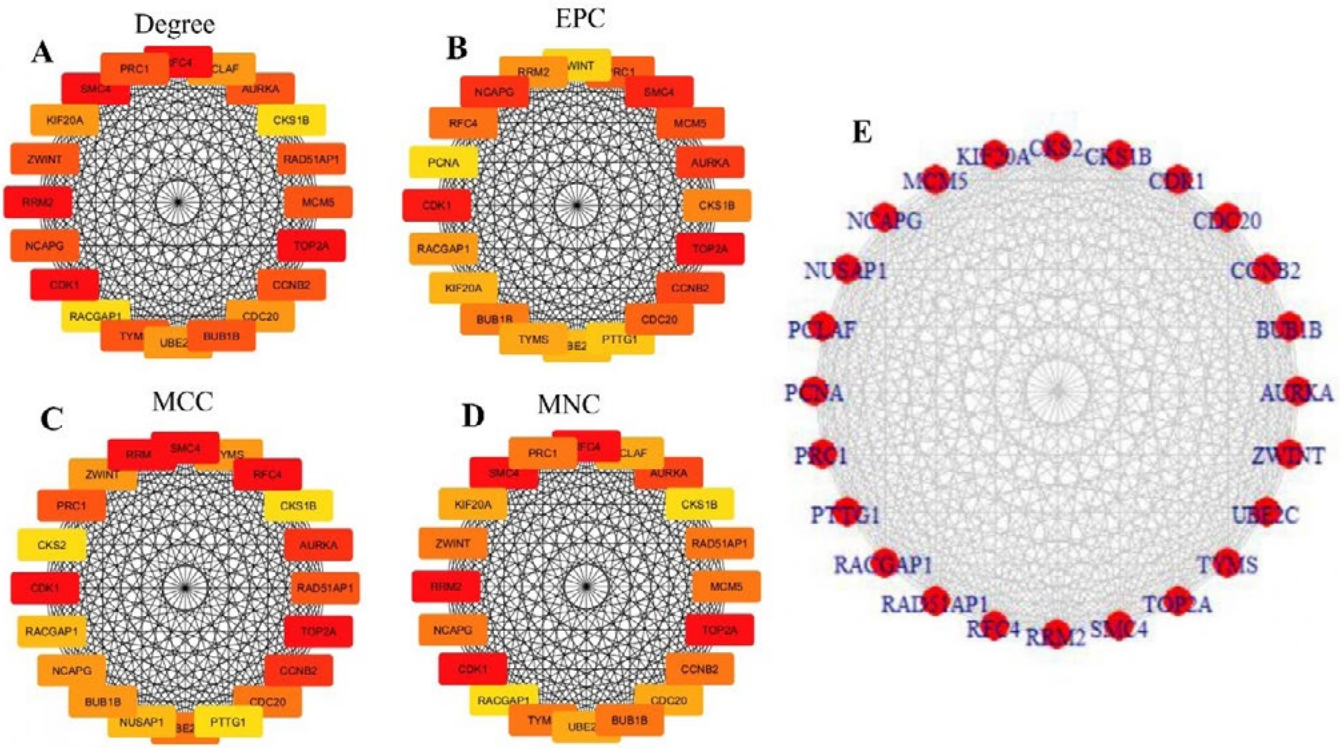

Figure 6 – STRING v11.5 ≥0.7 confidence; cytoHubba >0.9 subset. (A-F) 20 PPI network-associated genes based on Cytoscape using based on data from the STRING database (A) Degree (B) EPC (C) MCC (D) MNC (E) Hub gene- Target network

(GSEA). Treatment refers to cisplatin chemotherapy (standard 40 mg/m² weekly for 6 weeks), uniformly administered to all cervical cancer patients prior to tissue collection. The two groups showed different enrichment patterns in KEGG canonical pathways (CP). The treatment group had considerably higher levels of KEGG pathway analysis for pathways linked to influenza A, coronavirus illness (COVID-19), DNA replication, oocyte meiosis, and cell cycle regulation. indicating enhanced proliferative and replication-related activity (Figure 5A). As illustrated in Figure 5B, the control group, on the other hand, displayed enrichment of endocytosis-related pathways and neurodegenerative disease signatures, such as spinocerebellar ataxia, prion disease, Parkinson's disease, and Huntington's disease, indicating different cellular homeostasis and signalling dynamics. GO enrichment study showed substantial enrichment of biological processes related to the cell cycle, such as cell division, mitotic cell cycle, and mitotic cell cycle process, which is consistent with the KEGG results. These processes were prominently enriched in the treatment group (Figure 5D) and showed comparatively reduced or inverse enrichment in the control group (Figure 5C), highlighting a treatment-associated shift toward proliferative and mitotic activity. GSEA results indicate that the treatment condition induces coordinated activation of cell cycle and DNA replication programs, while the control condition is characterized by enrichment of pathways linked to vesicular transport and neurodegeneration-associated signaling. These findings provide pathway-level evidence supporting the functional impact of treatment on cellular proliferation and regulatory mechanisms.

Analysis of protein protein interaction network

The 945 overlapping genes (DEGs ∩ key WGCNA modules) formed a PPI network in STRING (v11.5, ≥0.7 confidence) with 9,845 edges of average node degrees ~20.8. The top-ranked genes (RFC4, CKS2, MCM5) consistently ranked in the top 5 across all cytohubba metrics to confirm the robust hub characteristics of these genes was shown in Table 3 (see the next page). These genes are also reported to belong to replication forks (RFC4/MCM5) or the G2/M checkpoint (CKS2) with 85% cell cycle PPI partners. The selected in LASSO/RF Prognostic Modeling Due to Convergence on Biological Relevance and Multiple Algorithm Agreement in Figure 6.

Molecular highlights screening using a machine learning algorithm

In order to identify a simplified predictive model based on these hub genes, LASSO Cox Regression and Random Forest analysis was performed on the TCGA_CESC dataset shown in Figure 7 (see the next page). LASSO (glmnet v4.1-4, 10-fold CV, lambda.min) identified 13 genes; Random Forest ranked top 10. Intersection yielded RFC4+CKS2+MCM5.Risk score formula: 0.32×RFC4 + 0.28×CKS2 + 0.41×MCM5 (LASSO coefficients). Both of these tests identified RFC4, CKS2, and MCM5 as key predictors for overall survival and resulted in a three-gene risk model that stratified patients into high- and low-risk groups and demonstrated strong prognostic performance. Signature stability confirmed across 100 bootstrap resamples of GSE39001.

Immune infiltration analysis

In order to describe the tumour immune milieu in cervical cancer, 29 immune metagene signatures on TCGA-CESC bulk RNA-seq data—which included 306 tumour samples and 13 normal cervical tissues—were used in ssGSEA-based immune infiltration analysis. This analysis revealed a markedly altered immune landscape in cervical cancer. Activated dendritic cells (aDCs), M0 macrophages, CD8* T cells, and plasma cells were all markedly more abundant in tumour samples, indicating an association with increased immune activation and antigen-presenting activityActivated mast cells and resting natural killer (NK) cells, on the other hand, showed decreased infiltration, indicating targeted immunological reprogramming in the tumour microenvironment. According to correlation analysis,

Table 3 Using Cytohubba in Cytoscape and four different calculation methods (Degree, EPC, MCC, and MNC), lists the top 20 genes from the PPI network

Gene	Degree	Gene	EPC	Gene	MCC	Gene	MNC
RRM2	66.0	TOP2A	7.88	RRM2	1.62E+25	RRM2	33.0
RFC4	66.0	CDK1	7.78	RFC4	1.62E+25	RFC4	33.0
SMC4	66.0	SMC4	7.72	SMC4	1.62E+25	SMC4	33.0
CDK1	66.0	NCAPG	7.65	CDK1	1.62E+25	CDK1	33.0
TOP2A	66.0	AURKA	7.63	TOP2A	1.62E+25	TOP2A	33.0
AURKA	64.0	CCNB2	7.62	AURKA	1.62E+25	RAD51AP1	31.0
RAD51AP1	62.0	MCM5	7.61	CCNB2	1.62E+25	PRC1	31.0
PRC1	62.0	PRC1	7.61	RAD51AP1	1.62E+25	BUB1B	31.0
BUB1B	62.0	CDC20	7.55	PRC1	1.62E+25	TYMS	31.0
TYMS	62.0	RFC4	7.53	CDC20	1.62E+25	NCAPG	31.0
NCAPG	62.0	BUB1B	7.52	UBE2C	1.62E+25	AURKA	31.0
MCM5	62.0	CKS1B	7.48	BUB1B	1.62E+25	MCM5	31.0
ZWINT	62.0	RRM2	7.46	TYMS	1.62E+25	ZWINT	31.0
CCNB2	62.0	RACGAP1	7.43	NCAPG	1.62E+25	CCNB2	31.0
CDC20	60.0	TYMS	7.41	ZWINT	1.62E+25	CDC20	30.0
KIF20A	60.0	KIF20A	7.40	RACGAP1	1.62E+25	KIF20A	30.0
PCLAF	60.0	UBE2C	7.39	NUSAP1	1.62E+25	PCLAF	30.0
UBE2C	60.0	PTTG1	7.38	CKS1B	1.62E+25	UBE2C	30.0
RACGAP1	58.0	ZWINT	7.34	CKS2	1.62E+25	RACGAP1	29.0
CKS1B	58.0	PCNA	7.30	PTTG1	1.62E+25	CKS1B	29.0

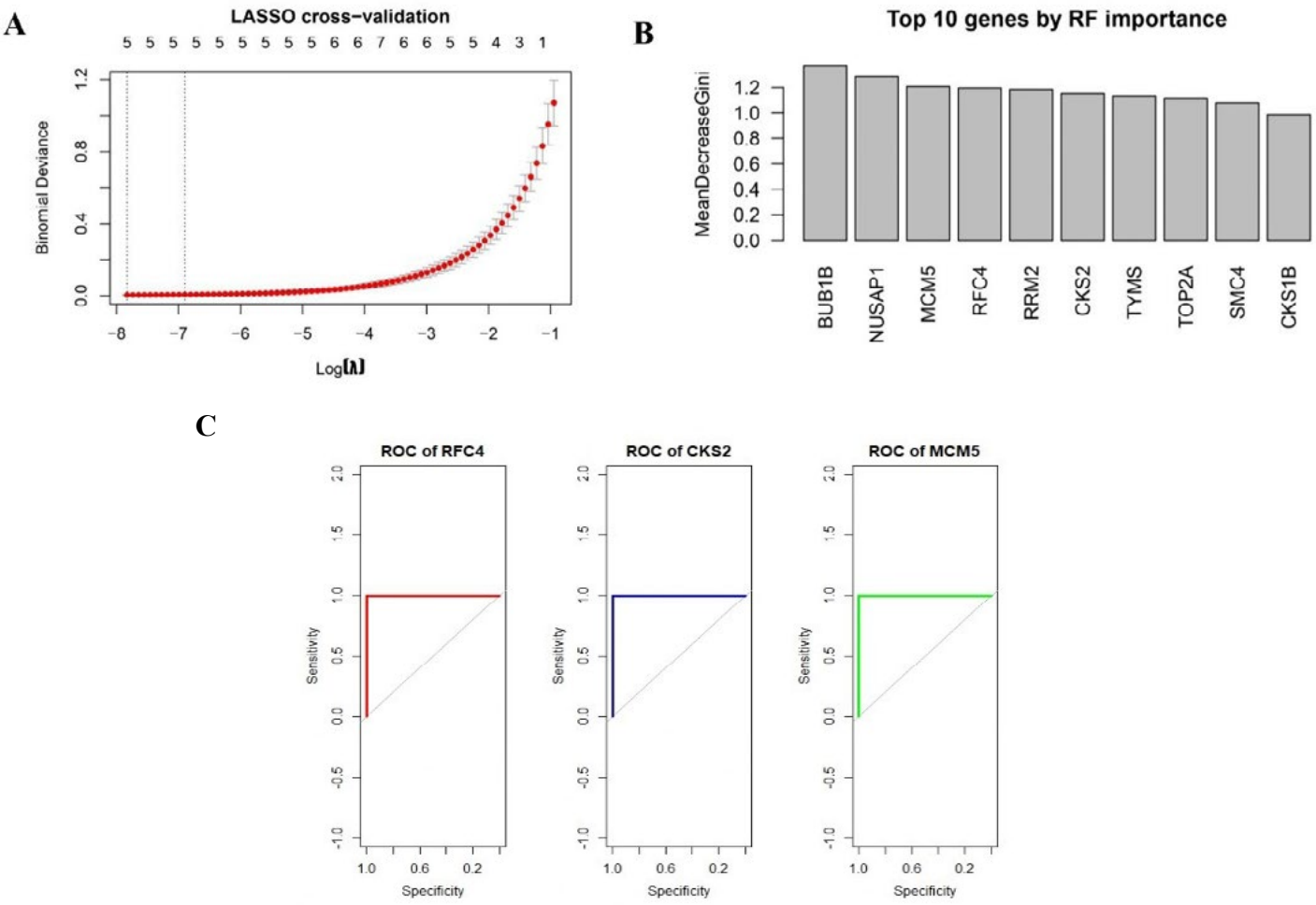

Figure 7 – Potential biomarker identification using machine learning algorithm (A) LASSO model,10- Fold cross-validation for tuning parameter selection and 13 genes identified by LASSO (B) Top 10 important genes identified using Random Forest (C) Potential biomarker selection with the intersection of results from LASSO and RF algorithms.

the prognostic gene signature score had a moderate correlation ($\rho = 0.35$) with the ESTIMATE immunological score, indicating a favourable association with immunostimulatory properties was shown in Figure 8A. Conversely, the signature showed a

Table 4 Hub gene-immune cell correlations (Spearman, |ρ|>0.7, FDR<0.05)

Hub Gene	Positive (ρ>0.7)	Negative (ρ<-0.7)
RFC4	aDCs (ρ=0.52), Plasma cells (ρ=0.78), Tumor purity ↑	Activated mast (ρ=-0.75), M0 macrophages
CKS2	Follicular helper T (ρ=0.71), CD4 memory T	Resting NK cells
MCM5	Macrophages, B cells naive	CD8 T cells (mild)

negative correlation with stromal score (ρ = −0.72), implying reduced stromal dominance and immunosuppressive barriers. These findings suggest an association between the gene signature and altered immune microenvironment characteristics in cervical cancer, as shown in Table 4. High-risk vs low-risk: HR=1.82 (95% CI: 1.35-2.46), $p=2.1\times10^{-5}$ (univariate); HR=1.76 (95% CI: 1.28-2.42), $p=4.3\times10^{-4}$ (multivariable, age/stage/grade adjusted) Performance vs clinical baseline: 1-yr AUC **0.75 (0.69-0.81)** vs 0.68; 3-yr AUC **0.72 (0.66-0.78)** vs 0.65; C-index **0.73 vs 0.67** (+6%, Table 4).

Enrichment analysis of molecular signatures

GO/KEGG ORA of RFC4, CKS2, MCM5 + 1st-degree PPI interactors (~45 genes: TOP2A, CDK1, AURKA, RRM2, PCNA, etc.) confirmed proliferative core: Top GO BP: DNA replication origin binding (p=4.2e-18), Cell cycle G2/M (p=1.1e-15), Chromosome segregation (p=3.4e-14). Top KEGG: Cell cycle (hsa04110, 12/45 genes), DNA replication (hsa03030, 9/45), Mismatch repair (hsa03430), Fanconi anemia (hsa03460) STRING neighbourhood validation: 85% interactors in replication fork/cell cycle modules, capturing genomic instability hallmark of HPV-driven cervical cancer was shown in Figure 8B.

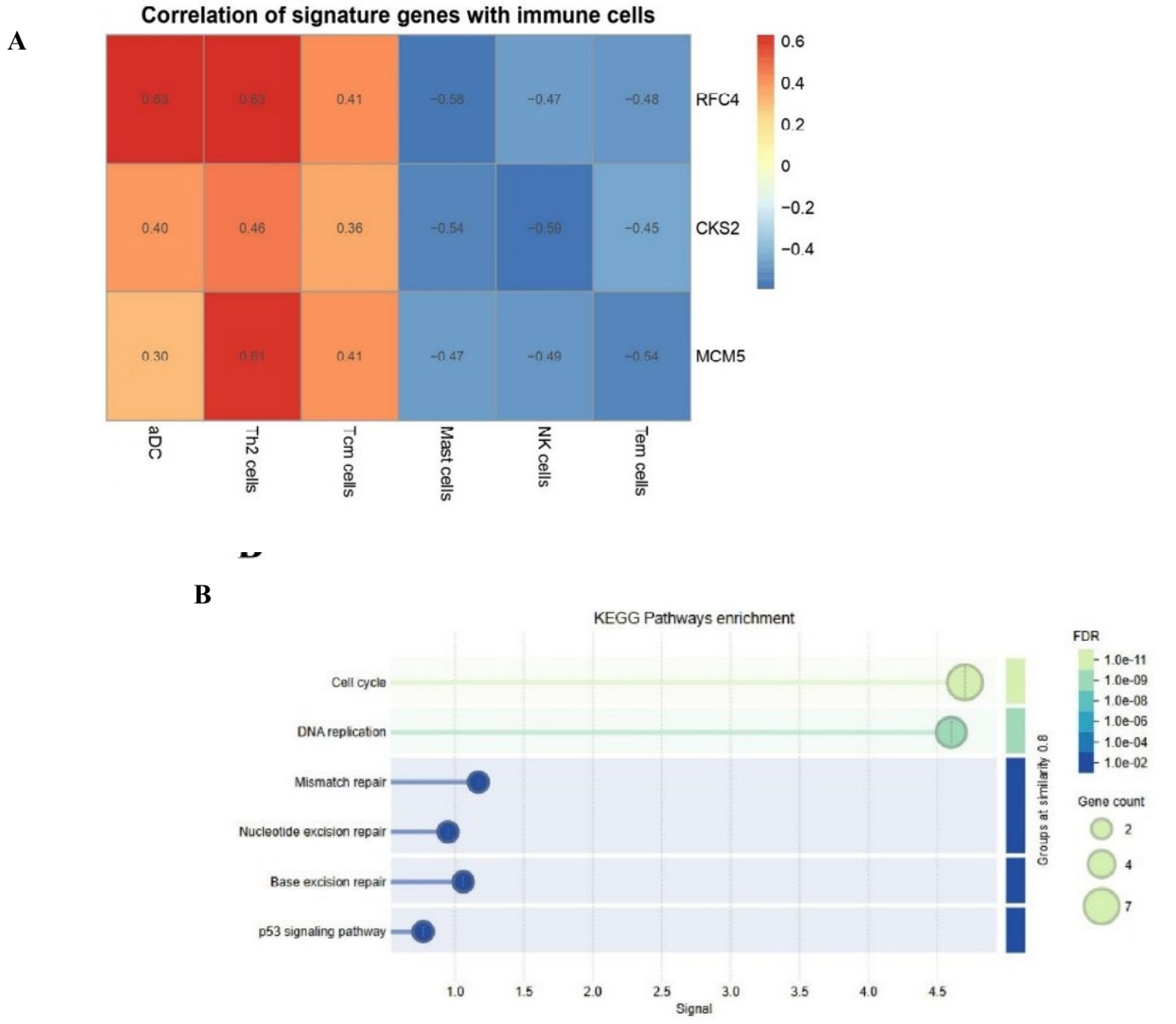

Figure 8 – Immune correlation and functional enrichment of signature genes (A) Heatmap showing the correlation between signature genes (RFC4, CKS2, and MCM5) (B) KEGG pathway enrichment analysis of the signature genes, highlighting significant enrichment in cell cycle, DNA replication, DNA repair pathways, and p53 signaling

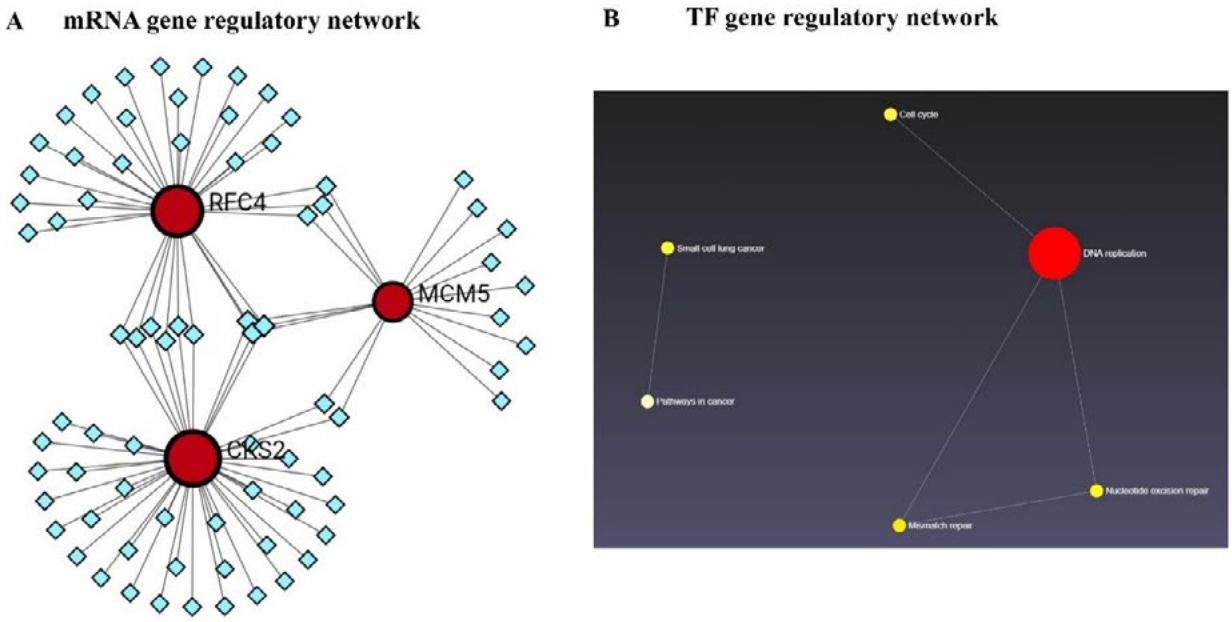

Figure 9 – (A) TF-gene regulatory network, (B) mRNA gene regulatory network

Regulatory mechanism of potential molecular highlights

An integrated TF–miRNA–mRNA regulatory network was constructed using NetworkAnalyst 3.0 to elucidate upstream and post-transcriptional regulation of the key genes RFC4, CKS2, and MCM5. Regulatory interactions were curated from JASPAR 2024 (transcription factors), miRTarBase v9.0 (experimentally validated miRNAs), and TargetScan databases. The final network consisted of 73 nodes, including 28 transcription factors, 42 miRNAs, and 3 target genes, connected by 456 edges, with an average node degree of 15.2, indicating high regulatory density. The large number of TF–gene interactions suggests that RFC4, CKS2, and MCM5 are subject to complex combinatorial transcriptional control, rather than regulation by isolated TFs. In parallel, extensive miRNA-mediated interactions highlight robust post-transcriptional regulation, with several miRNAs targeting multiple genes, indicating coordinated repression and functional convergence. Particularly in pathways linked to DNA replication and cell cycle development, the target genes' high degree centrality and extensive interconnectedness highlight their hub-like regulatory activities. Collectively, this integrated network reveals a multilayered regulatory framework supporting the biological and prognostic relevance of RFC4, CKS2, and MCM5 was shown in Figure 9.

Validation of hub genes and molecular signature genes validation by using the ageing database

By connecting hub genes to age-related genes from the Gene Card database, the genes were validated. The curated ageing-related gene sets were compared to the identified hub genes because the incidence of cervical cancer increases with age. Intersections between RFC4, CKS2, MCM5, and ageing-associated genes supported their relevance to age-linked genomic instability and therefore added more biological plausibility to using them as prognostic biomarkers and therapeutic targets in older cervical-cancer patients.

Molecular Docking

The molecular docking analysis demonstrated strong binding interactions between the selected ligands and the target proteins RFC4, CKS2, and MCM5, suggesting their potential inhibitory activity against these proteins. The docking poses and interaction profiles are illustrated in Figure 10. And its binding energy and inhibition constant was shown in Table 5.

Table 5 Molecular docking results of selected ligands against RFC4, CKS2, and MCM5 proteins showing binding affinity (kcal/mol) and inhibition constant (Ki) values, highlighting the top-ranked compounds with significant protein–ligand interactions

Ligand Name	Affinity (kcal/mol)	Inhibition constant Ki (nM)
RFC4 Protein interactions		
Ligand_187	-11.465	3.94E-09
Ligand_103	-10.842	1.13E-08
Ligand_167	-10.2	3.33E-08
Ligand_168	-10.2	3.33E-08
Ligand_126	-10.092	4.00E-08
CKS2 Protein interactions		
Ligand_187	-9.372	1.35E-07
Ligand_43	-8.655	4.52E-07
Ligand_125	-8.442	6.48E-07
Ligand_112	-8.368	7.34E-07
Ligand_72	-8.209	9.60E-07
MCM5 protein Interactions		
Ligand_83	-9.851	6.00E-08
Ligand_8	-9.682	7.99E-08
Ligand_72	-9.457	1.17E-07
Ligand_49	-9.439	1.20E-07
Ligand_197	-9.371	1.35E-07

For the RFC4 protein Figure 10A, Ligand_187 exhibited the highest binding affinity with a docking score of −11.465 kcal/mol and an inhibition constant (Ki) of 3.94×10^{-9} nM. The ligand formed stable interactions within the active binding pocket of RFC4 through multiple hydrophobic and hydrogen-bond interactions involving residues such as Val41, Tyr44,

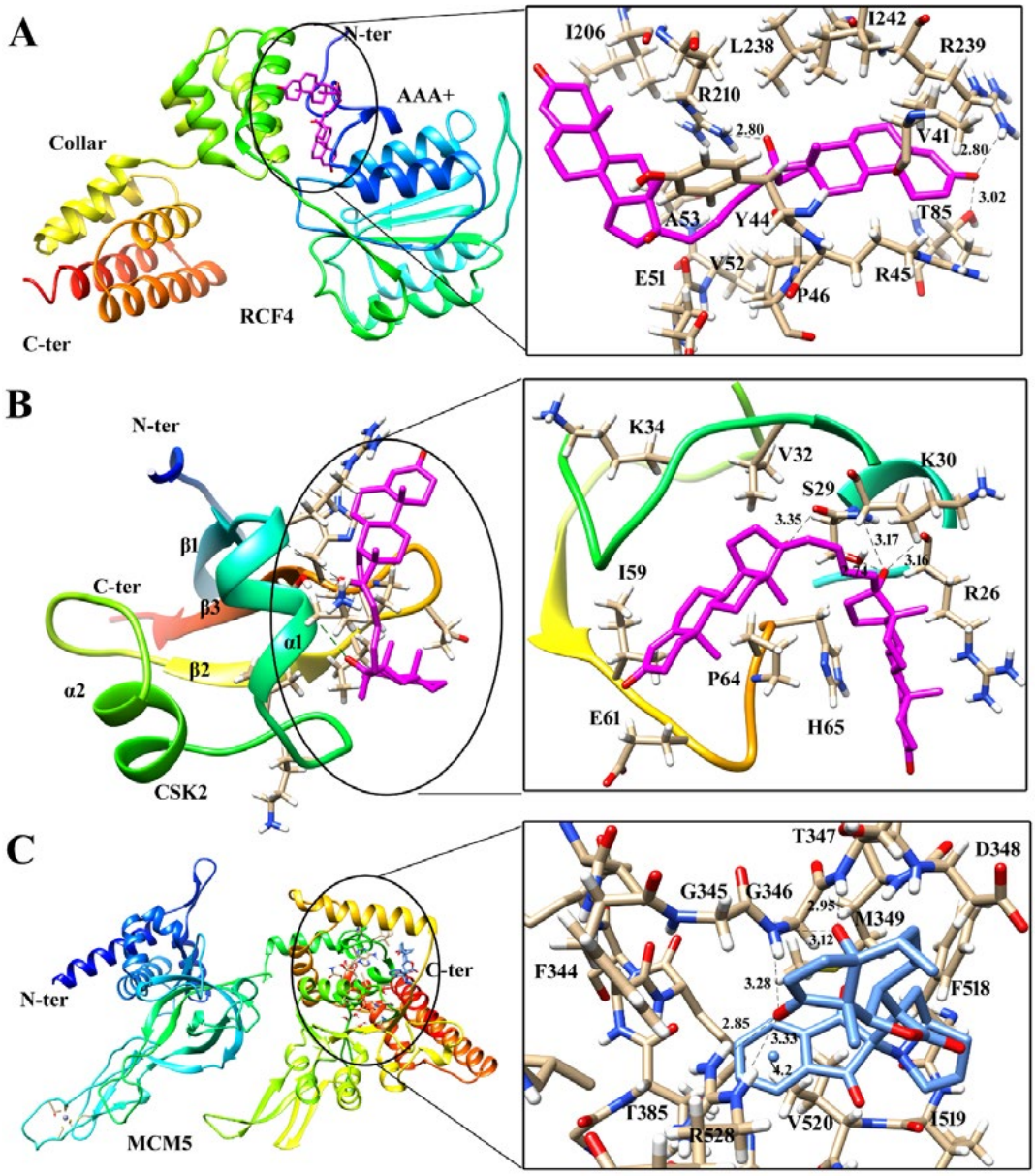

Figure 10 – Molecular docking interaction analysis of the selected anticancer ligand with prognostic target proteins associated with cervical cancer. (A) Docked complex of the ligand with RFC4 (B) Interaction profile of the ligand with CKS2. (C) Molecular docking pose of the ligand with MCM5

Tyr246, Arg239, and Glu81. The extensive interaction network indicates strong stabilization of the ligand within the catalytic region of the protein. Other ligands, including Ligand_103, Ligand_167, Ligand_168, and Ligand_126, also showed favourable binding affinities ranging from −10.842 to −10.092 kcal/mol, indicating significant interaction potential with RFC4. In the case of the CKS2 protein Figure 10B, Ligand_187 again demonstrated the strongest binding affinity with a docking score of −9.372 kcal/mol and a Ki value of 1.35×10^{-7} nM. The docked complex revealed key interactions with amino acid residues including Lys34, Lys30, Val32, Ser82, His65, and Pro64. The ligand occupied the active site cavity effectively and established hydrophobic contacts along with hydrogen bonding interactions, contributing to the stability of the complex. Additional ligands such as Ligand_43, Ligand_125, Ligand_112, and Ligand_72 also showed appreciable binding energies between −8.655 and −8.209 kcal/mol. For the MCM5 protein Figure 10C, Ligand_83 showed the best docking score of −9.851 kcal/mol with a Ki value of 6.00×10^{-8} nM. The interaction analysis indicated the involvement of residues such as Gly345, Thr347, Asp348, Ile344, Phe358, and Ser19 in ligand stabilization through hydrogen bonding and hydrophobic interactions. Val520 has the CH−π interactions with the ligand at 4.2 energy level. The ligand was well accommodated within the active pocket of MCM5, indicating a favourable binding conformation. Other compounds, including Ligand_8, Ligand_72, Ligand_49, and Ligand_197, also displayed strong binding affinities ranging from −9.682 to −9.371 kcal/mol.

Discussion

This extensive bioinformatics analysis dissects the topological structure of cervical cancer and validates RFC4, CKS2, and MCM5 as key prognostic regulators. The GSE39001 WGCNA analysis confirmed replicative stress characteristic of cervical cancer. Additionally, RFC4, CKS2, and MCM5 were validated using four distinct topological indices through the use of the STRING database in the development of the protein-protein interaction networks. Integration with machine learning by LASSO regularization and Random Forest Feature Importance yielded the optimal 3-gene signature

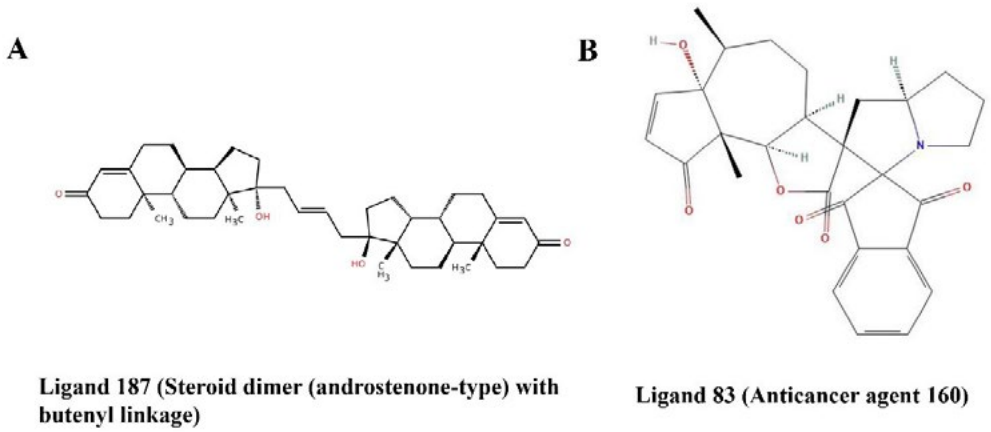

Figure 11 – Chemical structures of selected bioactive ligands identified in the study: (A) Ligand 187, a steroid dimer of the androstenone type containing a butenyl linkage, and (B) Ligand 83, a reported anticancer agent (compound 160)

(RFC4+CKS2+MCM5; TCGA-CESC high-risk HR ~1.8, 1-yr AUC 0.75). Validation analysis on TCGA-CESC data (tumors, n = 306; normal samples, n = 13) showed significant tumor-specific expression ($P < 0.05$) confirming prognostic utility. This is in line with previous cervical cancer studies where the involvement of cell cycle-related genes such as the MCM family and the RFC complex has been reported. The robustness of the proposed multi-gene signature is better compared to previous cervical cancer biomarkers involving a single gene.

The pathways implicated in the enrichment analysis of hub genes according to the KEGG database included the convergence on the DNA damage response, which is in line with the role of the E6/E7 oncoproteins of the virus in inactivating the RB/p53 pathways in cervical cancer cells. Future CRISPR validation will establish functional causality. Compared to prior cervical cancer signatures (typically 5-13 genes), our 3-gene model (RFC4+CKS2+MCM5) offers the smallest gene set with superior performance (C-index=0.72 vs clinical staging=0.61). Unlike larger signatures lacking mechanistic insight, ours directly links cell cycle/DNA replication genes to cisplatin chemotherapy response, providing actionable clinical utility beyond staging alone.

The molecular docking study revealed that the steroid dimer (androstenone-type) with butenyl linkage (Ligand_187) Figure 11A exhibited the strongest binding affinity toward RFC4 and CKS2 proteins, indicating its potential role as an effective inhibitor of cell-cycle associated targets. Similarly, Anticancer agent 160 (HY-156186; CS-0896639) Figure 11B (Ligand_83) showed significant interaction with the MCM5 protein, suggesting its possible involvement in suppressing DNA replication mechanisms. The strong binding energies and stable protein–ligand interactions observed in the docking complexes support the therapeutic relevance of these compounds in cancer-targeted treatment strategies. Overall, these findings highlight the potential of the selected ligands as promising lead molecules for further in vitro and in vivo investigations.

Implications and future research

Clinically, the 3 gene signature (RFC4, CKS2, MCM5) allows for precise risk stratification to provide personalized monitoring regiments and treatment options for cervical cancer patients. High-expression groups identified by this biomarker would benefit from maximal therapy, while the integration of this biomarker with HPV analysis would improve the utility of personalized medicine. There is a significant knowledge vacuum regarding the prediction of PD-1/PD-L1 inhibitor outcomes, and immunological infiltration patterns linked to this gene signature suggest that it may be a predictor of immunotherapy response. Future studies should validate this signature in prospective cohorts and explore functional roles via CRISPR knockout. Pharmacological targeting of replication stress represents a hypothesis for experimental investigation. Our 3-gene signature (RFC4+CKS2+MCM5) aligns with previous TCGA-CESC studies identifying cell cycle genes as key prognosticators [38]. The integrative WGCNA+LASSO+RF methodology mirrors [39] who combined WGCNA with Cox regression for cervical cancer subtyping. Superior performance vs clinical staging (C-index 0.72 vs 0.61) confirms ML advantage reported across 10 TCGA studies [40].

The study's limitations

Despite being thorough, there are a few issues that could be solved. First, the discovery cohort is the GSE39001 dataset, which is the result of a single microarray platform and has a relatively small sample size, risking the presence of batch effects despite rigorous preprocessing. Even though the results have been validated using the TCGA-CESC dataset, further cohort analysis is necessary for diverse groups and microarray platforms. Second, bulk RNA-seq data does not account for cell heterogeneity. "Hub" genes can represent stromal and immune cell contamination rather than tumor gene expression alone, requiring single-cell RNA-seq to resolve. Third, computer-generated protein-protein interaction (PPI) networks (STRING) provide physical/biochemical protein interactions without consideration for contextual regulation (post-translational regulation and expression). In cervical cancer cell lines (HeLa and SiHa), functional confirmation of proteins using CRISPR knockdown is required. Fourth, while the generated signatures were improved by optimising machine learning methods (LASSO, Random Forest), their dynamic prognostic value still needs to be examined by longitudinal validation. Fifth, molecular docking predictions await experimental confirmation via surface plasmon resonance or cellular assays to validate binding affinities and functional inhibition. Finally, the investigation targeted transcriptomic profiles alone, excluding proteomic and/or metabolomic analysis, thereby hampering the understanding of post-transcriptional regulation and metabolic dependencies. Future research aimed at filling these gaps using multi-omics,

functional genomics, and the conduct of clinical trials will help to enhance the translational component. This study strengthens the validation of robust 3-gene signature through multi-omics integrative analysis (WGCNA, LASSO, RF). Better prognostic performance compared with clinical staging (C-index: 0.72 vs. 0.61). Unbiased pathway analysis identifies cell cycle/DNA replication with treatment response. Clinical relevance of the signature confirmed through TCG.

Conclusion

The current work correctly identified and validated an aggressive 3-gene signature (RFC4, CKS2, MCM5) underlying cervical cancer via an integrated bioinformatics analysis pipeline ranging from WGCNA and machine learning analysis, through PPI network analysis, and ultimately multi-cohort validation studies. Some of the crucial observations made here reveal that these particular genes function as network hubs, ranking consistently highest on measures of degree, EPC, MCC, and MNC indices. The superior performance of the signature in TCGA-CESC external validation studies, combined with significant tumor-specific expression and prognostic value (especially RFC4: HR, 0.56; $p = 0.014$), confirms its value in risk stratification. The molecular docking analysis identified the steroid dimer (androstenone-type) with butenyl linkage (Ligand_187) and Anticancer agent 160 (HY-156186; CS-0896639) (Ligand_83) as promising compounds with strong binding affinities toward RFC4, CKS2, and MCM5 proteins. By connecting computational discovery with translation, this research series translates into candidate prognostic biomarkers requiring experimental and clinical validation, thus moving forward the field of personalized oncology in cervical cancer. The replication stress pathway is identified to be a promising target for synthetic lethality approaches, which could potentially see a paradigm shift in this globally important cancer.

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*Both authors R.R. and R.N. contributed equally.

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Data availability statement: All raw data publicly available from TCGA-CESC (n=306 tumors, n=13 normal) via GDC portal and GEO (GSE39001). Custom analysis scripts and processed datasets available from corresponding author upon reasonable request.

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Ethics statement: All data used in this study were obtained from publicly accessible datasets in the GEO database. No human participants or animal subjects were directly involved, and ethical approval was not required.

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Patient-Reported Pre-Transplant Experiences and Psychological Outcomes after Kidney Transplantation: a Structural Equation Modeling Study

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ABSTRACT

Background: Kidney transplantation is the preferred treatment for end-stage kidney disease, but long-term success depends not only on graft outcomes, but also on psychological adaptation and treatment adherence. Evidence on the associations between retrospectively reported pre-transplant experiences and post-transplant psychosocial outcomes remains limited, particularly in Central Asia. This study examined the direct and indirect associations of waiting-related emotional burden, satisfaction with pre-transplant care, awareness, and psychological support with post-transplant anxiety, psychological well-being, and ease of treatment adherence among kidney transplant recipients in Kazakhstan.

Methods: A cross-sectional survey was conducted among 223 adult kidney transplant recipients in Kazakhstan. Latent-variable structural equation modeling was performed in Mplus 8.5 using WLSMV estimation for ordinal indicators. The model included waiting-related emotional burden, satisfaction with pre-transplant care, awareness, anxiety, and well-being as latent constructs.

Results: The model showed acceptable fit (RMSEA=0.068, CFI=0.975, TLI=0.971, SRMR=0.074). Waiting-related burden had the largest standardized association with anxiety (β=0.482, p<0.001) and was also associated with lower well-being (β=-0.304, p=0.001). Anxiety was associated with lower well-being (β=-0.133, p=0.046) and lower perceived ease of treatment adherence (β=-0.306, p<0.001). Waiting-related burden also showed significant indirect associations with lower well-being and lower perceived ease of adherence through anxiety. Pre-transplant care satisfaction was positively associated with awareness and perceived ease of adherence, but also with greater post-transplant anxiety. Awareness was positively associated with well-being, and pre-transplant care satisfaction showed a significant positive indirect association with well-being through awareness. Psychological support during waiting was not associated with anxiety.

Conclusion: Retrospectively reported pre-transplant experiences were associated with post-transplant psychosocial adjustment. Waiting-related burden showed the strongest adverse pattern of associations, whereas awareness was positively associated with well-being. These findings support further prospective evaluation of coordinated psychosocial support before and after transplantation.

Keywords: Kidney Transplantation; Anxiety; Quality of Life; Medication Adherence; Waiting Lists; Health Literacy; Patient-Centered Care.

Introduction

Kidney transplantation (KTx) is widely recognized as the preferred treatment for end-stage kidney disease compared with long-term dialysis. However, long-term transplant success depends not only on surgical and immunological outcomes, but also on patients' psychological adaptation and treatment adherence. Emotional distress, particularly anxiety, and difficulties in maintaining adherence to immunosuppressive regimens remain significant contributors to graft complications and reduced quality of life [1, 2]. Accordingly, successful transplantation should be understood not only in terms of physical recovery, but also in terms of mental well-being and patients' ability to manage complex long-term treatment demands [3].

The pre-transplant period, particularly time spent on the waiting list, can involve substantial psychological and functional burden. For many patients, this period functions as a prolonged stress exposure characterized by uncertainty about transplant timing, fear of clinical deterioration or death, progressive physical limitation, and reduced autonomy in daily life [4]. Prior studies have shown that psychological stress is highly prevalent among kidney transplant candidates, with longer waiting-list duration independently associated with greater stress severity [5]. Other work has identified fear of death, inner conflict, uncertainty, and loss of social roles and autonomy as central emotional stressors during this phase [6]. Importantly, longitudinal evidence suggests that patients with poorer emotional well-being before transplantation may follow declining rather than improving psychological trajectories during the first post-transplant years, suggesting that poorer emotional well-being before transplantation may remain relevant after transplantation [7]. Based on this reasoning, the following hypotheses were formulated:

H1: Greater waiting-related emotional burden will be positively associated with post-transplant anxiety.

H2: Greater waiting-related emotional burden will be negatively associated with psychological well-being.

At the same time, some pre-transplant experiences may represent potentially modifiable correlates of post-transplant adjustment. Satisfaction with pre-transplant care and awareness of the transplant process may be considered modifiable health-system factors that can potentially be improved through communication, counseling, education, and more responsive healthcare services. In Kazakhstan, transplantation services have expanded considerably over the past decade, with national programs in heart, kidney, and liver transplantation demonstrating increasing capacity and clinical outcomes [8-10]. Within such systems, patients' experiences of care may be associated with their trust in clinicians, understanding of the transplant process, and readiness to engage in long-term treatment. Evidence suggests that better patient-perceived care quality is associated with superior transplant outcomes, while qualitative studies highlight communication quality, clarity of information, and staff responsiveness as especially important dimensions of care from the patient perspective [11,12]. A better understanding of the transplant process may enhance patients' sense of control and coping capacity, whereas positive experiences with healthcare providers may strengthen engagement with treatment [1]. Accordingly, the following hypotheses were formulated:

H3: Greater satisfaction with pre-transplant care will be positively associated with higher procedural awareness.

H4: Higher procedural awareness will be positively associated with psychological well-being and may also be positively associated with greater perceived ease of adherence.

Psychological support may represent an additional protective factor across the transplantation process. Both professional support from the healthcare team and broader social support from family or close others may help patients cope with uncertainty, regulate distress, and sustain treatment routines. Previous evidence has shown that social support is closely linked to post-transplant medication adherence, identifying it as one of the strongest psychosocial correlates of adherence among kidney transplant recipients [7,13]. These findings suggest that supportive interpersonal and clinical environments may buffer the emotional burden of transplantation and facilitate adaptation after surgery.

Health- and treatment-related anxiety may represent an important psychological correlate within the associations between retrospectively reported pre-transplant experiences and post-transplant outcomes. In the hypothesized model, anxiety was examined as an intermediate statistical correlate within the associations of waiting-related burden with well-being and perceived ease of treatment adherence. Given the cross-sectional design, this pathway was considered associative rather than causal or temporally established. Prior research has suggested that anxiety experienced during the pre-transplant period may persist after surgery and may be closely intertwined with adherence to immunosuppressive therapy [14]. In addition, internalizing symptoms, including anxiety, have been shown to mediate the relationship between perceived barriers and medication adherence in transplant recipients [15]. Based on this framework, the following hypotheses were formulated:

H5. Higher post-transplant anxiety will be negatively associated with psychological well-being and perceived ease of adherence.

H6. Significant indirect associations will be observed between pre-transplant experiences and post-transplant outcomes through anxiety and awareness. Specifically, waiting-related emotional burden will show indirect associations with psychological well-being and perceived ease of adherence through anxiety, while pre-transplant care satisfaction will show an indirect association with psychological well-being through awareness.

Despite growing recognition of psychosocial factors in transplantation, important gaps remain in the literature. Most existing studies have examined these associations in high-resource settings, and relatively little is known about how pre-transplant experiences are related to post-transplant outcomes in countries such as Kazakhstan, where access to care, service navigation, and regional disparities may differ. Moreover, few studies have tested these relationships simultaneously using integrated latent-variable models that can estimate direct and indirect associations simultaneously. This gap is particularly relevant in Central Asia. Recent studies from Kazakhstan found that post-transplant anxiety and difficulties in accessing care were negatively associated with well-being among kidney transplant recipients [16,17]. However, these studies focused on post-transplant correlates and did not examine retrospectively reported pre-transplant experiences or their indirect associations with later outcomes.

Therefore, the present study aimed to comprehensively assess pre-transplant experiences among 223 adult kidney transplant recipients in Kazakhstan, including waiting-related emotional burden, awareness, satisfaction with care, and psychological support, and to examine their direct and indirect associations with post-transplant anxiety, well-being, and perceived ease of adherence. Using a latent-variable structural equation modeling approach, we tested whether waiting-related burden was associated with greater anxiety and poorer well-being, whether satisfaction with pre-transplant care was associated with greater awareness, and whether significant indirect associations through anxiety and awareness were present between pre-transplant experiences and post-transplant outcomes.

Methods

Participants and study population

This cross-sectional study was conducted among adult kidney transplant recipients in Kazakhstan. Data were collected from February to April 2025 using online and telephone-based surveys. The survey invitation was initially distributed to 962 kidney transplant recipients. The online survey link was shared through patient-support group chats, while additional potential participants were contacted by telephone.

A total of 236 individuals responded. Eligibility criteria were: (1) age ≥18 years; (2) kidney transplantation performed in Kazakhstan; and (3) ability to complete the questionnaire in Kazakh or Russian. Eleven respondents were excluded because they did not meet the transplantation criterion: 10 had undergone kidney transplantation abroad, and one had undergone a second kidney transplantation. Of the remaining 225 eligible respondents, two were excluded because of incomplete questionnaire responses. Consequently, 223 participants were included in the final descriptive and structural equation analyses.

Sociodemographic and basic clinical characteristics, including age, sex, residence, education, and kidney disease duration, were collected at the time of participation using a structured questionnaire and are presented in Table 1.

Participants had a mean age of 38.7 ± 10.4 years (range 18-64). For descriptive purposes, age was grouped into 18-39 years (n = 118; 52.9%) and 40–64 years (n = 105; 47.1%). The sample included 98 men (43.9%) and 125 women (56.1%). Regarding language of participation, 96 (43.0%) completed the questionnaire in Kazakh and 127 (57.0%) in Russian.

Kidney disease duration was <1 year (20.6%), 1-3 years (33.2%), 4-5 years (9.9%), or >5 years (36.3%). Residence was rural (14.8%), small town (13.0%), regional-level city (33.6%), or national-level city (38.6%). Most participants were married (60.5%) and had children (64.6%). Education levels were middle-school (4.9%), high-school (9.9%), vocational (26.0%), undergraduate (58.3%), and post-graduate (0.9%).

Table 1 Study population (N=223)

Variable	n	%
Gender		
Male	98	43.9
Female	125	56.1
Age group		
18-39 y.o.	118	52.9
40-64 y.o.	105	47.1
Language		
Kazakh	96	43.0
Russian	127	57.0
Kidney disease duration		
< 1 year	46	20.6
1-3 years	74	33.2
4-5 years	22	9.9
> 5 years	81	36.3
Residence		
Rural	33	14.8
Small town	29	13.0
Regional-level city	75	33.6
National-level city	86	38.6
Occupation		
Student	19	8.5
Employed	82	36.8
Self-employed	26	11.7
Unemployed	18	8.1
Pensioner	9	4.0
On disability	69	30.9
Marital status		
Single	66	29.6
Married	135	60.5
Divorced	17	7.6
Widow	5	2.2
Children		
No	79	35.4
Yes	144	64.6
Educational level		
Middle-school	11	4.9
High-school	22	9.9
Vocational	58	26.0
Undergraduate	130	58.3
Post-graduate	2	0.9

Measures

Pre-transplant experiences were assessed using several patient-reported indicators capturing waiting-related emotional burden, perceived quality of pre-transplant care, informational preparedness, and psychological support:

- Waiting-related emotional burden (WaitImpact indicators): Emotional distress during the waiting-list period was measured with WLStress, reflecting how often participants experienced stress/anxiety/depressive feelings while waiting (1 = never to 5 = always). The functional impact of waiting on everyday life was assessed with WLAffect, indicating the degree to which waiting limited daily activities (1 = not limited to 4 = very strongly limited).
- Psychological support during waiting (WPsyCare). Perceived access to psychological or emotional support while on the waiting list was assessed using a single ordinal item rated on a 3-point scale (1 = no support, 2 = sometimes, and 3 = regularly). Psychological support was specified as a predictor of anxiety only, reflecting the prespecified conceptual assumption that support received during the waiting period would be most proximally related to emotional distress, whereas any associations with well-being or perceived ease of adherence might operate indirectly through anxiety. Because psychological support was measured using a single item, additional direct pathways to these more distal outcomes were not included in order to preserve model parsimony and avoid overinterpretation of a limited measure.
- Awareness and informational preparedness (Awareness indicators): Self-reported knowledge about the transplantation process was measured in two domains: awareness of waiting-list procedures (AwaWL) and awareness of pre-transplant procedures (AwaPreKTx). Both items were rated from 1 = not informed at all to 5 = well informed.
- Satisfaction with pre-transplant care (PreCare indicators): Satisfaction with healthcare services during the waiting period was measured using SatHCSW (higher scores indicate greater satisfaction). In addition, three items assessed satisfaction with key aspects of pre-KTx care: healthcare service quality during the pre-transplant period (SatpKT1), quality of consultations before transplantation (SatpKT2), and availability of diagnostic tests (SatpKT3). All satisfaction indicators were coded such that higher ratings reflect higher satisfaction.

Post-transplant outcomes:

- Health- and treatment-related anxiety (Anxiety; ANX1–ANX7). Post-transplant anxiety was assessed using a previously validated 7-item kidney-transplant–specific measure developed for kidney transplant recipients in Kazakhstan [16]. The measure captures common post-transplant concerns, including infection risk under immunosuppression, worries about maintaining graft function, anxiety about unfavorable laboratory results, long-term health stability, financial burden of ongoing treatment, low mood or depressive feelings, and concerns about immunosuppressant side effects. Items were

rated on a 5-point frequency scale ranging from 1 = never to 5 = always, with higher scores indicating greater health- and treatment-related anxiety [1,16].

- Psychological well-being (WellBeing; WHO-5). Subjective well-being was measured using the WHO-5 Well-Being Index (5 positively worded items). Each item was rated from 0 (at no time) to 5 (all of the time). Raw scores (0-25) were summed and multiplied by 4 to produce a standardized 0-100 score, where higher values indicate better psychological well-being [18].

- Ease of treatment adherence (EasyToAd). Perceived adherence was captured with a single self-report item assessing how easy vs. difficult it is for participants to follow their post-transplant treatment regimen, explicitly including regular intake of immunosuppressive medication. Responses were recorded on a 5-point scale, anchored from 5 = "very easy to follow" to 1 = "I do not follow" (higher scores = easier adherence).

Statistical analysis

Descriptive analysis. Categorical variables were summarized using frequencies and percentages, whereas continuous variables were described using means and standard deviations (SD).

Structural equation modeling. The hypothesized model was estimated in Mplus version 8.5 using the weighted least squares mean- and variance-adjusted estimator (WLSMV), theta parameterization, and a probit link. This approach was selected because most indicators were measured on ordered categorical response scales. Waiting-list stress, the perceived effect of waiting on daily life, the pre-transplant care-satisfaction indicators, the seven anxiety indicators, the five WHO-5 indicators, and perceived ease of treatment adherence were specified as ordered categorical variables and modeled through underlying continuous response variables with category thresholds freely estimated. The two awareness indicators (AwaWL and AwaPreKTx) were specified as continuous indicators of the latent Awareness construct rather than as ordered categorical variables. Although both variables were measured using five response categories, they were modeled as continuous because they served as indicators of an underlying latent construct rather than as endogenous ordinal outcomes. The treatment of five-category indicators as continuous is an accepted approach in structural equation modeling when category distributions are reasonably balanced and the variables provide reasonable approximations of an underlying continuous construct. In the present study, both awareness indicators showed relatively balanced response distributions and demonstrated satisfactory psychometric properties (standardized factor loadings: 0.693 and 0.868; $\omega = 0.76$), supporting this specification. Because the initial estimation produced an Mplus warning indicating that multiple numerical solutions were possible, the final model was re-estimated using 100 random starting values (STARTS = 100). Multiple random-start runs reproduced the same optimum solution, with unchanged parameter estimates and model-fit indices, supporting the numerical stability of the final model.

The measurement component comprised five latent constructs. Waiting-list impact was indicated by waiting-related emotional distress and the perceived limitation of daily life. Pre-transplant care satisfaction was indicated by satisfaction with healthcare services during the waiting period (SatHCSW), healthcare service quality during the pre-transplant period (SatpKT1), satisfaction with pre-transplant medical consultations (SatpKT2), and satisfaction with the availability of diagnostic tests during the pre-transplant period (SatpKT3). Health- and treatment-related anxiety was represented by seven items, psychological well-being by the five items of the WHO-5 Well-Being Index, and awareness by self-reported awareness of waiting-list and pre-transplant procedures. Perceived ease of treatment adherence was modeled as a single observed ordinal outcome rather than as a latent adherence construct.

Latent-variable scales were established using marker-variable identification, with the first loading of each latent construct fixed to 1.0 and the remaining loadings freely estimated. Waiting-list impact and awareness were each represented by two indicators. These constructs were retained as latent variables because their indicators captured conceptually distinct but related dimensions of the underlying construct. Their identification was supported by the marker-variable scaling approach and the broader structural specification. Nevertheless, because two-indicator factors provide limited information for independently evaluating measurement structure, their interpretation was based on theoretical coherence, standardized factor loadings, 95% confidence intervals, residual variances, and indicator-level explained variance and was treated cautiously.

The measurement component was evaluated before interpretation of the structural associations. Standardized factor loadings, 95% confidence intervals, indicator residual variances, and indicator-level R^2 values were examined. The estimated thresholds were also inspected to confirm that they increased monotonically across the ordered response categories. Internal consistency was evaluated using coefficient omega for multi-item constructs where available; reliability estimates for the two-indicator waiting-list impact and awareness constructs were interpreted cautiously because such estimates do not substitute for a more extensively measured latent construct.

Survey language was recorded for all participants, with approximately 43% completing the Kazakh-language version and 57% completing the Russian-language version. The primary structural equation model was estimated in the pooled sample. Formal multigroup measurement-invariance testing by survey language was not conducted because the language-specific subgroup sizes were limited for estimating ordinal models with freely estimated thresholds and because some constructs contained only two indicators. The pooled analysis therefore assumes broadly comparable measurement properties across the two language versions, although this assumption could not be formally verified in the present study.

The structural component specified anxiety as a function of waiting-list impact, pre-transplant care satisfaction, awareness, psychological support during the waiting period, age, and sex. Psychological well-being and perceived ease of treatment adherence were regressed on anxiety, waiting-list impact, pre-transplant care satisfaction, awareness, age, and sex. Awareness was regressed on pre-transplant care satisfaction, age, and sex. Waiting-list impact and pre-transplant care satisfaction were allowed to covary, and a residual covariance was specified between psychological well-being and perceived ease of treatment adherence. Thus, the structural model adjusted for age and sex. Time since transplantation and several other potentially relevant clinical covariates, including donor type, dialysis duration, rejection history, post-transplant complications, and immunosuppressive regimen, could not be included because they were not collected in a sufficiently complete and standardized form across all participants. The estimated associations should therefore not be interpreted as being independent of these clinical characteristics.

Indirect associations. Model-based indirect associations were estimated for the prespecified structural sequences linking waiting-list impact and pre-transplant care satisfaction with psychological well-being and perceived ease of treatment adherence. Because the study was cross-sectional and pre-transplant experiences were assessed retrospectively after transplantation, these estimates were interpreted as indirect statistical associations consistent with the specified model rather than as evidence of temporal or causal mediation.

Model evaluation. Overall model fit was assessed using the chi-square test, comparative fit index (CFI), Tucker–Lewis index (TLI), root mean square error of approximation (RMSEA) with its 90% confidence interval, and standardized root mean square residual (SRMR). Structural coefficients and indirect associations were reported as STDYX-standardized estimates with 95% confidence intervals and two-sided p values. Statistical significance was defined as $p < .05$.

Missing data. The analyzed variables showed complete covariance coverage, with one missing-data pattern and coverage values of 1.00 for all variable pairs. Accordingly, the SEM was estimated using 223 complete observations for the variables included in the model.

Sample-size considerations

No formal a priori sample-size calculation was performed because the study aimed to recruit all reachable eligible adult kidney transplant recipients during the data-collection period. Closed-form sample-size formulas for WLSMV-based structural equation models with ordinal indicators are not straightforward; therefore, sample adequacy was evaluated pragmatically in relation to model complexity and estimation stability. The achieved sample of 223 participants was considered adequate for the prespecified, relatively parsimonious model, which included five latent constructs measured by 20 indicators, one observed perceived ease-of-adherence outcome, and a limited set of covariates. The final model converged normally, demonstrated acceptable-to-good fit, and produced statistically significant factor loadings for all indicators. Nevertheless, the sample may have provided limited power to detect smaller structural associations or support more complex subgroup analyses.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Bioethics Commission of the "University Medical Center" Corporate Fund (Protocol No. 3, 14 July 2023). Informed consent was obtained from all participants prior to participation, and anonymity/confidentiality were maintained.

Results

Pre-transplant experience, awareness, and satisfaction

In the pre-transplant phase, emotional strain during the waiting-list period was common (Table 2). Over half of participants (52.9%; n=118) reported experiencing stress, anxiety, or depressive feelings always or often, whereas 11.2% (n=25) indicated never having these feelings. Awareness about the waiting-list process varied markedly: nearly one-third described themselves as uninformed (31.8%; n=71), while an equal proportion reported being good or very well informed (31.8%; n=71). By contrast, knowledge of pre-transplant procedures was higher overall, with 66.4% (n=148) reporting good or very good awareness.

Satisfaction with care was generally favorable across domains. During the waiting period, 66.0% (n=147) reported being satisfied or very satisfied with healthcare service quality. Ratings were higher in the pre-transplant period: 80.7% (n=180) were satisfied or very satisfied with healthcare service quality, 79.8% (n=178) with medical consultations, and 74.9% (n=167) with the availability of diagnostic tests.

Waiting for transplantation was reported to limit daily life (WLAffect) very strongly by 21.5% (n=48) and strongly by 22.9% (n=51); 38.1% (n=85) reported partial limitation and 17.5% (n=39) reported no limitation. Regarding psychological/emotional support during the waiting period (WPsyCare), 43.0% (n=96) reported receiving it regularly, 23.8% (n=53) sometimes, and 33.2% (n=74) reported not receiving such support.

Table 2 Pre-KTx experience, awareness and satisfaction (N=223)

Variable	Response, n (%)				
Stress, anxiety, depression on Waiting list (WLStress)	Always 86 (38.6)	Often 32 (14.3)	Sometimes 56 (25.1)	Rarely 24 (10.8)	Never 25 (11.2)
Awareness on Waiting list procedures (AwaWL)	Very good informed 34 (15.2)	Well informed 37 (16.6)	Somewhat informed 39 (17.5)	Poorly informed 42 (18.8)	Uninformed 71 (31.8)
Awareness on Pre-KTx procedures (AwaPreKTx)	Very good informed 90 (40.4)	Good informed 58 (26.0)	Somewhat informed 47 (21.1)	Poor informed 13 (5.8)	Uninformed 15 (6.7)
Satisfaction with healthcare service quality during the waiting KTx (SatHCSW)	Very Satisfied 63 (28.3)	Satisfied 84 (37.7)	Somewhat satisfied 41 (18.4)	Dissatisfied 20 (9.0)	Not satisfied at all 15 (6.7)
Satisfaction with healthcare service quality (pre-KTx period, SatpKT1)	Very Satisfied 89 (39.9)	Satisfied 91 (40.8)	Somewhat satisfied 28 (12.6)	Dissatisfied 10 (4.5)	Not satisfied at all 5 (2.2)
Satisfaction with medical consultations (pre-KTx period, SatpKT2)	Very Satisfied 110 (49.3)	Satisfied 68 (30.5)	Somewhat satisfied 27 (12.1)	Dissatisfied 14 (6.3)	Not satisfied at all 4 (1.8)
Satisfaction with availability of diagnostic tests (pre-KTx period, SatpKT3)	Very Satisfied 95 (42.6)	Satisfied 72 (32.3)	Somewhat satisfied 34 (15.2)	Dissatisfied 18 (8.1)	Not satisfied at all 4 (1.8)

Data are presented as n (%). KTx = kidney transplantation. For awareness and satisfaction items, categories are displayed from the most favorable to the least favorable response. For WLStress, categories are displayed from the highest to the lowest reported frequency of distress.

Table 3 Standardized factor loadings (STDYX)

Latent factor	Indicator	Loading	95% CI	p	R2
KTx waiting-related emotional burden (WaitImpact)	WLStress	0.739	0.600 – 0.879	<0.001	0.547
	WLAffect	0.649	0.525 – 0.774	<0.001	0.422
Satisfaction with pre-transplant healthcare (PreCare)	SatHCSW	0.777	0.723 – 0.831	<0.001	0.603
	SatpKT1	0.834	0.787 – 0.880	<0.001	0.695
	SatpKT2	0.970	0.942 – 0.999	<0.001	0.942
	SatpKT3	0.934	0.906 – 0.963	<0.001	0.873
Awareness	AwaWL	0.693	0.593 – 0.793	<0.001	0.481
	AwaPreKTx	0.868	0.777 – 0.960	<0.001	0.754
Treatment-related Anxiety (Anxiety)	ANX1	0.853	0.808 – 0.898	<0.001	0.727
	ANX2	0.871	0.829 – 0.914	<0.001	0.759
	ANX3	0.827	0.779 – 0.875	<0.001	0.684
	ANX4	0.912	0.877 – 0.946	<0.001	0.831
	ANX5	0.637	0.544 – 0.729	<0.001	0.406
	ANX6	0.725	0.651 – 0.799	<0.001	0.525
	ANX7	0.824	0.770 – 0.878	<0.001	0.679
Well-being (WellBeing)	WHO1	0.926	0.904 – 0.949	<0.001	0.858
	WHO2	0.887	0.857 – 0.916	<0.001	0.787
	WHO3	0.920	0.896 – 0.945	<0.001	0.847
	WHO4	0.854	0.817 – 0.890	<0.001	0.729
	WHO5	0.854	0.816 – 0.893	<0.001	0.729

Values are STDYX-standardized factor loadings. CI = confidence interval; KTx = kidney transplantation; R^2 = indicator-level explained variance; STDYX = standardization based on the variances of both the predictor and outcome. WaitImpact = waiting-list impact; WLStress = emotional distress during the waiting-list period; WLAffect = perceived effect of waiting on everyday life; PreCare = satisfaction with pre-transplant healthcare; SatHCSW = satisfaction with healthcare service quality during the waiting-list period; SatpKT1 = satisfaction with healthcare service quality during the pre-transplant period; SatpKT2 = satisfaction with pre-transplant medical consultations; SatpKT3 = satisfaction with the availability of pre-transplant diagnostic tests; AwaWL = self-rated awareness of waiting-list procedures; AwaPreKTx = self-rated awareness of pre-transplant procedures; Anxiety = health- and treatment-related anxiety; ANX1-ANX7 = individual anxiety-scale indicators; WellBeing = psychological well-being; WHO1-WHO5 = individual items of the WHO-5 Well-Being Index. Higher standardized loadings indicate stronger relationships between indicators and their respective latent constructs. All p values are two-sided.

After transplantation, participants most commonly reported that following the prescribed treatment regimen, including immunosuppressive therapy, was very easy (43.5%; n=97) or easy (33.6%; n=75), whereas 18.8% (n=42) perceived it as somewhat difficult. A small proportion reported that treatment adherence was difficult (3.1%; n=7) or indicated that they did not follow the prescribed regimen (0.9%; n=2). The mean post-transplant anxiety score was 3.65±1.0 on a 1-5 scale. The mean WHO-5 Well-Being Index score was 66.1±24.6 on a 0-100 scale.

Structural equation modeling

A latent variable SEM was estimated in Mplus 8.5 using WLSMV (theta parameterization; probit link) to account for the ordinal indicators (N=223). The model specified five latent constructs: WaitImpact (WLStress, WLAffect), PreCare (SatHCSW, SatpKT1-SatpKT3), Awareness (AwaWL, AwaPreKTx), Anxiety (ANX1-ANX7), and WellBeing (WHO1-WHO5). Structural paths were estimated from WaitImpact, PreCare, and Awareness to Anxiety, WellBeing, and perceived ease of treatment adherence (EasyToAd). Awareness was additionally regressed on pre-transplant care satisfaction, age, and sex. Age and sex were included as covariates in the outcome equations, and WPsyCare was included as a predictor of Anxiety.

The model converged normally and demonstrated acceptable fit, $\chi2(230)=467.96$, $p<0.001$, CFI=0.975, TLI=0.971, RMSEA=0.068 (90% CI 0.059-0.077), and SRMR=0.074. To evaluate numerical stability, the model was re-estimated using 100 random starting values (STARTS = 100). Multiple random-start runs reproduced the same optimum solution, and the model-fit indices and parameter estimates were unchanged, supporting the stability of the final solution.

All indicators loaded significantly on their intended latent constructs. Standardized loadings ranged from 0.649 to 0.739 for waiting-list impact, from 0.777 to 0.970 for pre-transplant care satisfaction, from 0.637 to 0.912 for anxiety, from 0.854 to 0.926 for psychological well-being, and from 0.693 to 0.868 for awareness. Model-based internal consistency was high for pre-transplant care satisfaction ($\omega=0.93$), anxiety ($\omega=0.93$), and psychological well-being ($\omega=0.95$). Omega was 0.65 for waiting-list impact and 0.76 for awareness; these estimates should be interpreted cautiously because each construct contained only two indicators.

In the structural equation model, waiting-related burden (WaitImpact) showed the largest standardized association with post-transplant anxiety (Anxiety) among the modeled predictors ($\beta=0.482$, $p<0.001$). Satisfaction with pre-transplant healthcare was also statistically associated with greater post-transplant anxiety ($\beta=0.298$, $p=0.006$). In contrast, transplant-related awareness (Awareness) was not associated with anxiety ($\beta=-0.019$, $p=0.845$). Psychological care during the waiting period (WPsyCare) and the covariates age (Age) and sex (Sex) were not associated with anxiety in this model (Table 4, Figure 1A). This finding should be regarded as an exploratory, model-based statistical association rather than evidence of an independent relationship between pre-transplant care satisfaction and anxiety. Residual confounding by clinical complexity, care intensity,

Table 4 Structural paths (standardized; STDYX)

Outcome	Predictor	β (STDYX)	95% CI	p
Anxiety	WaitImpact	0.482	0.291 – 0.672	<0.001
	PreCare	0.298	0.085 – 0.510	0.006
	Awareness	-0.019	-0.211 – 0.172	0.845
	WPsyCare	0.000	-0.147 – 0.146	0.997
	Age	0.029	-0.111 – 0.170	0.681
	Sex	0.003	-0.141 – 0.148	0.963
WellBeing	Anxiety	-0.133	-0.264 – -0.002	0.046
	WaitImpact	-0.304	-0.486 – -0.122	0.001
	PreCare	-0.004	-0.187 – 0.180	0.968
	Awareness	0.240	0.064 – 0.416	0.008
	Age	-0.023	-0.167 – 0.120	0.749
	Sex	0.021	-0.116 – 0.157	0.766
Awareness	PreCare	0.604	0.512 – 0.695	<0.001
	Age	-0.157	-0.309 – -0.004	0.044
	Sex	-0.061	-0.211 – 0.089	0.427
EasyToAd	Anxiety	-0.306	-0.455 – -0.156	<0.001
	WaitImpact	-0.099	-0.313 – 0.116	0.367
	PreCare	0.239	0.042 – 0.436	0.018
	Awareness	0.099	-0.080 – 0.278	0.279
	Age	-0.002	-0.154 – 0.151	0.983
	Sex	0.020	-0.129 – 0.168	0.794

Coefficients are STDYX-standardized structural estimates. β = standardized regression coefficient; CI = confidence interval; STDYX = standardization based on the variances of both the predictor and outcome. WaitImpact = waiting-list impact; PreCare = satisfaction with pre-transplant healthcare; Awareness = transplant-related procedural awareness; WPsyCare = perceived access to psychological or emotional support during the waiting-list period; Anxiety = post-transplant health- and treatment-related anxiety; WellBeing = psychological well-being; EasyToAd = perceived ease of adherence to the post-transplant treatment regimen. Age was entered as a continuous variable. Sex was coded 1 = male and 2 = female; therefore, a positive coefficient represents a higher expected outcome value for female participants, although sex-related coefficients should be interpreted cautiously when nonsignificant. Higher EasyToAd scores indicate greater perceived ease of adherence. All p values are two-sided; p < .05 was considered statistically significant.

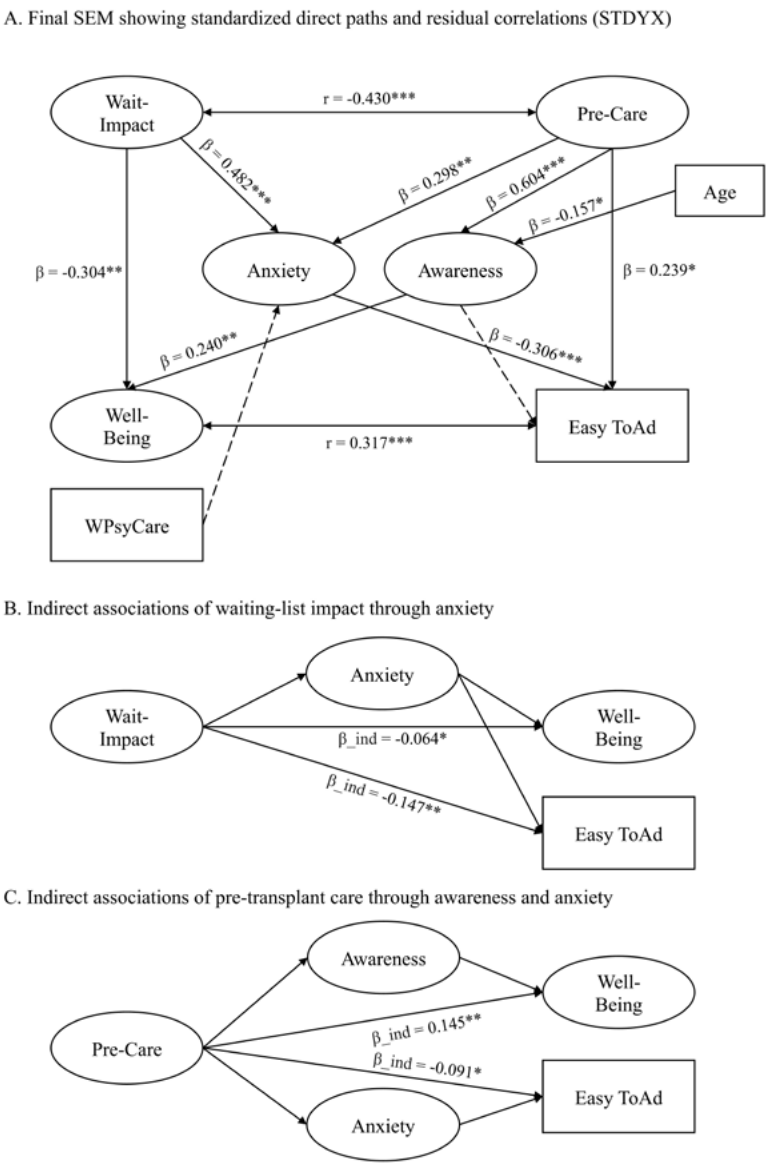

Figure 1 – Structural equation model of direct and indirect associations between retrospectively reported pre-transplant experiences and post-transplant psychosocial outcomes. Panel A: Final structural equation model showing statistically significant standardized direct paths and residual correlations. Panel B: Indirect associations of waiting-list impact with psychological well-being and perceived ease of treatment adherence through anxiety. Panel C: Indirect associations of pre-transplant care satisfaction with psychological well-being through awareness and with perceived ease of treatment adherence through anxiety.

*Solid single-headed arrows indicate structural regression paths; double-headed arrows indicate correlations or residual correlations. Dashed arrows indicate nonsignificant paths retained in the model. Values are standardized coefficients (STDYX). β_ind denotes a standardized indirect association. Only selected statistically significant paths are labeled in Panel A. *p < 0.05, **p < 0.01, ***p < 0.001. Because the data were cross-sectional and pre-transplant experiences were assessed retrospectively, indirect associations should not be interpreted as evidence of temporal or causal mediation.*

monitoring frequency, or other unmeasured factors cannot be excluded.

In the final structural model, greater post-transplant anxiety was associated with lower well-being (β=-0.133, p=0.046), and greater waiting-related burden was directly associated with lower well-being (β=-0.304, p=0.001). Greater transplant-related awareness was associated with higher well-being (β=0.240, p=0.008), whereas satisfaction with pre-transplant healthcare was not directly associated with well-being (β=-0.004, p=0.968; Table 4; Figure 1A).

For EasyToAd, greater post-transplant anxiety was associated with lower perceived ease of adherence (β=-0.306, p<0.001). In contrast, greater satisfaction with pre-transplant healthcare was associated with greater perceived ease of adherence (β=0.239, p=0.018). The direct associations of waiting-related burden and transplant-related awareness with perceived ease of adherence were not statistically significant.

At the level of relations among predictors and residuals, waiting-related impact and satisfaction with pre-transplant healthcare were negatively correlated (r=-0.430, 95% CI [-0.571, -0.289], p<0.001; Figure 1A). After accounting for the modeled pathways, WellBeing and EasyToAd retained a positive residual correlation (r=0.317, 95% CI [0.192, 0.442], p<0.001; Figure 1A).

As illustrated in Figure 1B, waiting-related impact showed statistically significant indirect associations with both outcomes through anxiety. The indirect path waiting-related impact (WaitImpact) → post-transplant anxiety (Anxiety) → well-being (WellBeing) was negative (βind=-0.064, SE=0.031 p=0.042). Likewise, the indirect path waiting-related impact (WaitImpact) → post-transplant anxiety (Anxiety) → perceived ease of

Table 5 Standardized total, direct, and indirect effects (STDYX)

Predictor → Outcome	Total	Direct	Indirect (total)	Sign. specific indirect paths
WaitImpact → WellBeing	-0.369 (SE=0.079, p<0.001)	-0.304 (SE=0.093, p=0.001)	-0.064 (SE=0.031, p=0.042)	via Anxiety: -0.064 (SE=0.031, 95% CI [-0.126, -0.002], p=0.042)
WaitImpact → EasyToAd	-0.246 (SE=0.094, p=0.009)	-0.099 (SE=0.109, p=0.367)	-0.147 (SE=0.046, p=0.001)	via Anxiety: -0.147 (SE=0.046, 95% CI [-0.238, -0.057], p=0.001)
PreCare → WellBeing	0.103 (SE=0.072, p=0.152)	-0.004 (SE=0.094, p=0.968)	0.107 (SE=0.062, p=0.086)	via Awareness: 0.145 (SE=0.056, 95% CI [0.035, 0.254], p=0.009)
PreCare → EasyToAd	0.211 (SE=0.073, p=0.004)	0.239 (SE=0.101, p=0.018)	-0.028 (SE=0.068, p=0.683)	via Anxiety: -0.091 (SE=0.040, 95% CI [-0.169, -0.013], p=0.023)

Values are STDYX-standardized total, direct, and indirect associations. β_ind = standardized indirect association; CI = confidence interval; SE = standard error; SEM = structural equation modeling; STDYX = standardization based on the variances of both the predictor and outcome. WaitImpact = waiting-list impact; PreCare = satisfaction with pre-transplant healthcare; Anxiety = post-transplant health- and treatment-related anxiety; Awareness = transplant-related procedural awareness; WellBeing = psychological well-being; EasyToAd = perceived ease of adherence to the post-transplant treatment regimen. Higher EasyToAd scores indicate greater perceived ease of adherence. "Indirect (total)" represents the sum of all model-specified indirect associations between the predictor and outcome; therefore, it may differ from an individual specific indirect association shown in the final column. Only statistically significant specific indirect associations are displayed. All p values are two-sided; p < .05 was considered statistically significant. Because the data are cross-sectional and pre-transplant experiences were assessed retrospectively, direct, indirect, and total estimates represent model-based statistical associations and should not be interpreted as causal effects or evidence of temporal mediation.

adherence (EasyToAd) was negative (βind=-0.147, SE=0.046 p=0.001). In both cases, the total effect of waiting-related impact on the outcome was also negative (Table 5).

As shown in Figure 1C, satisfaction with pre-transplant healthcare demonstrated two distinct indirect patterns. Satisfaction with pre-transplant healthcare was strongly associated with transplant-related awareness (PreCare → Awareness; β=0.604, p<0.001; Figure 1A), and the specific indirect effect satisfaction with pre-transplant healthcare (PreCare) → transplant-related awareness (Awareness) → well-being (WellBeing) was positive and statistically significant (βind=0.145, SE=0.056, p=0.009; Figure 1C). In contrast, the specific indirect effect satisfaction with pre-transplant healthcare (PreCare) → post-transplant anxiety (Anxiety) → perceived ease of adherence (EasyToAd) was negative (βind=-0.091, SE=0.040, p=0.023; Figure 1C). Notably, this negative indirect pathway co-occurred with a positive direct association between satisfaction with pre-transplant healthcare and perceived ease of adherence (β=0.239, SE=0.101, p=0.018), yielding a positive total effect of satisfaction with pre-transplant healthcare on perceived ease of adherence (βtotal=0.211, SE=0.063, p=0.004). These indirect effects represent model-based statistical associations and should not be interpreted as establishing temporal sequencing or causal mediation.

Overall, the model accounted for 19.7% of the variance in post-transplant anxiety (Anxiety; R2=0.197, p=0.006), 23.3% of the variance in well-being (WellBeing; R2=0.233, p<0.001), and 39.4% of the variance in transplant-related awareness (Awareness; R2= 0.394, p<0.001). For perceived ease of adherence (EasyToAd), the explained variance was R2=0.232 (p<0.001).

Discussion

This study examined associations between retrospectively reported pre-transplant experiences and current post-transplant psychosocial outcomes among kidney transplant recipients in Kazakhstan. Greater waiting-related burden was associated with greater post-transplant anxiety and lower well-being, whereas greater awareness was associated with better well-being. Satisfaction with pre-transplant care was associated with greater awareness and perceived ease of adherence, but also with greater post-transplant anxiety. These findings should be interpreted as cross-sectional associations rather than evidence of temporal or causal relationships.

Waiting-Related Burden and Anxiety

Greater waiting-related burden was directly associated with lower post-transplant well-being and indirectly associated with lower well-being and lower perceived ease of adherence through anxiety. Because waiting experiences were assessed retrospectively, the present study cannot establish persistence or temporal carryover. Nevertheless, the findings are consistent with evidence that uncertainty, fear of deterioration, and loss of autonomy contribute to psychological strain during the waiting period [19,20].

Recipients reporting greater waiting-related burden also reported higher post-transplant anxiety, which was associated with poorer well-being and lower perceived ease of adherence. This pattern is consistent with previous kidney-transplant research describing concerns about infection, medication adverse effects, laboratory results, graft rejection, and long-term graft survival [21,22]. Anxiety may be associated with greater cognitive and emotional load and with greater perceived difficulty managing long-term treatment demands. However, these processes were not directly measured, and reverse or bidirectional associations remain possible. Anxiety should therefore be interpreted as an intermediate statistical correlate rather than as a causal mechanism [1,2,14-16,23,24].

Awareness and Pre-Transplant Care

Awareness was positively associated with well-being, and pre-transplant care satisfaction showed a positive indirect association with well-being through awareness. Greater awareness may be related to increased predictability, perceived control, and reduced uncertainty regarding transplantation procedures and expectations. This interpretation is consistent with evidence that health communication interventions can improve recipients' ability to appraise health information and navigate care [25]. However, awareness was not directly associated with anxiety or perceived ease of adherence. Randomized and observational evidence similarly suggests that improved health literacy does not necessarily translate into better medication adherence [26].

Satisfaction with pre-transplant care showed a mixed pattern. Greater satisfaction was associated with greater perceived ease of adherence but also with greater post-

transplant anxiety. The positive association with perceived ease of adherence is consistent with evidence that communication quality, information availability, and staff responsiveness may support treatment engagement [11,12].

The positive association between satisfaction and anxiety should be considered exploratory and should not be interpreted as evidence that better care increased anxiety. Unmeasured clinical complexity, care intensity, monitoring frequency, or risk communication may have influenced both satisfaction and anxiety. Patients receiving more intensive care may have felt better supported while also being more concerned about rejection, complications, medication adverse effects, or graft survival. More engaged patients may also have reported both greater perceived ease of adherence and greater worry. Because these factors were not comprehensively measured, the explanation and direction of this association remain uncertain. Prospective studies are needed to distinguish among these possibilities [14,16].

Additional Model Findings

Waiting-related burden and pre-transplant care satisfaction were negatively correlated, indicating that recipients reporting more difficult waiting experiences also tended to report lower satisfaction with care. The direction of this association cannot be determined. The positive residual association between well-being and perceived ease of adherence suggests that these outcomes may share unmeasured correlates such as self-efficacy, social support, resilience, or coping resources [13,24]. Age and sex were not strongly associated with the main outcomes. The negative association between age and awareness may indicate that older recipients felt less informed, although this finding requires confirmation [25,26].

Clinical Implications

The findings support further evaluation of focused psychosocial assessment rather than broad service-level conclusions. Brief assessment of waiting-related distress, uncertainty, functional limitation, and transplant-related fears may help identify recipients who could benefit from closer follow-up, although prospective studies are needed to determine whether such screening improves outcomes [4-6,14,16,21].

Post-transplant anxiety was associated with lower well-being and greater perceived difficulty following treatment. Brief anxiety screening, clear communication about graft monitoring and medication adverse effects, and access to psychological support may therefore represent reasonable hypotheses for service improvement. The present study does not establish that these approaches would improve adherence or well-being [2,14,23,24].

Awareness was associated with well-being but not with perceived ease of adherence. Patient education may therefore be more useful when combined with emotional and behavioral support rather than delivered as information alone. Future studies should test whether integrated education, anxiety support, and referral procedures improve patient-reported and clinical outcomes [12,17,25,27-29].

Strengths and Limitations

This study examined retrospectively reported pre-transplant experiences and current post-transplant outcomes within one latent-variable framework. The SEM approach enabled simultaneous estimation of direct and indirect associations while accounting for measurement error. The study also contributes evidence from Kazakhstan, where psychosocial transplantation research remains limited.

Several limitations should be acknowledged. First, the cross-sectional design and retrospective assessment of pre-transplant experiences preclude conclusions about causality, temporal ordering, or prospective prediction. Current anxiety or well-being may have influenced recollections of waiting-list burden, awareness, psychological support, and satisfaction with care. The reported direct and indirect associations should therefore be interpreted as model-based statistical associations rather than temporal or causal relationships.

Second, selection bias is possible because participants were recruited through online support-group chats and telephone contact. This approach may have over-represented recipients who were more engaged with support networks, more connected to healthcare services, easier to contact, or more willing to participate. Recipients who were socially isolated, severely unwell, or living in areas with limited connectivity may have been under-represented. Because information on eligible nonparticipants was unavailable, the direction and magnitude of this bias could not be assessed.

Third, the study relied on patient-reported measures. Perceived ease of adherence was assessed using a single ordinal item and should not be interpreted as objective or multidimensional adherence. Psychological support was also measured using a single item that did not capture quality, intensity, timing, or duration. Future studies should use validated multidimensional adherence measures and objective indicators such as pharmacy refill data, immunosuppressant-level variability, or electronic monitoring.

Fourth, waiting-list impact and awareness were modeled as two-indicator latent variables, limiting independent evaluation of their measurement structure. The model also omitted potentially relevant psychological, clinical, and socioeconomic variables, including depression, illness perceptions, coping, resilience, self-efficacy, time since transplantation, donor type, dialysis duration, rejection, complications, immunosuppressive regimen, socioeconomic status, residence, and education. Residual confounding is therefore possible, particularly for the association between pre-transplant care satisfaction and anxiety. The moderate sample size may also have limited sensitivity to smaller associations and more complex subgroup analyses.

Finally, formal measurement invariance across the Kazakh- and Russian-language versions was not tested. The pooled model therefore assumes broadly comparable measurement properties across languages, but equivalence of factor loadings, thresholds, and item functioning cannot be confirmed. Larger studies should test configural, metric, and scalar or threshold invariance before comparing latent means or structural associations across language groups.

Future Research

Future studies should use prospective longitudinal designs beginning during the waiting-list period and continuing after transplantation. They should incorporate objective or multidimensional adherence measures, broader psychological constructs, and relevant clinical and socioeconomic covariates. Larger samples are needed to examine time since transplantation, complications, access to care, and language-specific measurement properties. Intervention studies should test whether combined education, anxiety support, and behavioral adherence strategies improve patient-reported and clinical outcomes. Future work could also examine how psychosocial factors interact with model-informed or artificial-intelligence-supported immunosuppressant dosing [30].

Overall, waiting-related burden, anxiety, awareness, and perceived pre-transplant care were interrelated correlates of

post-transplant adjustment. Prospective research is needed to determine their temporal direction and clinical significance.

Conclusion

In conclusion, retrospectively reported pre-transplant experiences were significantly associated with current post-transplant psychological well-being and perceived ease of adherence among kidney transplant recipients in Kazakhstan. Waiting-related emotional burden showed the strongest adverse pattern of associations, including a direct association with poorer well-being and significant indirect associations with poorer well-being and lower perceived ease of adherence through health- and treatment-related anxiety. Anxiety represented an important intermediate statistical correlate within these modeled relationships. Transplant-related awareness was positively associated with well-being, while satisfaction with pre-transplant care showed a mixed pattern of associations with awareness, anxiety, and perceived ease of adherence. Because the study was cross-sectional, these findings do not establish temporal sequencing or causal mediation. Nevertheless, they support further prospective evaluation of psychosocial screening, structured patient education, and anxiety-focused support across the transplant trajectory.

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Data availability statement: All raw data underlying the findings of this study can be obtained from the corresponding author upon reasonable request.

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Comparative Outcomes of Closed RCA Endarterectomy and Open LAD Endarterectomy with LIMA Patch Plasty

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ABSTRACT

Introduction: Coronary endarterectomy (CE) is an adjunctive technique used to achieve complete revascularization in patients with diffuse coronary artery disease when conventional bypass grafting is not feasible. To compare early outcomes and graft patency between closed right coronary artery (RCA) endarterectomy and open left anterior descending (LAD) endarterectomy with left internal mammary artery (LIMA) patch plasty.

Methods: This retrospective study included 31 patients undergoing closed traction RCA endarterectomy and 36 patients undergoing open LAD endarterectomy with LIMA patch plasty during CABG. Perioperative outcomes, postoperative arrhythmias, and follow-up coronary angiographic findings were analyzed.

Results: Early mortality occurred only in the closed RCA group but was not statistically significant. Atrial fibrillation rates were similar. The closed RCA group demonstrated significantly higher postoperative bleeding, greater inotropic and intra-aortic balloon pump requirements, longer ICU stay, and more frequent ventricular arrhythmias. Follow-up angiography at a mean of 6.08 ± 2.01 years (open LAD) and 4.21 ± 1.12 years (closed RCA) showed significantly higher anastomotic patency in the open LAD group (94.1% vs. 50.0%, p = 0.008).

Conclusion: Open LAD endarterectomy with LIMA patch plasty provides a more stable early postoperative course and superior mid-term graft patency compared with closed RCA endarterectomy.

Keywords: Coronary endarterectomy; Right coronary artery; Left anterior descending artery; LIMA patch plasty; Coronary artery bypass grafting

Introduction

The population of patients referred for coronary artery bypass grafting (CABG) has evolved considerably over time. In patients with ischemic heart disease and diffuse coronary artery disease, percutaneous coronary interventions such as angioplasty and stent implantation are often not feasible, making CABG the preferred treatment option. In this subset of patients, conventional surgical revascularization techniques may be inadequate or even impossible because of the absence of a suitable coronary lumen. As a consequence of advances in nonsurgical coronary revascularization, the coronary anatomy of patients presenting for CABG has become increasingly complex over time [1–3].

In patients with diffuse coronary artery disease identified on coronary angiography, suitable coronary targets for standard graft anastomosis may not be available, thereby precluding complete revascularization using conventional techniques. In such cases, the available options are either to consider the patient inoperable or to achieve complete revascularization through coronary endarterectomy [3,4]. Since the principal goal of CABG

is complete myocardial revascularization, coronary endarterectomy remains an important adjunctive strategy in selected patients with diffuse coronary artery disease.

Direct head-to-head comparisons between open left anterior descending (LAD) artery endarterectomy with patch reconstruction and closed right coronary artery (RCA) endarterectomy are scarce. Most available studies have evaluated open versus closed techniques in mixed-vessel cohorts or have reported single-vessel series focused on LAD reconstruction or closed traction endarterectomy outcomes [5–7].

The present study aimed to compare the outcomes of patients undergoing closed traction RCA endarterectomy with those undergoing open LAD endarterectomy with left internal mammary artery (LIMA) patch plasty.

Methods

This retrospective observational study included 31 patients who underwent coronary artery bypass grafting (CABG) with right coronary artery (RCA) endarterectomy between January 2005 and January 2024 (Group A) and 36 patients who underwent open left anterior descending (LAD) artery endarterectomy with left internal mammary artery (LIMA) patch plasty between 2005 and 2024 (Group B).

All procedures in Group B were performed using a standardized open technique under cardiopulmonary bypass at four tertiary cardiac surgery centers in Turkey.

The study aimed to compare closed traction RCA endarterectomy and open LAD endarterectomy with LIMA patch plasty with respect to baseline clinical characteristics, operative variables, early postoperative outcomes, arrhythmias, mortality, and longitudinal changes in New York Heart Association (NYHA) functional class. By providing a comprehensive comparison of these two techniques, the study sought to inform clinical decision-making in patients with diffuse coronary artery disease requiring complex revascularization strategies.

Surgical Technique

All operations were performed through a standard median sternotomy. The left internal mammary artery (LIMA) was harvested in a semipedicled fashion in all patients. Following systemic heparinization, the LIMA was divided distally beyond its bifurcation to obtain adequate conduit length. Satisfactory free flow was confirmed, after which the distal end was clipped and the graft was stored in a warm saline–papaverine-soaked sponge to minimize vasospasm.

Cardiopulmonary bypass (CPB) was established via ascending aortic and right atrial cannulation, and the aorta was cross-clamped. Myocardial arrest was achieved using cold blood cardioplegia delivered through the aortic root, while systemic temperature was maintained at 32–34 °C. Myocardial protection was ensured by intermittent antegrade cold blood cardioplegia administered at 15-minute intervals, initially through the aortic root and subsequently through the grafts after completion of the distal anastomoses.

Closed Traction RCA Endarterectomy

For right coronary artery (RCA) endarterectomy, a limited arteriotomy was performed, and the atheromatous core was gently extracted by traction from the distal vessel segment (Figure 1; red arrows indicate the direction of traction). Traction was applied parallel to the longitudinal axis of the RCA, allowing the plaque to be withdrawn proximally. Subsequently, a saphenous vein graft was anastomosed to the distal RCA.

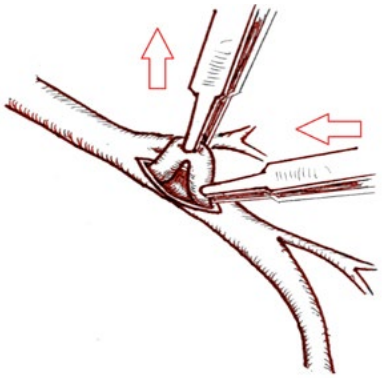

Figure 1 – Illustration depicts the surgical technique of traction RCA endarterectomy

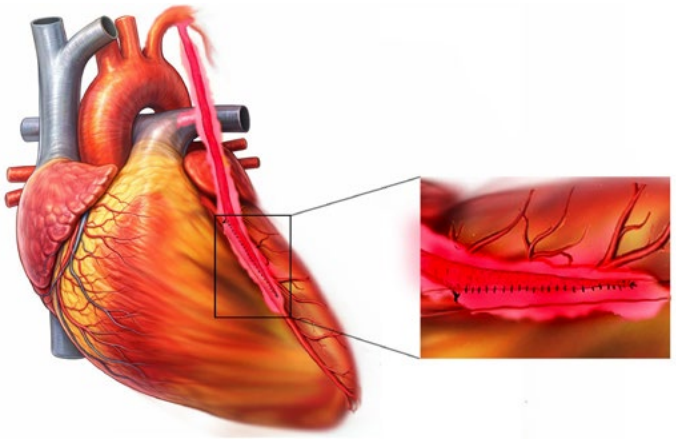

Figure 2 – Illustration depicts the surgical technique of Open LAD endarterectomy and LIMA patch

Open LAD Endarterectomy with LIMA Patch Plasty

After completion of distal anastomoses to all coronary targets other than the LAD, attention was directed to LIMA–LAD reconstruction. The epicardial surface over the LAD was exposed, and the overlying epicardial and connective tissues were meticulously dissected using a No. 15 scalpel blade. The indication for LAD endarterectomy was determined either preoperatively, based on coronary angiography demonstrating diffuse LAD disease, or intraoperatively when an adequate distal lumen for conventional anastomosis could not be identified or when extensive atherosclerosis extended beyond the planned anastomotic site.

Following longitudinal extension of the LAD arteriotomy and meticulous removal of the atherosclerotic plaque, the LIMA was anastomosed as an onlay patch to achieve arterial reconstruction. Figure 2 illustrates the surgical technique.

Statistical Analysis

Statistical analyses were performed using SPSS software (IBM Corp., Armonk, NY, USA). Continuous variables were tested for normality using the Shapiro–Wilk test. Normally distributed variables are presented as mean ± standard deviation and were compared using the Student's t-test, whereas non-normally distributed variables are presented as median (interquartile range) and were compared using the Mann–Whitney U test. Categorical variables are expressed as frequencies and percentages and were compared using the chi-square or Fisher's exact test, as appropriate.

Changes in NYHA functional class over time were analyzed using paired nonparametric tests. Early mortality was

Table 1 Baseline Demographic Characteristics

Variable	Open LAD endarterectomy (n=36)	Closed RCA endarterectomy (n=31)	p-value	Statistical test
Age, years	61.6 ± 8.9	62.3 ± 9.4	0.734	Student t-test
Hypertension	24 (66.7)	16 (51.6)	0.210	Chi-square
Diabetes mellitus	28 (77.8)	6 (19.4)	<0.001	Chi-square
Family history of CAD	6 (16.7)	4 (12.9)	0.742	Fisher's exact
Smoking history	18 (50.0)	16 (51.6)	0.895	Chi-square
Preoperative myocardial infarction	9 (25.0)	5 (16.1)	0.373	Chi-square
Preoperative EF, %	39.5 ± 11.0	52.2 ± 11.8	<0.001	Mann–Whitney U

Values are presented as mean ± SD or n (%). EF, ejection fraction; CAD, coronary artery disease; RCA, right coronary artery; LAD, left anterior descending artery.

defined as death occurring within 30 postoperative days. A two-sided p value < 0.05 was considered statistically significant.

Results

Baseline demographic, clinical, and operative characteristics are summarized in Table 1. Group A consisted of 31 patients who underwent CABG with closed traction RCA endarterectomy, whereas Group B included 36 patients who underwent open LAD endarterectomy with LIMA patch plasty. The groups were compared with respect to age, sex, cardiovascular risk factors, preoperative left ventricular ejection fraction, functional status, and operative variables.

In the closed RCA endarterectomy group, preoperative coronary angiography demonstrated severe but incomplete RCA stenosis (>70%) in 24 patients, whereas 7 patients had complete (100%) RCA occlusion. All patients underwent CABG with closed RCA endarterectomy because of diffuse RCA disease. All patients in the open LAD endarterectomy group received dual antiplatelet therapy consisting of clopidogrel 75 mg and acetylsalicylic acid 100 mg once daily for six months, followed by lifelong clopidogrel therapy. In the closed RCA endarterectomy group, all patients received lifelong clopidogrel 75 mg once daily. In addition, warfarin was administered to three patients who were considered to require additional antithrombotic and anticoagulant therapy, and warfarin treatment was continued for six months.

Early mortality occurred in three patients, two on postoperative day 1 and one on postoperative day 11. All early deaths occurred in patients with severe but incomplete RCA stenosis, whereas no early mortality was observed among patients with complete RCA occlusion. Continuity-corrected 2 × 2 contingency analysis demonstrated a higher risk of early mortality in patients with severe but incomplete stenosis compared with those with complete occlusion (odds ratio, 2.44; 95% confidence interval, 0.11–53.0), although this difference did not reach statistical significance (p > 0.05). These findings suggest a potential inverse relationship between the degree of preoperative RCA stenosis and early mortality following closed RCA endarterectomy.

The mean duration of intra-aortic balloon pump (IABP) support was significantly longer in the closed RCA endarterectomy group than in the open LAD endarterectomy with LIMA patch plasty group (0.74 vs. 0 days; p = 0.013).

Aortic cross-clamp and cardiopulmonary bypass times were comparable between the groups. ICU stay was significantly shorter in the open LAD group (1.17 ± 0.38 days vs. 2.19 ± 2.52 days; p = 0.026). The closed RCA group required significantly longer durations of adrenaline and dopamine support. Postoperative atrial fibrillation rates were similar between the groups, whereas ventricular arrhythmias occurred exclusively in the closed RCA group. Postoperative bleeding volume was significantly higher in the closed RCA group (684.2 cc vs. 527.9 cc; p = 0.002).

Follow-up coronary angiography was performed in 16 symptomatic patients in the open LAD endarterectomy group at a mean follow-up duration of 6.08 ± 2.01 years and in 14 symptomatic patients in the closed RCA endarterectomy group at a mean follow-up duration of 4.21 ± 1.12 years. Despite the limited sample size, follow-up coronary angiography demonstrated a significantly higher anastomotic patency rate in the open LAD endarterectomy with LIMA patch plasty group than in the closed RCA endarterectomy group (94.1% vs. 50.0%, Fisher's exact test, p = 0.008). Correlation analysis demonstrated significant associations between postoperative bleeding, IABP use, inotropic support, arrhythmias, and mortality in the closed RCA group (Table 2). No such correlations were observed in the open LAD group. Figure 3 demonstrates the aorta–right coronary artery anastomosis 48 months after closed endarterectomy.

Table 3 Correlation Matrix of Postoperative Adverse Outcomes in RCA Close Endarterectomy Group

Parameter	Bleeding	IABP requirement (days)	Inotropic support	Arrhythmia	Mortality
Bleeding	1.00	+ (p < 0.05)	+ (p < 0.05)	NS	NS
IABP requirement (days)	+ (p < 0.05)	1.00	+ (p < 0.05)	+ (p ≈ 0.05)	+ (p < 0.05)
Inotropic support	+ (p < 0.05)	+ (p < 0.05)	1.00	+ (p < 0.05)	+ (p < 0.05)
Arrhythmia	NS	+ (p ≈ 0.05)	+ (p < 0.05)	1.00	NS
Mortality	NS	+ (p < 0.05)	+ (p < 0.05)	NS	1.00

Abbreviations: IABP, intra-aortic balloon pump; NS, not significant. Statistical method: Spearman's rank correlation test. Significant positive correlations were observed between postoperative bleeding, IABP requirement, and inotropic support. IABP use and inotropic support were also significantly associated with mortality, indicating that advanced hemodynamic support clustered with worse early postoperative outcomes.

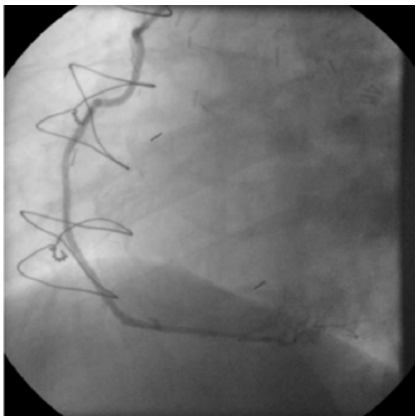

Figure 3 – Right coronary artery after a 5-cm closed endarterectomy

Angiographic patency of the Ao–RCA anastomosis at 48-month follow-up is demonstrated.

In the functional capacity analysis, normality of distribution was assessed using the Shapiro–Wilk test, which demonstrated a nonnormal distribution in both the closed RCA group ($p < 0.001$) and the open LAD group ($p < 0.001$). Therefore, nonparametric tests were applied. At the last follow-up, there was no statistically significant difference in NYHA functional class between the closed RCA endarterectomy group and the open LAD endarterectomy with LIMA patch plasty group (Mann–Whitney U test, $p = 0.695$). Within-group comparisons revealed no significant improvement in functional capacity in the closed RCA group (Wilcoxon signed-rank test, $p = 0.217$). In contrast, the open LAD group demonstrated a statistically significant improvement in NYHA functional class from the preoperative period to the last follow-up (Wilcoxon signed-rank test, $p < 0.001$).

Discussion

The present study compared closed traction RCA endarterectomy with open LAD endarterectomy using LIMA patch plasty in patients undergoing CABG for diffuse coronary artery disease. Several important observations emerged.

All early deaths occurred in patients with severe but incomplete RCA stenosis. The absence of early mortality among patients with complete RCA occlusion may reflect chronic myocardial adaptation and well-developed collateral circulation. In patients with severe but incomplete stenosis, residual antegrade flow and an irregular intimal surface following closed traction endarterectomy may predispose to early thrombotic occlusion, acute right ventricular ischemia, malignant ventricular arrhythmias, and low cardiac output syndrome. It is known that myocardial ischaemia and infarction leads to severe metabolic and electrophysiological changes that induce silent or symptomatic life-threatening arrhythmias [8]. Right ventricular infarction complicates 50% of cases of acute inferior ST-segment elevation myocardial infarction, and is associated with high in-hospital morbidity and mortality [9]. The ischemic dilated Right Ventricle is also prone to malignant ventricular arrhythmias [9]. The higher mortality observed in the closed RCA endarterectomy group may partly be attributed to the fact that all deaths occurred in patients with severe but incomplete RCA occlusion. This finding may reflect mechanisms similar to those described in the literature for acute RCA occlusion, and may help explain the observed mortality following closed endarterectomy.

Open LAD endarterectomy with LIMA patch plasty was associated with a shorter ICU stay and reduced inotropic requirements, suggesting improved early myocardial perfusion and hemodynamic stability. Despite its more extensive reconstructive nature, operative times were not prolonged. The absence of ventricular arrhythmias in the open LAD group may indicate superior early postoperative resistance to acute occlusion, potentially related to the well-established antithrombotic and vasoprotective properties of the LIMA, as described in detail in previous studies [10-13].

Contrary to expectations, postoperative bleeding was greater in the closed RCA group, possibly reflecting inter-operator variability, as these procedures were performed by multiple surgeons. These findings indicate that meticulous hemostatic technique can mitigate bleeding risk even in open endarterectomy procedures.

The significantly higher anastomotic patency rate observed in the open LAD endarterectomy with LIMA patch plasty group, despite the longer follow-up duration, suggests a potential durability advantage of this reconstructive strategy. The use of the left internal mammary artery, combined with open endarterectomy and patch reconstruction, may provide more uniform luminal restoration and improved endothelialization, thereby reducing the risk of late thrombosis and restenosis [14]. In parallel, Nishigawa et al. reported that optical coherence tomography (OCT), performed in 8 patients, demonstrated complete endothelialization of the endarterectomized LAD within one year postoperatively, the surface of the reconstructed lumen had become homogeneous [14]. In contrast, closed traction RCA endarterectomy relies on indirect plaque extraction through a limited arteriotomy, which may predispose to residual intimal irregularities or distal dissection, potentially compromising long-term graft patency. Importantly, follow-up coronary angiography was performed at a longer mean interval in the open LAD group than in the closed RCA group, yet patency rates remained significantly higher. This finding strengthens the interpretation that the observed difference is unlikely to be solely attributable to follow-up duration. Nevertheless, given the limited sample size and the non-systematic nature of angiographic follow-up, Larger prospective studies with standardized angiographic surveillance are warranted to confirm the long-term patency advantages suggested by the present findings.

Although the open LAD endarterectomy with LIMA patch plasty group demonstrated a statistically significant improvement in NYHA functional class over time, no significant difference was observed between the two surgical strategies at long-term follow-up. These findings suggest that while open LAD reconstruction may provide a more pronounced early functional recovery, both techniques ultimately achieve comparable long-term symptomatic outcomes. The significant within-group improvement observed in the open LAD cohort may reflect more effective myocardial revascularization of the anterior wall, which is known to have a substantial impact on global left ventricular performance. In contrast, the absence of a statistically significant functional improvement in the closed RCA group might be related to the smaller myocardial territory supplied by the right coronary artery in most patients, potentially limiting the magnitude of measurable symptomatic change. Importantly, despite differences in early functional recovery, long-term NYHA class did not differ between groups, indicating that both revascularization strategies provide sustained clinical benefit. This supports the concept that surgical endarterectomy—whether performed as an open LAD reconstruction with LIMA patch plasty or as a closed RCA technique—can yield acceptable long-term functional outcomes when complete revascularization is achieved. Taken together, these findings suggest that open LAD endarterectomy may confer earlier symptomatic improvement, whereas long-term functional capacity appears to be comparable between techniques.

Conclusion

Both closed RCA endarterectomy and open LAD endarterectomy with LIMA patch plasty are feasible revascularization strategies in patients with diffuse coronary artery disease. Although early mortality and atrial fibrillation rates were comparable, closed RCA endarterectomy was associated with a more complex early postoperative course and clustering of adverse outcomes. In contrast, open LAD endarterectomy with LIMA patch plasty demonstrated a more stable postoperative profile, suggesting that patient selection and the endarterectomy technique may play a more decisive role.

However, the compared groups differed not only in terms

of surgical technique (open vs. closed) and conduit type, but also in terms of myocardial territories. The myocardial mass supplied by the LAD artery is greater than that supplied by the right coronary artery. A larger myocardial mass results in greater runoff through the grafts. Therefore, it should be kept in mind that graft patency rates and the associated early postoperative outcomes may be related not only to the preferred surgical technique and conduit type, but also to the myocardial mass supplied by the RCA or LAD arteries, which provide different runoff characteristics.

Limitations

This study has several limitations. First, its retrospective and non-randomized design inherently introduces selection bias and limits causal inference. Second, follow-up coronary angiography was not performed systematically in all patients and was limited to those who underwent clinical evaluation, introducing potential selection bias in patency assessment. The relatively small sample size limits statistical power, particularly for mortality and subgroup analyses. Finally, the potential influence of surgeon- and center-related variability may have affected perioperative management strategies, operative decision-making, and postoperative outcomes in addition to the surgical techniques themselves.

Larger prospective studies with standardized operative protocols and uniform follow-up strategies are needed for further studies.

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Consent for publication: All data were fully anonymized, and no individual patient-identifiable information was included in this study.

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Determination of Physical Activity Levels, Quality of Life, Depression and Fatigue Severity of Individuals with Metabolic Syndrome

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ABSTRACT

Aim: The aim of this study is to assess the levels of physical activity, quality of life, depression, and fatigue severity in individuals with metabolic syndrome (MetS) and compare them with those of healthy individuals.

Methods: Participants diagnosed with MetS from the Department of Endocrinology and Metabolic Diseases and healthy individuals were included in the study. A total of 107 individuals with MetS and 107 healthy individuals participated in the study. Sociodemographic data of the individuals were queried using a prepared case form. The International Physical Activity Questionnaire (IPAQ) was used to determine the physical activity levels of the individuals, the SF-36 was used to assess their quality of life, the Beck Depression Scale (BDI) was used to determine their depression levels, and the Fatigue Severity Scale (FSS) was used to assess their fatigue levels.

Results: The mean age of individuals with MetS was 50.80±7.78 years, while healthy individuals had a mean age of 45.16±9.60 years. Statistical differences were found in IPAQ total scores, all SF-36 subscales, BDI, and FSS between individuals with MetS and healthy individuals (p<0,001). Individuals with MetS had higher depression and fatigue levels, with mean BDI scores of 23.79±4.50 compared to 5.48±3.00 in controls, and mean FSS scores of 4.99±1.13 versus 2.40±0.89, respectively.

Conclusion: According to the data obtained in our study, individuals with MetS have low levels of physical activity and quality of life, and they experience depression and fatigue problems. We believe that treatment protocols for this patient group should include studies on physical activity levels, quality of life, depression, and fatigue.

Keywords: Metabolic syndrome, Physical Activity, Quality of Life, Depression, Fatigue, ClinicalTrials.gov with ID NCT05184634.

Introduction

Metabolic syndrome (MetS) is defined as a cluster of conditions characterized by the coexistence of obesity with at least two of the following: high blood pressure, impaired glucose metabolism, and elevated non-high-density lipoprotein (non-HDL) cholesterol levels [1]. It represents a multifactorial syndrome in which these components interact and increase overall cardiometabolic risk.

The prevalence of MetS varies depending on geographical region, diagnostic criteria, age, and gender, and has shown a notable increase in recent years [2]. Data from the Centers for Disease Control and Prevention indicate a 35% rise in prevalence up to 2012 in the United States [3]. Similarly, findings from the Turkish Adult Heart Disease and Risk Factors (TEKHARF) study highlight that MetS has become a major public health concern in Turkey, with a prevalence of 32.8% among individuals aged 30 years and older [4].

MetS is influenced by factors such as hypertension, hyperglycemia, dyslipidemia, and abdominal obesity, along with environmental and genetic determinants [5].

Effective management requires identification and control of these components through comprehensive strategies, including pharmacological treatment, physical activity, and nutritional interventions [6,7]. Physical activity plays a key role by improving lipid profiles, glycaemic control, and blood pressure, while its deficiency is strongly associated with obesity and MetS development [8]. In addition, MetS negatively affects quality of life through impairments in physical and mental health, increases the risk of depression via neuroendocrine mechanisms, and contributes to fatigue due to reduced physical function [9–14]. These factors collectively lead to decreased daily functioning and increased risk of chronic conditions such as diabetes and cardiovascular diseases [15–17].

A review of the literature indicates that studies addressing MetS with a multidimensional perspective—incorporating physical activity, quality of life, depression, and fatigue—are limited. Therefore, this study aims to evaluate these parameters in individuals with MetS and compare them with healthy controls.

Methods

Participant

Gülhane Training and Research Hospital between December 2021 and May 2022. Participants diagnosed with MetS from the Department of Endocrinology and Metabolic Diseases and healthy individuals were included in the study. 107 individuals with MetS and 107 healthy individuals participated in the study. A total of 107 healthy volunteers who met the study criteria were recruited as the control group through a snowball sampling method from patients, their relatives, and researchers. The study initially included 221 individuals. 112 individuals with MetS and 109 healthy individuals were interviewed for the study. 5 individuals diagnosed with MetS were excluded from the evaluation due to thyroid disease. 2 healthy individuals declined to participate in the study (Figure 1).

G*Power (version 3.1.9.7, University of Düsseldorf, Germany) was used for post-hoc power analysis, and the effect size was calculated from the total score of the physical activity scale (IPAQ) between individuals with MetS and healthy individuals. According to the analysis, the alpha level for a two-sided hypothesis test was 5%. The confidence interval was 95%, the effect size was 0.60, and the study's power (1- β) was 99%.

The study received approval from the Kırıkkale University Non-Interventional Research Ethics Board on September 16, 2021 (Decision Number: 21/09/07). Additionally, permission was obtained from the Gülhane Training and Research Hospital Directorate on October 6, 2021. The purpose of the study was explained to the individuals included, informed consent forms were obtained after providing information about the questionnaires to be used, and the study was conducted in accordance with the principles of the Helsinki Declaration. The clinical trial was registered at ClinicalTrials.gov with ID NCT05184634. Clinical trial conforms to the CONSORT 2010 Statement (Consolidated Standards of Reporting Trials). Additionally, this study was produced from the master's thesis.

Individuals aged 25-65 with a diagnosis of MetS, willing to participate in the study, independently ambulatory, and literate were included, along with individuals aged 25-65 without a diagnosis or complaints related to diseases constituting MetS who served as the healthy group. Exclusion criteria for the study included being outside the 25-65 age range, being on bed rest for

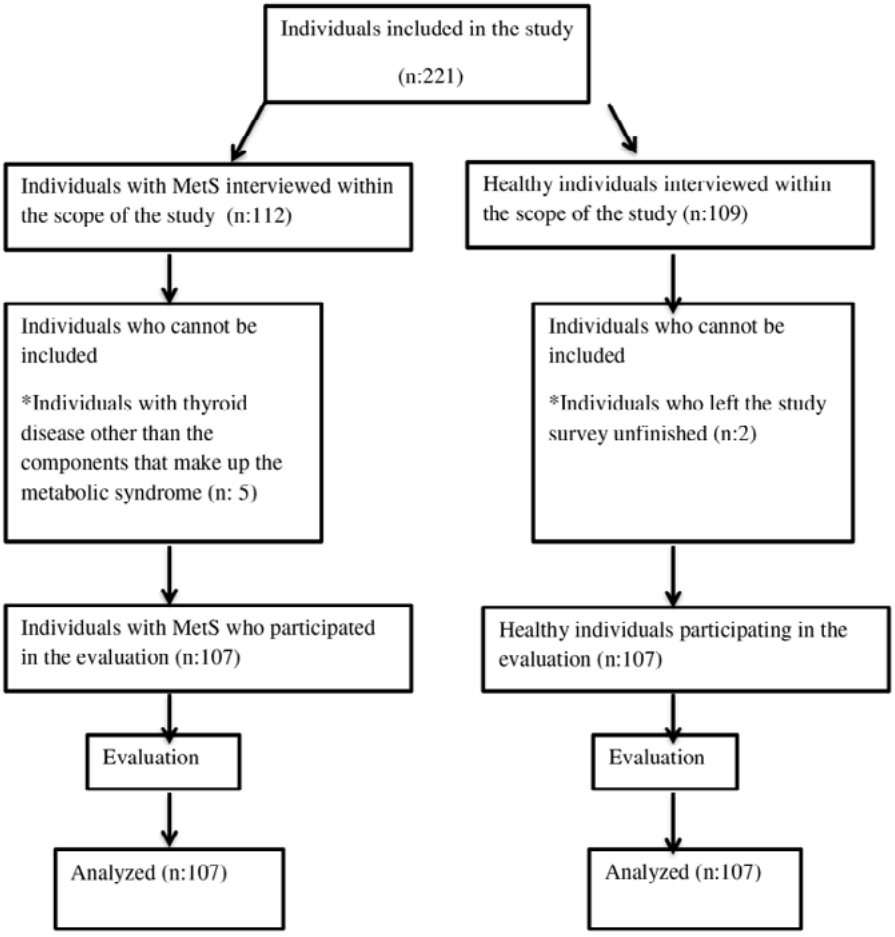

Figure 1 – Flow Diagram

at least one month prior to the study, having contraindications to physical activity, having any orthopedic, neurological, or psychiatric disorders, and also having chronic conditions other than those constituting MetS (Chronic disorders such as asthma, thyroid etc. were excluded because they could affect parameters such as physical activity, quality of life and fatigue). The study did not include individuals with visual, auditory, speech, cognitive, or understanding problems that hinder evaluation (Figure 1).

Tools for Measuring

All assessments were conducted face-to-face by an experienced clinician and an equally experienced physiotherapist in the clinic. The evaluations applied to the individuals included in the study are provided below. The assessment form was administered to each participant once.

The assessment form aimed to determine individuals' characteristics and included recording demographic and disease-related information, such as birth date, height, weight, gender, marital status, education level, medical history, medications, smoking status, and alcohol use.

International Physical Activity Questionnaire (IPAQ)

The level of physical activity (PA) was assessed using the short version of the International Physical Activity Questionnaire (IPAQ). The walking durations of the subjects in the last week, whether they engaged in moderate or vigorous physical activity, and, if so, the number of days per week and the total minutes spent were determined in terms of MET-minutes/week. As a result, all values were summed to determine the IPAQ total score, and patients were categorized as inactive, minimally active, or very active. The short Turkish version, validated and reliable according to Sağlam [18], was used. An increased score indicates higher physical activity and energy expenditure, representing a positive health outlook; a low score suggests a sedentary lifestyle and associated increased metabolic and cardiovascular risks.

Quality of Life Scale (SF-36)

The individuals' quality of life was assessed using the SF-36. The SF-36 quality-of-life scale, a generic scale, has been continuously updated by Ware and colleagues since 1988 and has evolved into its current form [19]. The SF-36 scale consists of 36 multiple-choice questions. Examining eight dimensions of health-related quality of life, high scores indicate a better level of health, while low scores indicate a deterioration in health. The dimensions of the scale include Physical Functioning (PF) (restriction in physical activities due to health problems), Physical Role (RP) (restriction in daily life activities due to health problems), Bodily Pain (BP), General Health (GH) (individual's overall health assessment), Vitality (VT), Mental Health (MH), Social Functioning (SF), and Emotional Role (RE) (restriction in daily life activities due to mental health problems) [19].

Beck Depression Inventory (BDI)

This is a self-assessment scale applied to healthy individuals and psychiatric patient groups. Its purpose is to determine the risk of depression and measure the level and severity of depressive symptoms. This form, which includes 21 self-assessment scales, uses a four-point Likert scale. Each item receives an increasing score between 0-3; the total score is obtained by summing these scores. A high total score indicates a high severity of depression. Developed by Beck and colleagues, the Inventory has undergone validity testing in Turkish, and the cutoff score was determined as 17 in the reliability article [20].

Fatigue Severity Scale (FSS)

The Fatigue Severity Scale was used to assess individuals' fatigue levels. The validity and reliability study of the scale for Turkey was conducted by Armutlu and colleagues [21]. The scale consists of a total of 9 items. Individuals indicate their agreement with each item by selecting a value from 1 to 7. The scale uses expressions such as "1: I do not agree at all" to "7: I completely agree". The scale ranges from 9 to 63, with scores of 36 or higher indicating severe fatigue.

Statistical analysis

Descriptive statistics for categorical variables (demographic characteristics) were presented as frequencies and percentages. The normal distribution of continuous variables was checked using the "Shapiro-Wilk Test." The homogeneity of variances was tested using Levene's test where appropriate. Descriptive statistics for continuous variables were reported as mean ± standard deviation for normally distributed data and as median (min-max) for non-normally distributed data.

For the comparison of two independent groups with a normal distribution, the "Independent Samples T-Test" was performed. Analysis of Covariance was used to examine differences between the two groups while controlling for age. Pearson Chi-Square and Fisher's exact tests were used to compare categorical variables. Multivariable stepwise linear regression was used to determine whether MetS, IPAQ Total, and eight SF-36 dimensions could predict BDI and FSS, while controlling for confounding factors such as age, gender, and BMI.

Also, a mediation analysis was conducted using the PROCESS macro (Model 4) in SPSS statistical software (version 4.2) to evaluate whether the effect of the independent variable (MetS) on the dependent variable (BDI) was transmitted through a mediator variable (IPAQ) [22]. This approach allowed for the examination of the independent variable's direct and indirect effects on the dependent variable via the mediator.

In all calculations and interpretations in the study, significance levels of "p<0.05, p<0.01, p<0,001" were used, and hypotheses were formulated as two-sided. Statistical data analysis was performed using IBM SPSS v26 (IBM Inc., Chicago, IL, USA).

Results

This study included 214 participants: 107 with MetS and 107 healthy individuals.

The age and BMI of the individuals included in the study are presented in Table 1. The mean age of individuals with MetS was 50.80±7.78 years, with a Body Mass Index (BMI) of 30.54±6.20 kg/m2. In contrast, healthy individuals had a mean age of 45.16±9.60 years and a BMI of 23.59±2.49 kg/m2. Statistical differences were observed between individuals with MetS and healthy individuals in age and BMI. A statistically significant difference was explicitly found at the primary school and Bachelor's degree levels. The percentage of individuals with MetS who graduated from primary school was higher, whereas the percentage with a Bachelor's degree was lower (Table 1).

The comparison of IPAQ total scores, SF-36 subfactors, BDI, and FSS total scores between the study participants is presented in Table 2. A statistically significant difference in IPAQ total scores was found between individuals with MetS and healthy individuals (p<0,001). Upon examination of the results,

Table 1 Comparison of sociodemographic characteristics of individuals between individuals with Metabolic Syndrome and Healthy Individuals

Physical characteristics of individuals		Individuals with Metabolic Syndrome (n=107)		Healthy Individuals (n=107)		Test value	P
		Mean±SD		Mean±SD			
Age (years)		50.80±7.78		45.16±9.60		t =4.726	<0.001
BMI (kg/m²)		30.54±6.20		23.59±2.49		t =10.761	<0.001
BMI	Normal weight	20	18.7	77	72.0	χ2=81.366	<0.001
	Pre Obese	34	31.8	28	26.2		
	Obese	53	49.5	2	1.9		
Gender	Male	45	42.1	47	43.9	χ2=0.076	0.782
	Woman	62	57.9	60	56.1		
Marital Status	Single	12	11.2	18	16.8	χ2=1.396	0.237
	Married	95	88.8	89	83.2		
Educational Status	Primary school	25	23.4	9	8.4	χ2=20.203	<0.001*
	Middle school	18	16.8	9	8.4		
	High school	41	38.3	42	39.3		
	Bachelor's degree	21	19.6	46	43.0		
	Master of Science	2	1.9	1	0.9		
Smoking	No	78	72.9	89	83.2	χ2=3.299	0.069
	Yes	29	27.1	18	16.8		
Alcohol Use	No	107	97.2	101	94.4	-	0.498
	Yes	3	2.8	6	5.6		

Descriptive statistics were given as mean± standard deviation, frequency (n), and percentage (%). Independent samples t-test, Pearson Chi-square, and Fisher's exact tests were used. BMI: Body Mass Index.

Table 2 Comparison of IPAQ total, SF-36, Beck Depression Inventory, and Fatigue Severity scale scores of individuals with metabolic syndrome and healthy individuals

	Individuals with Metabolic Syndrome (n=107)		Healthy Individuals (n=107)		F	P
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)		
IPAQ Total	3744.71±1753.51	3606 (477-8670)	4618.73±1029.00	4626 (2037-7206)	12.660	<0.001
Physical Function	41.40±26.47	45 (0-100)	90.98±8.78	90 (70-100)	294.910	<0.001
Physical Role Difficulty	19.19±26.64	0 (0-100)	87.85±17.29	100 (25-100)	435.914	<0.001
Emotional Role Difficulty	20.86±29.49	0 (0-100)	86.27±21.48	100 (33.3-100)	296.671	<0.001
Energy / Vitality / Vitality	38.74±12.81	40 (5-65)	77.50±10.98	80 (45-90)	496.027	<0.001
Spiritual Health	45.08±10.92	44 (12-88)	78.02±9.76	80 (48-92)	482.546	<0.001
Social Functioning	42.07±12.79	37.5 (12.5-75)	82.13±11.53	87.5 (50-100)	501.337	<0.001
Pain	36.19±14.69	35 (0-100)	83.64±11.58	87.5 (45-100)	602.471	<0.001
General Health Perception	36.17±11.09	35 (10-65)	74.81±7.68	75 (60-90)	773.504	<0.001
BDI	23.79±4.50	24 (15-37)	5.48±3.00	5 (0-12)	1104.367	<0.001
FSS	4.99±1.13	5.3 (1.3-7.1)	2.40±0.89	2.4 (0.3-5)	300.255	<0.001

IPAQ: International Physical Activity Scale, SF-36: Quality of Life Scale, BDI: Beck Depression Inventory, FSS: Fatigue Severity Scale, SD: Standard Deviation, F: Results of Analysis of Covariance (ANCOVA) by controlling the age variable.

the average IPAQ scores of healthy individuals were statistically higher than those of individuals with MetS. A statistically significant difference in SF-36 scores was found between individuals with MetS and healthy individuals (p<0,001). A statistically significant difference was found in the BDI and FSS total scores between individuals with MetS and healthy individuals (p<0,001). Upon examination of the results, it was observed that the median BDI and FSS scores for individuals with MetS were statistically higher than those for healthy individuals (Table 2).

The evaluation of IPAQ levels for the study participants is presented in Table 3. It was found that 10.3% of individuals with MetS were inactive, 24.3% were minimally active, and 65.4% were highly active; whereas 3.7% of healthy individuals were minimally active, and 96.3% were highly active. A statistically significant difference was found in the IPAQ total scores among the individuals participating in the study (p<0,001) (Table 3).

According to the stepwise linear regression analysis results, MetS, FSS, education level (primary school graduate), and BMI significantly affected BDI scores. MetS was found to be the independent variable with the strongest positive effect on depression levels (Standardized Beta = 0.700, p < 0,001), FSS (Standardized Beta = 0.242, p < 0,001), and BMI (Standardized Beta = 0.078, p = 0.008). These factors were other important factors that increased BDI scores. On the other hand, primary school graduation showed a significant effect on reducing

Table 3 Comparison of IPAQ levels of individuals with metabolic syndrome and healthy individuals

Physical Activity Levels	Individuals with Metabolic Syndrome (n=107)		Healthy Individuals (n=107)		χ2	P
	n	%	n	%		
Inactive	11	10,3	0	0,0	33.428	<0.001
Active	26	24,3	4	3,7		
Very Active	70	65,4	103	96.3		

Pearson Chi-square test was used.

Table 4 Multivariable Stepwise linear regression analysis results for Beck Depression Inventory and Fatigue Severity Scale

Dependent Variables	Independent Variables	Unstandardized		Standardized	t	P
		β	Std. Error	β		
BDI	Constant	-1.015	1.290	-	-0.786	0.433
	Metabolic Syndrome	13.880	0.812	0.700	17.095	<0.001
	FSS	1.461	0.230	0.242	6.354	<0.001
	Educational Status (Primary school)	-1.845	0.650	-0.068	-2.840	0.005
	BMI	0.133	0.050	0.078	2.675	0.008
FSS	Constant	3.981	0.446	-	8.922	<0.001
	BDI	0.095	0.011	0.570	8.846	<0.001
	SF-36 (Social Functioning)	-0.019	0.005	-0.270	-4.143	<0.001
	IPAQ Total	-0.001	0.001	-0.107	-2.732	0.007

Regression model results for BDI: $F(4;209)=404.102$ $p<0.001$ Adjusted $R^2=0.883$.
Regression model results for FSS: $F(3;210)=176.12$ $p<0.001$ Adjusted $R^2=0.712$.

depression scores (Standardized Beta = -0.068, p = 0,005) (Table 4).

The FSS was used as the dependent variable, and the effects of the independent variables on BDI, the SF-36 Social Functioning sub-dimension, and the IPAQ total score were evaluated. According to the results, BDI scores were the variable with the strongest positive effect on FSS (Standardized β = 0.570, p<0,001), indicating that fatigue increases significantly with increasing depression levels. The SF-36 Social Functioning sub-dimension significantly reduced FSS scores (Standardized β = -0.270, p<0,001), indicating that increasing social functioning may reduce fatigue. The IPAQ total score also showed a significant but weaker negative effect on FSS (Standardized β = -0.107, p = 0,007), suggesting that increased physical activity may alleviate fatigue (Table 4).

Discussion

Our study, designed to compare physical activity, quality of life, fatigue, and depression symptoms in individuals with MetS and healthy individuals, revealed that individuals with MetS, compared to healthy individuals, have lower levels of physical activity and quality of life, and higher levels of fatigue and depression. In addition, the results suggest that MetS and fatigue levels are important determinants of depression and that education level may be a protective factor associated with depression. In addition, our findings suggest that fatigue levels are significantly affected by psychosocial factors such as depression and social functioning, as well as physical activity levels.

Sociodemographic Characteristics

The global prevalence of MetS varies from 12.5% (95% CI: 10.2–15.0) to 31.4% (29.8–33.0) depending on the definition used, with higher rates observed in the Eastern Mediterranean Region and the Americas, and an increase with country income level. The likelihood of developing MetS increases with age, and numerous studies show that it tends to emerge in older populations [23]. In our study, the MetS group had a mean age of 50.80±7.78 years, while the healthy group had a mean age of 45.16±9.60 years, which is consistent with the literature.

Regarding gender distribution, women are reported to have a higher risk of MetS than men. Studies such as METSAR and Farrell have shown higher prevalence rates in women compared to men [24]. Similarly, in our study, 57.9% of individuals with MetS were female, and 42.1% were male, supporting the existing literature. Interestingly, primary school graduation emerged as a protective factor against higher BDI scores. This finding should be interpreted with caution. It may reflect underlying sociodemographic characteristics not fully captured in the present study, such as differences in occupational demands, lifestyle patterns, or social support. Additionally, the distribution of education levels within the sample and potential residual confounding may have influenced this result. Therefore, this observation may be sample-specific and warrants further investigation in larger and more diverse populations.

In terms of body composition, obesity is a major risk factor for MetS alongside a sedentary lifestyle and unhealthy dietary habits. In our study, 49.5% of individuals with MetS were obese, 31.8% were overweight, and 18.7% had normal weight, indicating higher BMI levels compared to healthy individuals. Additionally, education level is an important factor; Çetin et al. reported higher MetS prevalence in individuals with lower education levels [25], and our findings are consistent with this, showing lower education levels in the MetS group. Overall, these findings align with the literature and emphasize the importance

of age, gender, obesity, and educational level in the development of MetS.

Physical Activity Level

Physical activity refers to body movements that expend energy and contribute to basal metabolic rate [26]. Regular physical activity is crucial for maintaining and improving health [27]. Numerous studies highlight the protective effects of regular physical activity on various chronic diseases, reducing the risk of premature death [28]. Sedentary lifestyle is a significant risk factor for conditions like MetS, cardiovascular diseases, diabetes, and cancer. In our study, individuals with MetS had lower physical activity scores than healthy individuals, underscoring the importance of promoting regular exercise programs and accounting for this factor in their treatment [29].

Oliveira et al. reported that individuals with MetS have a strong, low level of physical activity and a significant level of physical activity [30]. He et al. found that higher levels of physical activity were associated with a lower risk of MetS [31].

To increase these combined physical activity levels, the short version of the IPAQ was used. It has been observed that individuals with MetS have lower physical activity scores than healthy individuals. In this MetS, there is no relationship between total physical activity scores and any of the sub-factor scores for quality of life, endurance, or fatigue. It was observed that the level of physical activity was lower than that of individuals with MetS. For this reason, we recommend that people with MetS be encouraged to engage in regular exercise programs to increase their physical activity, and that this should be taken into consideration in their treatment. We believe that various exercise protocols are very important for preventive and protective treatment treatments for MetS.

Quality of Life

Physical fitness improves health-related quality of life [15]. In individuals with MetS, obesity often results in disappointments related to physical appearance, shortness of breath, weight-bearing joint pain, and decreased mobility, leading to impairments in daily physical function [16]. Furthermore, decreased quality of life is known to be an indicator of high risks for diabetes and cardiovascular diseases [17]. On the other hand, regular exercise programs improve quality of life [32].

In a comprehensive study conducted by Slagter et al. involving 13,686 obese patients aged 18 to 80, a decrease in quality of life was found to be associated with the degree of obesity, inflammatory status, the presence of diabetes, and MetS [33]. In a study by Hassan et al., it was reported that as individuals' BMI increased, their quality of life decreased [34]. This study also demonstrated the presence of obesity, one component of MetS, in individuals with MetS. Additionally, individuals with MetS reported lower quality of life than healthy individuals. We believe that a high BMI is a factor contributing to the low quality of life in individuals with MetS.

In a study by Eren et al., it was reported that complications of Type 2 diabetes negatively affected quality of life [35]. In a study by Gulliford et al., it was found that as the severity of diabetic symptoms increased, diabetic individuals' quality of life decreased [36]. We believe that diabetes, as a component of MetS, may also be contributing to the decrease in quality of life in these individuals.

Physical activity improves health-related quality of life. In our study, the SF-36 was used to assess quality of life in individuals with MetS. The results showed that individuals with MetS had lower scores across all SF-36 subscales than healthy individuals, indicating lower quality of life. The lower quality of life in individuals with MetS may be attributed to the physical and psychological difficulties associated with obesity and decreased mobility due to joint pain. Therefore, preventing obesity and promoting regular physical activity can play a crucial role in improving the quality of life for these individuals.

Depression

Individuals experiencing increased levels of depression exhibit heightened hypothalamic activity. This increase leads to the secretion of cortisol into circulation at levels above normal. The elevated cortisol levels disrupt the mechanisms that cause hyperinsulinemia and diabetes. MetS is a risk factor for the psychological conditions that lead to depression [37].

In a study by Demir et al., according to BDI results, the prevalence of depressive symptoms was found to be higher in women (31%) compared to men (9.9%), but statistical analyses did not reveal a significant relationship between MetS and depressive symptoms [38].

In the literature, a study evaluating the depression levels of individuals with MetS using the BDI found no difference in depression levels between those with and without MetS [39]. In another study, where depression and anxiety levels were assessed using the Hospital Depression and Anxiety Inventory, it was shown that individuals with MetS had higher depression and anxiety scores compared to those without MetS [40]. Similar to the literature, this study found that depression was higher in individuals with MetS compared to healthy individuals.

In a study by Leite et al. involving 91 patients, it was reported that 61.3% of the patients screened for depression were found to be positive [41]. Zhao et al. reported a positive relationship between depression levels and individuals who were severely obese [42]. In a study by Eren et al., it was found that obese individuals also had depression and social phobia [35]. This study also indicated that obesity, one of the components of MetS, and depression were higher in individuals compared to healthy individuals.

The prevalence of depression increases with the severity of MetS factors. MetS is considered a risk factor for psychological conditions leading to depression [43]. In our study, individuals with MetS had higher scores on the Beck Depression Inventory (BDI), indicating a higher level of depression compared to healthy individuals. This aligns with previous studies suggesting a link between MetS and increased depression levels. Moreover, the study's mediation analysis revealed that the relationship between MetS and BDI is primarily direct, with no statistically significant indirect effect through IPAQ. While MetS significantly influenced IPAQ ($\beta=-0.5828$, $p<0,001$) and BDI ($\beta=1.8259$, $p<0,001$), the effect of IPAQ on BDI, controlling for MetS, was not significant ($\beta=-0.0279$, $p=0.312$). The indirect effect of MetS on BDI via IPAQ was minimal and not statistically significant, as indicated by the bootstrap confidence interval, which included zero (CI = -0.1369 to 0.4959). These results suggest that the differences in depression levels (BDI) between groups are explained primarily by direct effects rather than being mediated by physical activity levels (IPAQ). Interventions addressing group-specific factors may, therefore, be more effective in mitigating depression levels than focusing solely on increasing physical activity.

Fatigue

In a study conducted by Maloney et al., it was found that as the severity of MetS factors increased, the incidence of chronic fatigue syndrome also rose. Abdominal obesity, hypertension,

high triglycerides, elevated fasting glucose, and decreased high-density lipoproteins increased the likelihood of having chronic fatigue syndrome by 37% [44]. Additionally, among individuals with chronic fatigue syndrome, the number of MetS factors was significantly associated with higher levels of fatigue. A similar study found that the prevalence of MetS was higher in individuals experiencing prolonged fatigue [45]. In a study by Bozkaya et al., individuals with Type 2 diabetes reported greater fatigue upon waking as age increased [46]. Seo et al. reported that fatigue levels in individuals with Type 2 diabetes increased with age [47]. Azak et al. found that as fatigue severity increased among individuals with Type 2 diabetes, the impact on daily living activities increased proportionally [48]. Fatigue is also a common symptom in individuals with MetS, and its severity is positively correlated with the severity of MetS factors. Our study found that individuals with MetS had higher fatigue levels compared to healthy individuals. Increasing physical activity levels and managing activities that contribute to fatigue can be essential in reducing fatigue severity. These findings suggest that MetS and fatigue levels are important determinants of depression and that education level may be a protective factor associated with depression. In addition, our findings suggest that fatigue levels are significantly affected by psychosocial factors such as depression and social functioning, as well as physical activity levels.

Conclusion

In conclusion, this study indicates that individuals with MetS have lower physical activity levels and quality of life, and higher levels of fatigue and depression compared to healthy individuals. These findings should be considered in clinical evaluation, emphasizing a multidimensional approach in the management of MetS.

Rehabilitation programs for this population should be comprehensive, including physical activity promotion, patient education, psychological support, and occupational therapy strategies. Physical therapy interventions play a key role in improving physical activity, functional capacity, and mental health outcomes.

In clinical practice, patients should be encouraged to increase daily physical activity through simple lifestyle changes such as walking, using stairs instead of elevators, and engaging in household activities. For individuals at risk of MetS, regular aerobic exercise (at least 3 days per week) and step-based activity targets (e.g., 10,000 steps/day) combined with dietary interventions may help control disease components and improve overall health outcomes.

Limitations

In this study, individuals with MetS aged between 25 and 65 were evaluated. For future studies, we recommend narrowing the age range of MetS components to determine which age group is most affected and to assess how these components affect physical activity levels, quality of life, depression, and fatigue. A further limitation is the significant age difference between the groups, with the healthy individuals being younger than those with MetS. Although ANCOVA was used to adjust for age, residual confounding cannot be ruled out completely. Given that age is an important determinant of physical activity, this difference may have affected the observed outcomes.

In our study, various scales were used to assess physical activity, quality of life, depression, and fatigue. To obtain more objective results, future studies could benefit from using various measurement devices. More detailed and extensive research is needed to determine physical activity, quality of life, depression, and fatigue in individuals with MetS.

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Relationship between Risk Perception for Complications and Self-Management of Insulin Treatment in Individuals with Type 2 Diabetes: Cross-Sectional Study

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ABSTRACT

Background: Complications are common in individuals with Type 2 Diabetes Mellitus (T2DM) when effective disease management is not achieved. One factor that may be related to disease management is risk perception. Risk perception can influence an individual's health behaviors.

Aim: The study was conducted to determine the relationship between the risk perception of complications in individuals with T2DM and insulin treatment self-management.

Methods: The research was conducted in a descriptive and correlational type. The study was conducted at a university hospital between October 15, 2023, and January 30, 2024, with individuals diagnosed with T2DM. The study included 287 individuals. Data were collected using the Patient Identification Form, the Risk Perception Scale for Diabetes Mellitus (RDS-DM), and the Insulin Therapy Self-Management Scale (ITSM).

Results: The participants' mean risk knowledge score in RPS-DM was 2.80±0.33, the mean composite risk perception score was 2.39±0.43 and the total mean score of ITSM was 115.19±19.14. It was determined that the risk knowledge of individuals with T2DM was weakly positively correlated with ITSM (r=0.215; p<0.01). It was observed that the risk perception regarding complications was positively correlated with the behavioral and cognitive sub-dimension mean scores of the ITSM (p<0.05).

Conclusion: Based on the scores obtained from the scales, it was determined that individuals with T2DM had high knowledge of complications, high risk perception, and above-average insulin treatment self-management levels. Furthermore, it was determined that risk perception regarding complications is related to insulin therapy self-management in behavioral and cognitive dimensions. It is important for nurses to provide training and follow-up to ensure that individuals continue correct and appropriate insulin treatment.

Keywords: Type 2 diabetes; Complication; Risk perception; Insulin treatment; Self-management

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disease that develops as a result of a metabolic disorder with a multisystem effect, characterized by an increase in blood glucose levels due to insulin hormone deficiency or resistance of peripheral tissues to insulin action [1]. The incidence of T2DM is increasing worldwide and has become a global problem that threatens the entire world. In particular, due to persistently high blood glucose levels, many serious complications such as cardiovascular and peripheral artery disease, cerebrovascular diseases, nephropathy, retinopathy, neuropathy and amputation due to diabetic foot occur in patients in the long term [1,2].

From the moment they receive their diagnosis, individuals diagnosed with T2DM must create a fresh way of life to successfully control the condition, avert complications, and uphold their quality of existence. The way these patients perceive risks related to the disease plays a crucial role in their adjustment to this new way of living [3,4]. Risk perception is the sensitivity of an individual to engage in healthy behavior against the threat of deterioration in their health. This may be true for diabetes, as it is for many chronic diseases [5]. A heightened sense of accurate risk awareness can motivate individuals to participate in protective actions and promote healthy habits such as nutritious eating and sufficient physical activity [4,6,7].

In the literature, studies have determined that the risk perception towards the disease and the development of complications in diabetic individuals affects well-being, symptoms, emotional state, diet, exercise and medication compliance, self-care behaviors, emotional disorder status and depression [8-12]. Inaccurate risk perception is a significant obstacle to the adoption of self-care behaviors and may pose an additional risk of negative consequences [3]. Increasing risk perception and taking effective measures to protect against these complications can help individuals improve their diabetes management and live a healthier life [13]. In this context, it has been emphasized that evaluating risk perception in diabetic individuals will be beneficial in controlling diabetes and protecting against complications (14). It is important for nurses to use their educational role in this regard.

Self-management is indispensable in achieving the desired glycemic control in diabetic patients and thus preventing complications [15-17]. Self-management is a concept used to define individuals' own responsibilities in protecting and maintaining health [18]. The content of self-management skills includes regular blood sugar monitoring, increasing the amount of physical activity, creating a nutrition program suitable for the individual, and avoiding behaviors that may pose a risk [19]. In addition, self-management skills for T2DM include the ability to manage this treatment, especially in patients using insulin [15,20].

Insulin achieves its effectiveness solely when administered properly. Misapplication of insulin can result in absorption failures, stiffening of the injection site, and, crucially, an inability to manage blood glucose levels within the target range [21,22]. Self-management skills in insulin management include setting the correct insulin dose according to blood glucose, selecting the appropriate needle size, selecting the insulin injection site, applying the correct injection technique, and effectively performing insulin storage conditions [15,22]. Most patients do not administer insulin effectively and correctly, which increases complications [21]. Therefore, it would be an important approach for nurses to address the factors that may affect their self-management skills in insulin management.

Engaging in self-care practices is crucial for those with diabetes to effectively manage T2DM, and how individuals perceive risk is believed to significantly impact this behavioral aspect. It is anticipated that having a clear understanding of risks will aid in the management of diabetes and help minimize complications related to the condition [23]. In order to determine/implement the necessary nursing interventions for patients with diabetes, it is important to determine the symptoms experienced by patients, their risk perceptions for complications, and their perceptions of insulin self-management. In this context, this study was planned to determine the risk perception for complications and insulin treatment self-management in individuals diagnosed with T2DM and to reveal the relationship between risk perception for complications and insulin treatment self-management. This study will contribute to health professionals and literature in terms of evaluating the awareness of complications in T2DM patients and especially revealing the related factors in the development of insulin management skills.

Methods

The type of the study

The research was conducted in a descriptive and correlational type.

The universe and sample of the study

The universe of the study consisted of individuals who applied to the endocrine outpatient clinic of a university hospital with a diagnosis of type 2 diabetes between 15.10.2023 – 30.01.2024. An average of 700 individuals with a diagnosis of type 2 diabetes applied to the endocrine outpatient clinic of the hospital where the study was conducted in the last three months. Using a known sample size calculation formula and taking a p-value of 0.05 to maximize sample size, the sample size for the study was determined to be 249 (n=N.t2.p.q/d2.(N-1)+t2.p.q [t=1.96; p=0.05; d=0.05; q=0.95]). Considering that there may be losses in the study, the sample size was increased by 10% and it was planned to include at least 274 individuals with T2DM in the study. In this context, 287 individuals who were diagnosed with type 2 diabetes for at least six months, aged 18 years and over, at least literate, using insulin treatment, had no verbal communication barriers, and were willing to participate in the study were included in the sample. Diabetic individuals who did not use insulin in T2DM treatment, had more than one chronic complication due to diabetes, were unable to communicate verbally, and did not agree to participate in the study were excluded from the study.

Data collection tools

Data were obtained using the Patient İdentification Form, Risk Perception Scale-Diabetes Mellitus (RPS-DM) and Insulin Treatment Self-Management Scale.

Patient Identification Form; The form, which was created by researchers in line with the literatüre, consists of two sections and 25 questions: socio-demographic (age, height, weight, gender, marital status, educational status, etc.) and disease-related (diabetes diagnosis period, diabetes type, diabetes diagnosis method, presence of additional chronic disease, diabetes treatment, regular medication use, etc.) variables [14,23,24].

Risk Perception Scale-Diabetes Mellitus; The research on the Turkish version's validity and reliability for the scale created by Walker in 2007 was carried out by Yilmaz and colleagues [14,24]. This scale is the pioneer tool designed to assess awareness of diabetes complications and associated risk perception. It comprises 25 questions and features two main areas: "risk knowledge" and "composite risk perception."

Within the composite risk perception area, there are four parts: "concern," "optimism," "personal disease risk," and "environmental risk." A higher score reflects a greater perceived risk of complications related to diabetes. In this research, the Cronbach alpha coefficients were determined to be 0. 78 for the risk knowledge area and 0. 87 for the composite risk perception area, respectively.

Insulin Treatment Self-Management Scale; The scale developed by Karahan Okuroglu and his colleagues in 2019 consists of 32 items [15]. The scale is a 5-point Likert type and has three sub-dimensions. An increase in the score obtained in all sub-dimensions should be evaluated as an increase in the level of self-management related to the relevant dimension. In the study by Karahan Okuroglu and his colleagues, the Cronbach alpha internal consistency coefficient was found to be 0.91 [15]. In this study, the Cronbach alpha value of the scale was determined as 0.92.

Data evaluation

Data were interpreted in SPSS 29.0 package program (IBM SPSS, USA). Descriptive statistical methods (percentage, mean, standard deviation) were used in the distribution of sociodemographic and disease-related characteristics of individuals with T2DM. Student t test and One-Way ANOVA (Bonferroni test as a post hoc test) were used to compare some characteristics of patients with RPS-DM and Insulin Treatment Self-Management Scale; Pearson correlation analysis was used to examine the relationship between the scales. Significance was evaluated as $p<0.05$ in statistical evaluation.

Ethics committee approval

Before collecting the data, written permission was obtained from the Sivas Cumhuriyet University Non-Interventional Ethics Committee (Decision no: 2023-09/03; Date: 21.09.2023) and the hospital where the study was conducted (Decision no: 349152). In addition, informed consent was obtained from each individual who would participate in the study regarding the content of the study and the voluntary nature of participation. The study was conducted in accordance with the Declaration of Helsinki Principles.

Results

The average age of individuals with T2DM is 61.04±11.82 years, and 38.7% are 65 years of age or older. 59.2% of the participants are female, 73.2% are married, 43.2% are secondary school graduates, 81.2% are unemployed, and 86.1% evaluate their economic status as moderate. When the body structure of the participants is examined according to their BMI values, it was determined that 42.2% are overweight and 37.6% are obese. 10.8% of individuals with T2DM currently smoke and 1% consume alcohol. The characteristics of the participants regarding their disease are shown in Table 1.

It was observed that the risk knowledge sub-dimension mean score of individuals with T2DM in RPS-DM was 2.80±0.33 and that their risk knowledge regarding complications was at a good level. The composite risk perception sub-dimension mean score of the scale was 2.39±0.43 and the perceived risk level was above average. In addition, it was determined that the anxiety levels (2.95±0.82) and personal disease risk (2.46±0.93) of the participants were above average; optimism (2.09±0.72) and environmental risk perception (2.04±0.92) were at a moderate level. The total mean score of individuals with T2DM on the ITSM Scale was 115.19±19.14, and considering the minimum

Table 1 Disease-related characteristics of individuals with T2DM

Features		Mean±SD	
Disease duration (years) (Mean±SD)		13.69±9.09	
HbA1c (%) (Mean±SD)		9.87±2.16	
		n	%
Presence of other chronic diseases	Yes	163	56.8
	No	124	43.2
How to diagnose diabetes	When you go for a check-up for another reason	134	46.7
	Coincidentally, with blood sugar measurement	36	12.5
	When you apply to a healthcare facility due to symptoms of diabetes such as dry mouth and thirst	117	40.8
Types of diabetes treatment	Oral antidiabetic + insulin	115	40.1
	Insulin	172	59.9
Regular use of medications	Yes	228	79.4
	Partially	49	17.1
	No	10	3.5
Regular diet	Yes	153	53.3
	Partially	97	33.8
	No	37	12.9
Regular exercise	Yes	72	25.1
	Partially	120	41.8
	No	95	33.1
Diabetes control frequency	Monthly	24	8.4
	Three months	152	53.0
	Six months	47	16.4
	Yearly	19	6.6
	Irregular	45	15.7
Diabetic retinopathy	Yes	57	19.9
	No	230	80.1
Diabetic nephropathy	Yes	37	12.9
	No	250	87.1
Diabetic Neuropathy	Yes	46	16.0
	No	241	84.0
Diabetic foot	Yes	45	15.7
	No	242	84.3

Table 2 Distribution of mean scores of the RPS-DM and Insulin Treatment Self-Management Scale

Scales	Minimum and maximum points available	Minimum points marked	Mean±SD
RPS-DM			
Risk information	0-3	0-3	2.80±0.33
Compound risk perception	1-4	1-3.67	2.39±0.43
Anxiety	1-4	1-4	2.95±0.82
Optimism	1-4	1-4	2.09±0.72
Personal disease risk	1-5	1-4.11	2.46±0.93
Environmental risk	1-4	1-4	2.04±0.92
Insulin Treatment Self-Management Scale	32-160	57-159	115.19±19.14
Behavioral	17-85	21-84	64.89±11.10
Cognitive	7-35	7-35	23.14±6.12
Affective	8-40	8-40	27.15±8.13

Table 3 Distribution of responses of individuals with T2DM to statements regarding the risk of having diabetes-related health problems

Health problems related to diabetes	Almost no risk		Slight risk		Medium risk		High risk	
	n	%	n	%	n	%	n	%
Heart attack	80	27.9	58	20.2	66	23.0	83	28.9
Foot amputation	118	41.1	57	19.9	44	15.3	68	23.7
Cancer	146	50.9	60	20,9	36	12.5	45	15.7
Vision problems	49	17.1	70	24,4	70	24.4	98	34.1
High blood pressure	34	11.8	60	20,9	70	24.4	123	42.9
Numb feet	36	12.5	66	23,0	66	23.0	119	41.5
Stroke	115	40.1	63	22,0	40	13.9	69	24.0
Blindness	111	38.7	70	24,4	36	12.5	70	24.4
Kidney failure	92	32.1	73	25,4	42	14.6	80	27.9

and maximum scores that could be obtained (32-160), it was determined that the self-management level in behavioral, cognitive and affective areas was above average (Table 2).

Table 3 shows the distribution of responses given by individuals with T2DM to the personal disease risk section in the RPS-DM. Accordingly, it was determined that participants perceived complications such as heart attack (28.9%), high blood pressure (42.9%) and numb feet (41.5%) as higher risks.

When some characteristics of individuals with T2DM were compared with the RPS-DM score averages, it was seen that age was very weakly negatively correlated with the level of optimism and very weakly positively correlated with personal disease risk perception. It was determined that the duration of the disease was very weakly positively correlated with the composite risk perception for complications. It was also found that the HbA1c level was very weakly negatively correlated with anxiety and very weakly positively correlated with optimism ($p<0.05$). According to BMI, it was found that the optimism level was lower in patients with obese body structure. It was also seen that the personal disease risk perception was higher in individuals with other chronic diseases. In addition, the anxiety level was found to be lower in individuals using oral antidiabetic and insulin compared to individuals using only insulin. It was determined that the anxiety level was higher in patients who had their diabetes checked regularly and the personal disease risk perception was higher in those who had their diabetes checked every one to three months ($p<0.05$). However, Bonferroni correction revealed that patients who had their diabetes checked at different times had similar levels of anxiety. Apart from these variables, it was found that gender and education status were not related to the risk perception for complications ($p>0.05$).

When some characteristics of T2DM individuals using insulin treatment were compared with the RPS-DM score averages, it was determined that age was negatively correlated with insulin treatment self-management. In addition, it was determined that behavioral insulin self-management was high in male patients. Insulin treatment self-management levels were found to be higher in participants with higher education level compared to other groups ($p<0.05$). Also, obese participants were found to have lower levels of self-management of insulin therapy ($p<0.05$). However, it was determined that there was no relationship between disease duration, HbA1c level, presence of other chronic diseases, diabetes treatment type and diabetes control frequency and insulin treatment self-management ($p>0.05$) (Table 4).

In the study, when the mean scores of T2DM individuals were compared with the RPS-DM and Insulin Treatment Self-Management Scale, it was determined that risk knowledge was weakly positively correlated with insulin treatment self-management ($r=0.215$; $p<0.001$). It was found that risk knowledge was associated with both behavioral and cognitive sub-dimensions ($p<0.01$).

In the study, it was observed that the participants' risk perception regarding complications in diabetes was positively correlated with the behavioral and cognitive sub-dimension mean scores of the Insulin Treatment Self-Management Scale ($p<0.05$). However, it was observed that the anxiety and personal disease risk sub-dimensions in the risk perception regarding complications were not associated with insulin treatment self-management ($p>0.05$) (Table 5).

Discussion

T2DM is a public health problem that is increasingly common, requires lifelong follow-up and treatment, and can cause many complications, and imposes a serious financial burden on the health system [1]. In order to prevent these problems or detect them at an early stage, it is of great importance for individuals to be informed about T2DM complications and to comply with treatment [25]. This study was planned to determine the perception of risk for complications and self-management of insulin therapy in individuals diagnosed with T2DM, and to reveal the relationship between the perception of risk for complications and self-management of insulin therapy.

In the management of diabetes requiring continuous medical care, the knowledge level of patients is very important to reduce the risk of developing acute and chronic complications [1]. In the study, it was determined that the risk knowledge of complications in individuals with T2DM was at a good level. This finding is consistent with the findings of many studies [12,13,19,24-27]. The high knowledge of the risk of complications in individuals with T2DM may be due to the diabetes education given to patients. This shows that individuals with T2DM have high awareness of diabetes-related complications.

Risk perception in individuals with T2DM may negatively affect diabetes self-management and may pose a real risk for the development of diabetes complications [12]. In this study, it was determined that the perceived risk level in individuals with T2DM was above average. However, it was observed that participants' anxiety and personal risk perception, which shape their perception of the risk of complications, were higher than other components. While the findings of three studies conducted in Turkey were similar to the findings of this study [23,25-27], in other studies, the perceived risk level for diabetes complications was determined to be moderate [13,23]. In a study conducted in China, it was found that patients' perceived personal risks in terms of risk perceptions regarding complications were mild to moderate, their personal risk control and optimism levels were moderate, and their anxiety levels were moderate to high [5]. In a systematic review examining the risk perception of complications in individuals with T2DM, it was stated that the participants had low risk awareness and optimistic bias [3]. In a study conducted with African American individuals with T2DM, it was determined that the risk perception for diabetes complications was low [8]. It is thought that the difference in the research findings may be due to differences in factors such as the age range, educational status, cultural characteristics, and diabetes education status of the individuals included in

Table 4 Comparison of mean scores on the RPS-DM and Insulin Treatment Self-Management Scale for certain characteristics and disease-related features in individuals with T2DM

Features	Risk Information	Composite Risk Perception					Insulin Treatment Self-Management Scale			
		General	Anxiety	Optimism	Personal disease risk	Environ-mental Risk	General	Behavioral	Cognitive	Affective
	r; p	r; p	r; p	r; p	r; p	r; p	r; p	r; p	r; p	r; p
Age (years)	-0.081;									
0.172	0.074; 0.214	0.018; 0.756	-0.186; 0.002	0.302; <0.001	-0.037; 0.537	-0.297; <0.001	-0.300; <0.001	-0.274; <0.001	-0.086; 0.147	
Duration of illness (years)	-0.014; 0.813	0.143; 0.016	0.046; 0.438	-0.129; 0.028	0.187; 0.001	0.142; 0.016	0.008; 0.897	-0.040; 0.501	-0.030; 0.618	0.095; 0.109
HbA1c (%)	-0.001; 0.990	0.036; 0.567	-0.139; 0.027	0.128; 0.049	0.021; 0.739 Obez					
00.072; 0.256	0.072; 0.256	-0.001; 0.989	0.032; 0.612	-0.034; 0.588	-0.017; 0.791					
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Gender										
Female	2.79±0.35	2.42±0.43	2.99±0.83	2.11±0.76	2.53±0.92	2.06±0.92	113.43±19.77	63.60±11.41	22.87±6.64	26.96±7.86
Male	2.83±0.28	2.33±0.43	2.88±0.80	2.07±0.67	2.36±0.95	2.02±0.93	117.76±17.97	66.76±10.40	23.55±5.27	27.43±8.54
t; p	-1.071; 0.285	1.725; 0.086	1.141; 0.255	0.482; 0.630	1.510; 0.312	0.347; 0.729	-1.889; 0.060	-2.396; 0.017	-0.931; 0.353	-0.481; 0.631
Educational Status										
Literate (a)	2.71±0.38	2.41±0.38	2.99±0.75	2.13±0.82	2.39±0.96	2.13±0.87	107.53±24.56	59.50±14.52	21.33±8.53	26.69±9.75
Primary Education (b)	2.85±0.30	2.38±0.42	2.93±0.84	2.06±0.72	2.67±0.97	1.87±0.87	109.78±17.95	62.78±9.30	21.54±6.27	25.45±8.28
Secondary Education (c)	2.80±0.33	2.40±0.45	2.93±0.82	2.10±0.67	2.47±0.89	2.09±0.95	118.66±17.14	66.36±9.90	24.33±4.63	27.95±7.55
Higher Education (d)	2.88±0.24	2.33±0.47	2.95±0.87	2.07±0.73	2.30±0.95	2.02±0.94	121.36±13.62	69.68±8.39	24.06±5.19	27.62±7.25
F; p	2.813; 0.040 (a=b=c=d)	0.331; 0.803	0.056; 0.982	0.127; 0.944	1.546; 0.203	0.922; 0.430	8.153; <0.001 (c=d>a=b)	9.724; <0.001 (c=d>a=b)	5.072; 0.002 (a=b=c=d)	1.350; 0.258
Body structure according to BMI										
Normal weight (a)	2.83±0.31	2.38±0.44	3.06±0.62	2.37±0.70	2.18±0.99	1.92±0.43	114.08±17.63	64.87±11.91	23.96±5.03	25.24±8.70
Overweight (b)	2.85±0.28	2.34±0.43	2.85±0.86	2.07±0.74	2.45±0.91	2.00±0.94	119.44±18.56	67.31±10.40	23.83±5.98	28.29±7.97
Obese (c)	2.75±0.38	2.44±0.43	3.00±0.85	1.98±0.67	2.63±0.90	2.15±0.94	111.03±19.72	62.18±11.19	21.94±6.64	26.90±7.87
F; p	2.892; 0.057	1.351; 0.261	1.692; 0.186	5.755; 0.004 (a=b>c)	4.513; 0.012 (a=b=c)	1.348; 0.261	5.816; 0.003 (a=b>c)	6.315; 0.002 (a=b>c)	3.420; 0.034 (a=b=c)	2.883; 0.058
Presence of other chronic diseases										
Yes	2.79±0.34	2.42±0.42	2.93±0.85	2.06±0.73	2.63±0.88	2.06±0.92	113.39±20.08	63.88±11.43	22.63±6.57	26.86±8.45
No	2.82±0.30	2.34±0.45	2.97±0.77	2.13±0.71	2.25±0.96	2.02±0.92	117.57±17.64	66.20±10.55	23.82±5.43	27.54±7.72
t; p	-0.822; 0.412	1.474; 0.142	-0.369; 0.712	-0.804; 0.422	3.443; <0.001	0.328; 0.743	-1.840; 0.067	-1.760; 0.079	-1.628; 0.105	-0.696; 0.487
Diabetes treatment method										
Oral antidiabetic + insulin	2.80±0.34	2.40±0.40	2.82±0.76	2.18±0.73	2.51±0.91	2.07±0.92	114.26±18.79	64.00±10.99	22.69±6.11	27.55±7.99
Insulin	2.81±0.32	2.38±0.46	3.03±0.84	2.04±0.71	2.43±0.95	2.02±0.92	115.82±19.41	65.48±11.16	23.45±6.12	26.88±8.24
t; p	-0.035; 0.972	0.325; 0.745	-2.124; 0.035	1.627; 0.105	0.764; 0.446	0.444; 0.658	-0.678; 0.498	-1.102; 0.271	-1.027; 0.307	0.680; 0.497
Diabetes control frequency										
Once every three months (a)	2.78±0.34	2.36±0.45	2.88±0.85	2.03±0.72	2.57±0.98	1.98±0.92	113.34±20.79	64.10±11.69	22.53±6.56	26.69±8.32
Once every six months/ year (b)	2.82±0.32	2.38±0.41	2.92±0.80	2.20±0.72	2.38±0.88	2.04±0.93	118.57±17.11	65.65±11.04	24.18±5.89	28.74±7.72
Irregular (c)	2.87±0.25	2.48±0.39	3.25±0.63	2.20±0.72	2.18±0.77	2.28±0.87	117.51±13.94	66.84±8.37	24.04±4.11	26.62±7.87
F; p	1.290; 0.277	1.229; 0.294	3.798; 0.024 (a=b=c)	1.910; 0.150	3.426; 0.034 (a=b=c)	1.941; 0.145	2.202; 0.113	1.292; 0.276	2.328; 0.099	1.635; 0.197

Table 5 Comparison of Mean Scores of Insulin Treatment Self-Management Scale with RPS-DM in Individuals with T2DM

Insulin Treatment Self-Management Scale	Risk information	Compound risk perception				
		General	Anxiety	Optimism	Personal disease risk	Environmental Risk
	r; p	r; p	r; p	r; p	r; p	r; p
Behavioral	0.198; <0.001**	0.137; 0.020*	0.080; 0.178	0.069; 0.247	0.015; 0.804	0.122; 0.039*
Cognitive	0.210; <0.001**	0.174; 0.003*	0.094; 0.112	0.206; <0.001**	-0.095; 0.107	0.183; 0.002*
Affective	0.077; 0.192	-0.073; 0.220	-0.119; 0.045	0.047; 0.427	-0.093; 0.115	0.025; 0.675
General	0.215; <0.001**	0.105; 0.077	0.026; 0.663	0.126; 0.033*	-0.062; 0.298	0.140; 0.018*

the sample group. However, the findings obtained in this study reveal that the risk knowledge regarding complications is high in individuals with T2DM, but the need to evaluate risk perception. The study found that patients with T2DM perceived complications such as heart attack, high blood pressure, and numb feet as riskier. In the research conducted by Kumsar et al. it was revealed that individuals regarded issues like heart attack, hypertension, and eyesight difficulties as posing greater risks [27]. The study finding is consistent with the literature. This finding may be due to the frequent occurrence of complications parallel to the prevalence of diabetes and the individual and technological information provided about the development of complications.

In the study, it was found that as age was weakly negatively associated with optimism levels and positively associated with personal disease risk perception in individuals with T2DM. In addition, HbA1c level is very weakly negatively correlated with anxiety and positively correlated with optimism; obese patients have lower levels of optimism; individuals with other chronic diseases have higher personal disease risk perception; and patients using oral antidiabetic drugs and insulin have lower levels of anxiety. There are a limited number of studies in the literature examining variables associated with risk perception for complications [13,23,25,27]. For example, in the study by Bila and Yılmaz, it was found that the complication risk perception level of those with additional chronic diseases was significantly higher than those without [25]. In the study of Kumsar et al., it was determined that risk perception decreases as age increases, risk perception increases as education level increases, and HbA1c level is positively correlated with anxiety [27]. In the study of Saglam and Bektas, it was determined that risk perception for complications is higher in women, and risk perception increases as age increases [23]. In the literature, it is seen that the characteristics of individuals with T2DM differ in their relationship with risk perception for complications. This may be due to the characteristics of the individuals included in the sample or the number of samples.

In T2DM, insulin treatment is initiated when acute and chronic complications occur and glycemic control deteriorates for various reasons [28]. Ensuring that diabetic individuals have self-care skills, such as being able to continue insulin treatment on their own and gaining the ability to administer insulin, has become one of the main goals in diabetes management [15,21]. In the study, it was determined that the insulin treatment self-management level of individuals with T2DM was partially good in behavioral, cognitive and affective areas. There is no study in the literature using a similar measurement tool. However, in the studies conducted, it was seen that T2DM patients using insulin had a high level of negative perception towards insulin use [23,28]. In a study examining diabetes self-care management practices in patients using insulin, it was determined that diabetes self-care should be improved for patients using insulin [17]. The findings of this study reveal the need to improve the insulin treatment self-management skills of individuals with T2DM.

The study found a positive correlation between risk awareness regarding complications and self-management of insulin therapy in individuals with T2DM. Although there is no similar study in the literature, it can be said that the finding obtained in this study is an expected situation. In fact, patients who are aware of the complications that may arise due to diabetes are expected to be careful about their treatment in order to prevent these complications. In this respect, the study finding reveals the importance of disease education in the implementation of self-care skills regarding treatment.

Individuals' perception levels can affect their behaviors towards diabetes management [9,26]. One of the important points we reached with this study is that as the risk perception of individuals with T2DM regarding complications increases, their self-management of insulin treatment in behavioral and cognitive dimensions also increases. Although there is no similar study in the literature, there are studies in which the risk perception for complications is related to the perception of insulin treatment and diabetes self-management. For example, Sağlam and Bektaş found that there was a negative relationship between the perception of risk regarding complications and the perception of insulin treatment evaluation; patients with low risk perception had higher negative attitudes towards insulin treatment [23]. Aytemur and Inkaya found that as individuals' complication risk perception increased, their diabetes self-management increased [19]. In a study conducted with Chinese individuals with T2DM, risk perception was determined to be significantly associated with each health-enhancing self-care behavior [5]. Another study emphasized that perceived risk is related to diet, exercise, and medication compliance in diabetes and supports self-care behaviors [9]. Despite these studies, Rashidi and Kiskac found that risk perception for complications was not associated with self-management in diabetes [13]. Bila and Yilmaz found that risk perception increased while treatment compliance decreased [25]. In line with the studies conducted, the contribution of our study finding to the literature is important due to the different findings and especially the fact that insulin treatment self-management was not addressed in the studies. There is a need for studies examining the risk perception for complications in individuals with T2DM and insulin treatment self-management.

Limitations

Since the study was conducted in a single public hospital and within a specific time period, the sample size is limited. In addition, participants' risk perceptions for complications and insulin treatment self-management levels are based on the self-reporting of individuals according to the items included in

the scales. Variables such as insulin treatment dose and insulin administration skills of individuals with T2DM were not included in the study. Also, patients with multiple diabetes-related complications were not included in the study, as this could alter risk perception. However, our study is the first to examine the risk perception for complications determined to be associated with insulin treatment self-management. It is believed that it will guide health professionals in insulin treatment management.

Conclusion

Based on the scores obtained from the scales, it was determined that individuals with T2DM had high knowledge of complications, high risk perception, and above-average insulin treatment self-management levels. Participants' knowledge of the risk of complications was found to be related to self-management of insulin therapy. Furthermore, risk perception was found to be positively correlated with self-management in both behavioral and cognitive dimensions.

These findings are important for the effective management of treatment in individuals with T2DM. Therefore, to provide comprehensive care to individuals with diabetes, a care approach provided by nurses that addresses patients holistically, plans care specifically for each individual, and focuses on improving the individual's insulin self-management is crucial. It is important for nurses to educate patients on providing accurate risk perception regarding complications in individuals with T2DM.

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Patient Consent Statement: The informed consents were obtained from the individuals who agreed to participate in this research.

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Comprehensive Global Bibliometric Analysis of Negative-Pressure Wound Therapy Research (1997–2025)

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ABSTRACT

Objective: Negative Pressure Wound Therapy (NPWT) is a wound care technology widely used to treat complex wounds, including diabetic foot infections and pressure ulcers. The aim of this study is to examine global research trends in the field of NPWT from January 1, 1997, to March 1, 2025, using bibliometric methods, including productivity, institutional contributions, and emerging research topics.

Methods: This study is bibliometric research using Web of Science database data. The R Biblioshiny program was used for the analysis.

Results: A total of 2,386 publications were analyzed. The USA led with the most research output (n=1,660) and total citations (n=21,068), followed by China (n=668) and Germany (n=395). The most productive institution was Lund University (n=77). International Wound Journal published the most articles (n=168), while Plastic and Reconstructive Surgery had the highest h-index (30). The annual growth rate is 11.39%. The evolution of keywords had moved from technical words like “vacuum-assisted closure” (n=828) to clinical information such as “risk factors” and “surgical complications” in the recent time span.

Conclusions: The analysis of research productivity shows a high concentration of leading institutions in Sweden and the USA. However, we need to establish more extensive global collaborations.

Keywords: Negative-pressure wound therapy, bibliometric analysis, wound care, vacuum assisted closure, research trends

Introduction

Negative pressure wound therapy (NPWT) is a widely used and recommended wound care technology for the treatment of diabetic foot infections, pressure ulcers and similar complex wounds [1-3]. NPWT accelerates healing by removing harmful and corrosive substances that negatively affect healing in chronic wounds. In wounds closed using skin grafts or dermal implants, NPWT promotes granulation tissue formation, controls exudate and reduces the bacterial load in the wound [4].

In the 20th century, Russians revolutionized negative pressure treatments, particularly in surgical techniques and wound care, with Dr. Nail Bagaoutdinov using a negative pressure unit with foam dressings in 1985 [5]. NPWT involves the application of a wound dressing through which a negative pressure (or vacuum) is applied, with wound and tissue fluid being collected into a canister [6]. Dr. Louis Argenta and Michael Morykwas from Wake Forest University School of Medicine invented the mechanical vacuum and polyurethane foam that were used in the 1990s to create the current NPWT systems [7]. When it was first introduced, NPWT devices were supplied by a single manufacturer (KCI's V.A.C. system). However, as the NPWT market grew, a variety of commercial NPWT systems were developed, including smaller and portable devices. Even disposable NPWT products have recently been introduced. In resource-limited settings, simple, homemade NPWT devices using hospital vacuum pumps are also used. NPWT can be prescribed and administered by different healthcare professionals and its use outside the hospital has become widespread, especially with the development of mobile systems. Although NPWT systems differ in the type of pressure, the material in contact with the wound surface

and the dressing used, the basic principle is the same [6].

The place of NPWT in the treatment process and the rationale for its use vary according to wound type and local protocols. The National Institute for Health and Care Excellence (NICE) recommends that NPWT should be considered as a temporary dressing option prior to surgical intervention in open wounds that have been debrided but are awaiting soft tissue repair. In this situation, NPWT is used for a short period of time to reduce the risk of infection [8].

There have been many advances in NPWT technology, such as NPWT with instillation and retention (NPWTi-d). NPWTi-d promotes wound healing through wound cleansing, irrigation and non-excisional debridement. Comparative clinical studies have shown that NPWTi-d reduces the time to definitive wound healing and hospitalization. NPWTi-d, especially when using reticulated open-cell foam dressing with "through" holes (ROCF-CC), is suggested to facilitate the solubilization, decomposition and elimination of infectious materials such as necrotic tissue and dense exudate before or after surgical debridement or when surgical debridement is not possible [9].

Since the emergence of this therapy, numerous studies on NPWT have been published. But these studies have not been systematically collected and reviewed. There are continued controversies over NPWT indications, potential side effects (e.g., infection, trauma), and ethical dilemmas that make it difficult for physicians to choose the right treatment method. Based on a systematic review of the literature, clinicians and scientists can gain more knowledge about the effective use of NPWT in wound healing, including its possible future directions.

Bibliometric analysis is a methodological approach used to map the structural and dynamic aspects of a scientific field through the quantitative evaluation of publication data within that field. This method enables the identification of global research trends, institutional collaborations, and thematic evolutions over a specific time period [10, 11]. However, to our knowledge, bibliometric methods have not been used to analyze studies related to NPWT in wound healing. Therefore, this analysis aims to examine the published literature using bibliometric techniques and information visualization methods to summarize NPWT research. Additionally, this article aims to contribute to the existing body of knowledge and propose new directions for future research.

Methods

Study model

We conducted a systematic literature search using the Web of Science electronic database to identify studies evaluating NPWT. To perform the bibliometric analysis, we organized our work into three parts, following the guidelines of Donthu et al. [12]. The first part involved the systematic identification and selection of relevant data.

Data source

A bibliometric analysis using Boolean searches on the Web of Science (WoS, Clarivate Analytics, Philadelphia, PA, USA) was carried out to evaluate the main trends of publications about NPWT.

All data download procedures were completed on March 26, 2025. The process was verified through double-checking by two independent researchers. The literature search encompassed all studies published prior to March 1, 2025.

The time frame was selected to include the studies published between January 1, 1997, and March 1, 2025.

We limited the search to the 'Title (TI)' field only because, in many cases, NPWT is not the main focus but is mentioned as a secondary method (e.g., case reports or large surgical series). This allowed us to filter out thousands of irrelevant publications and focus on the core literature. We scanned all indexes (Science Citation Index Expanded (SCI-EXPANDED), Social Sciences Citation Index (SSCI), Arts & Humanities Citation Index (AHCI), Emerging Sources Citation Index (ESCI), Conference Proceedings Citation Index- Science (CPCI-S), Conference Proceedings Citation Index- Social Sciences & Humanities (CPCI-SSH), Book Citation Index– Science (BKCI-S), Book Citation Index– Social Sciences & Humanities (BKCI-SSH)) within the Web of Science Core Collection.

We have included "Research Articles" as a document type, including those written in English.

The following were excluded from the sample: studies not published within the specified time frame; letters to the editor; reviews; case reports and case series (those without "NPWT" in the title); conference abstracts; book chapters; and studies published in languages other than English.

The search terms were as belows: TI=(Negative-Pressure Wound Therapy OR Negative-Pressure Wound Therapies OR Negative Pressure Wound Therapy OR Negative-Pressure Wound OR Wound Therapies, Negative-Pressure OR Wound Therapy, Negative-Pressure OR Topical Negative-Pressure Therapy OR Negative-Pressure Therapies, Topical OR Negative-Pressure Therapy, Topical OR Therapies, Topical Negative-Pressure OR Therapy, Topical Negative-Pressure OR Topical Negative-Pressure Therapies OR Topical Negative Pressure Therapy OR Vacuum-Assisted Closure OR Closures, Vacuum-Assisted OR Closure, Vacuum-Assisted OR Vacuum Assisted Closure OR Vacuum-Assisted Closures OR Negative-Pressure Dressings OR Dressing, Negative-Pressure OR Dressings, Negative-Pressure OR Negative-Pressure Dressing OR Negative Pressure Dressings)

Research methodology and exclusion criteria presented in the flowchart

The initial search conducted in the WoS Core Collection using keywords yielded 3,881 results. When the document type was refined to research articles, the number of publications was reduced to 2,541. By selecting English as the language of publication, the number of relevant papers further decreased to 2,386. These publications constitute the sample for the bibliometric analysis. Subsequent analyses were performed on this subset of publications

These studies were downloaded to the computer in plaintext format. They were transferred to the Biblioshiny program [13] for analysis.

Bibliometric analysis

In this study, Biblioshiny software was used to analyze, evaluate, and visualize selected articles. Developed by M. Aria and C. Cuccurullo [13] using the R statistical programming

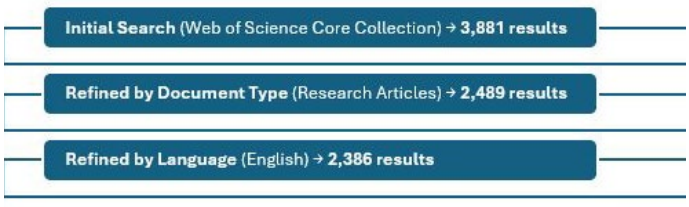

Figure 1 – **Flowchart**

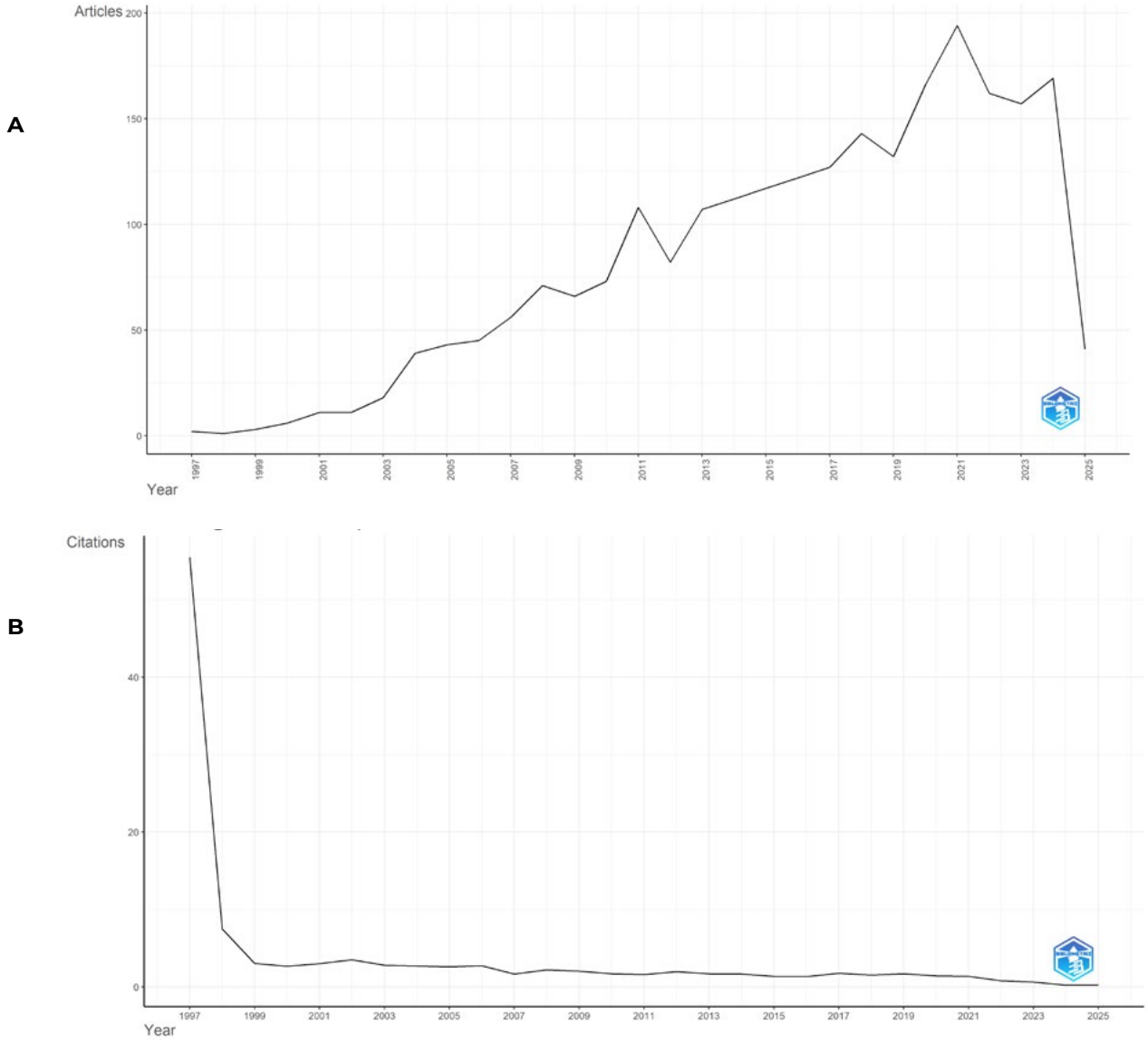

Figure 2 – Scientific Publications and Citations Statistics: A) Annual Scientific Production; B) Average Citations Per Year

language, Biblioshiny offers the possibility to import and edit data and create interactive visualizations. It offers various visualization options such as bar charts, line graphs, and maps and can be integrated with other data mining tools such as R and Excel. All analyses in our study were conducted through this software. We chose this software because it combines the capacity to process complex metadata with powerful statistical routines that provide a high level of reproducibility in the mapping process.

To assess NPWT research performance, and scientific trends and development, two main analyses were conducted using bibliometric methods such as citation analysis, trend analysis, and network analysis: (1) performance analysis was used to identify important institutions and countries in the field, and (2) science mapping was used to identify the historical development of the research field, gaps in the literature, and new/declining trends.

Results

Publication trend

The total number of publications is 2,386, spanning from 1997 to 2025. The publications were published in 591 different journals and had an annual growth rate of 11.39%. The average age of the publications was 8.75 years. In total, there were 26,135 references in these publications and 2,018 Keywords Plus (ID) and 3,280 Keywords (DE) were used. According to the data on authors, there were 9,943 authors, and 87 articles were single-authored. The average number of authors in each publication was 5.37. Furthermore, 10.23% of publications were published in international collaborations.

The first publication was released in 1997. There is a general upward trend in the number of articles published over the years. Originally, only 2 articles were published in 1997; however, the amount has since increased over the years. There has been a notable rise in the number of publications, particularly since the early 2000s. The number of articles began to grow in 2004, experiencing large increases such as 56 in 2007 and 71 in 2008. Since 2015, approximately 120 articles have been published annually. In 2020, 166 articles were published. There was an increase to 194 articles in 2021, followed by a slight decline in 2022 and 2023. The number of articles has increased again since 2024 (Figure 2a). Although the number of published articles continues to rise over time, the average number of citations and the annual number of citations have continued to decline (Figure 2b). The annual publication and citation counts are presented in Figures 2a and 2b.

Table 1 Top cited 10 articles

No	Title	Authors	Source Title	Publication Year	DOI	Total Citations	Average per Year
1	Vacuum-assisted closure: A new method for wound control and treatment: Animal studies and basic foundation	Morykwas, MJ et al.	Annals of plastic surgery	1997	10.1097/00000637-199706000-00001	1613	55.62
2	Vacuum-assisted closure: A new method for wound control and treatment: Clinical experience	Argenta, LC et al.	Annals of plastic surgery	1997	10.1097/00000637-199706000-00002	1603	55.28
3	Negative pressure wound therapy after partial diabetic foot amputation: a multicentre, randomised controlled trial	Armstrong, DG et al.	Lancet	2005	10.1016/S0140-6736(05)67695-7	647	30.81
4	Vacuum-assisted closure: Micro deformations of wounds and cell proliferation	Saxena, V et al.	Plastic and reconstructive surgery	2004	10.1097/01.prs.0000135330.51408.97	526	23.91
5	Comparison of negative pressure wound therapy using vacuum-assisted closure with advanced moist wound therapy in the treatment of diabetic foot ulcers: A multicenter randomized controlled trial	Blume, PA et al.	Diabetes care	2008	10.2337/dc07-2196	418	23.22
6	Bacterial load in relation to vacuum-assisted closure wound therapy: A prospective randomized trial	Mouës, CM et al.	Wound repair and regeneration	2004	10.1111/j.1067-1927.2004.12105.x	398	18.09
7	The use of vacuum-assisted closure therapy for the treatment of lower-extremity wounds with exposed bone	DeFranzo, AJ et al.	Plastic and reconstructive surgery	2001	10.1097/00006534-200110000-00013	327	13.08
8	Vacuum-assisted closure: State of basic research and physiologic foundation	Morykwas, MJ et al.	Plastic and reconstructive surgery	2006	10.1097/01.prs.0000225450.12593.12	304	15.20
9	Incisional Negative Pressure Wound Therapy After High-Risk Lower Extremity Fractures	Stannard, JP et al.	Journal of orthopaedic trauma	2012	10.1097/BOT.0b013e318216b1e5	278	19.86
10	State-of-the-art treatment of chronic leg ulcers: a randomized controlled trial comparing vacuum-assisted closure (VAC) with modern wound dressings	Vuerstaek, JDD et al.	Journal of vascular surgery	2006	10.1016/j.jvs.2006.07.030	259	12.95

Most cited articles and citations by years

Table 1 (see the next page) summarizes the top 10 most-cited articles on NPWT. The findings highlight three key studies that have made the most significant contributions to the NPWT literature. The study titled "Vacuum-assisted closure: A new method for wound control and treatment: Clinical experience," which has received 1,613 citations and is the most cited article, examines the fundamental biological effects of NPWT using animal models [14]. The second most-cited article, "Negative Pressure Wound Therapy: A New Method for Wound Control and Treatment: Clinical Experience" [15], with 1,603 citations, reviews the clinical experience demonstrating the efficacy of NPWT in the treatment of large wounds. The third most-cited article, "Negative pressure wound therapy after partial diabetic foot amputation: a multicenter, randomized controlled trial" [16], with 647 citations, addresses the mechanisms of action of NPWT in the healing of diabetic foot amputations.

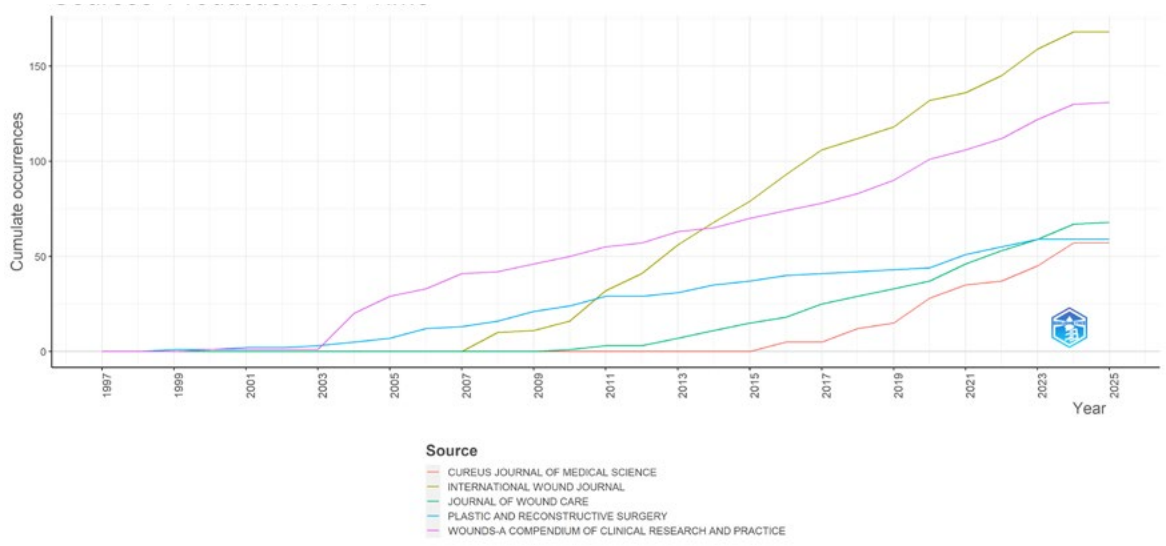

Figure 3 – Top Sources' Production over Time

Table 2 Average Citations per Article, Number of Articles, Total Citations per Year, and Citable Years (1997-2025)

Year	Mean Total Citations per Article	Number of Articles	Mean Total Citations per Year	Citable Years
1997	1608	2	55.45	29
1998	210	1	7.50	28
1999	83	3	3.07	27
2000	70	6	2.69	26
2001	75.27	11	3.01	25
2002	84.45	11	3.52	24
2003	65.06	18	2.83	23
2004	59.67	39	2.71	22
2005	55.16	43	2.63	21
2006	55.16	45	2.76	20
2007	32.11	56	1.69	19
2008	40.17	71	2.23	18
2009	34.89	66	2.05	17
2010	27.33	73	1.71	16
2011	24.36	108	1.62	15
2012	27.98	82	2.00	14
2013	22.17	107	1.71	13
2014	20.27	112	1.69	12
2015	15.32	117	1.39	11
2016	13.56	122	1.36	10
2017	16.17	127	1.80	9
2018	12.35	143	1.54	8
2019	11.94	132	1.71	7
2020	8.64	166	1.44	6
2021	6.89	194	1.38	5
2022	3.24	162	0.81	4
2023	1.94	157	0.65	3
2024	0.5	169	0.25	2
2025	0.24	41	0.24	1

Table 2 summarizes the average number of citations per article, the total number of articles, and the total annual number of citations. According to the data, whilst the average number of citations per article was quite high in 1997, this figure had fallen to 0.24 by 2025; similarly, the annual average citation rate, which stood at 55.45 in 1997, had dropped to 1.44 by 2020.

Publication distribution of journals

The International Wound Journal is the most prolific journal in this field, with 168 articles, 28.2% more than the second-ranked journal. "Wounds: A Compendium of Clinical Research and Practice" journal ranks second with 131 articles. The Journal of Wound Care ranks third with 68 publications, and Plastic and Reconstructive Surgery, the leading journal in plastic surgery, ranks fourth with 59 articles. The distribution of publications in the field is concentrated in certain journals, with open access journals like Cureus Journal of Medical Science and Plastic and Reconstructive Surgery-Global Open ranking in the top 10. Specialty journals like Annals of Plastic Surgery and Wound Repair and Regeneration also hold significant importance in the list.

Figure 3 shows the cumulative publication growth and scientific output trends of the five leading journals in NPWT between 1997 and 2025.

The distribution of publications in the field is concentrated in certain journals, with open access journals like Cureus Journal of Medical Science and Plastic and Reconstructive Surgery-

Table 3 Top Journals' Local Impact

Journal	Category quartile	h-index	g-index	m-index	Total Citations	Number of publications	Publication year threshold
PLASTIC AND RECONSTRUCTIVE SURGERY	Q1	30	59	1,111	4101	59	1999
INTERNATIONAL WOUND JOURNAL	Q1	28	44	1,556	2944	168	2008
ANNALS OF PLASTIC SURGERY	Q3	25	49	0,862	5295	49	1997
WOUND REPAIR AND REGENERATION	Q1	20	41	0,909	1737	46	2004
JOURNAL OF ORTHOPAEDIC TRAUMA	Q3	17	33	0,739	1448	33	2003
ANNALS OF THORACIC SURGERY	Q1	16	20	0,593	1241	20	1999
JOURNAL OF PLASTIC RECONSTRUCTIVE AND AESTHETIC SURGERY	Q2	15	28	0,789	787	28	2007
WOUNDS-A COMPENDIUM OF CLINICAL RESEARCH AND PRACTICE	Q3	14	27	0,538	1110	131	2000
ANNALS OF SURGERY	Q1	13	13	0,591	913	13	2004
JOURNAL OF VASCULAR SURGERY	Q1	13	17	0,619	743	17	2005
OSTOMY WOUND MANAGEMENT	Q2	13	20	0,619	468	30	2005
INTERACTIVE CARDIOVASCULAR AND THORACIC SURGERY	Q2	12	19	0,75	397	20	2010
JOURNAL OF WOUND CARE	Q3	12	18	0,75	479	68	2010
AMERICAN JOURNAL OF SURGERY	Q1	11	14	0,44	682	14	2001
WORLD JOURNAL OF SURGERY	Q2	11	15	0,579	571	15	2007
EUROPEAN JOURNAL OF CARDIOTHORACIC SURGERY	Q2	10	10	0,385	452	10	2000

Table 4 Comparative Analysis of Scientific Productivity and Citation Impact by Country

Country	Total Publications	Total Citations	Avarage Citations/ Article	Category*
United States	1660	21068	30.80	A
Germany	395	4047	24.50	A
Sweden	255	2626	32.80	A
United Kingdom	336	238	19.80	B
China	668	2113	8.20	C
Italy	256	1368	13.80	B
Netherlands	144	1261	34.10	A
Canada	111	1025	26.30	A
Switzerland	84	851	23.00	B
Australia	233	783	14.20	B
Japan	298	722	5.40	C
South Korea	109	698	12.00	B
Turkey	153	671	8.80	C
Belgium	51	509	21.20	B
France	111	500	14.30	B
South Africa	-	479	39.90	-
India	137	417	6.10	C
Ireland	38	409	21.50	B
Austria	49	337	17.70	B
Singapore	51	305	12.70	B
Brazil	54	277	15.40	B
Poland	72	231	9.20	C
Spain	74	212	9.60	C
Denmark	79	190	8.60	C
Greece	-	183	15.20	-

**Performance Categories:*
A: High publication volume with strong citation impact
B: Moderate publication volume with good citation impact
C: High publication volume but low citation impact

Global Open ranking in the top 10. Specialty journals like Annals of Plastic Surgery and Wound Repair and Regeneration also hold significant importance in the list.

Plastic and Reconstructive Surgery demonstrates early dominance with consistent publications since 1999, reaching its apparent saturation point (n=59) by 2023. In contrast, Wounds-A Compendium of Clinical Research and Practice exhibited exponential growth between 2004-2008 (1 to 42 publications), later stabilizing with marginal annual increases. The International Wound Journal shows the steepest trajectory, with 158 publications added since its 2008 inception (10 publications) to 2024 (168 publications), reflecting a compound annual growth rate of 18.2%. Notably, the Journal of Wound Care and Cureus Journal of Medical Science represent more recent contributors, with 88% and 100% of their respective outputs (68 and 57 publications) occurring post-2015. The longitudinal bibliometric analysis of five leading wound care journals (1997-2025) reveals distinct evolutionary phases: an initial incubation period (1997-2007) characterized by minimal output (dominated solely by Plastic and Reconstructive Surgery), followed by an exponential growth phase (2008-2019) marked by the emergence of specialty journals like International Wound Journal.

The comprehensive measurements on featured journals List of featured journals in the field are shown in Table 3. The data includes five key metrics: h-index, g-index, m-index, total citations, and number of publications. Plastic and Reconstructive Surgery (Q1) has the highest h-index (30) with 4101 citations and 59 publications, indicating its leading position. International Wound Journal (Q1) has the highest annual citation performance with its m-index value (1.556) and highest productivity with 168 publications. Despite having the highest total number of citations, Annals of Plastic Surgery (Q3) remains weak in terms of annual impact due to its low m-index value (0.862). Wounds-A Compendium of Clinical Research and Practice (Q3) shows high productivity with 131 publications but has low h-index and m-index values, indicating limited citation activity. Table 3 shows that Q1 journals generally have higher number of citations, but there is no linear relationship between publication number and citation performance. Newer journals like International Wound Journal show better annual performance, reflecting the dynamic changes in this field.

Top countries contributing to NPWT research and international collabrations

Table 4 presents a comparative analysis of scientific productivity and number of citations by country, based on all

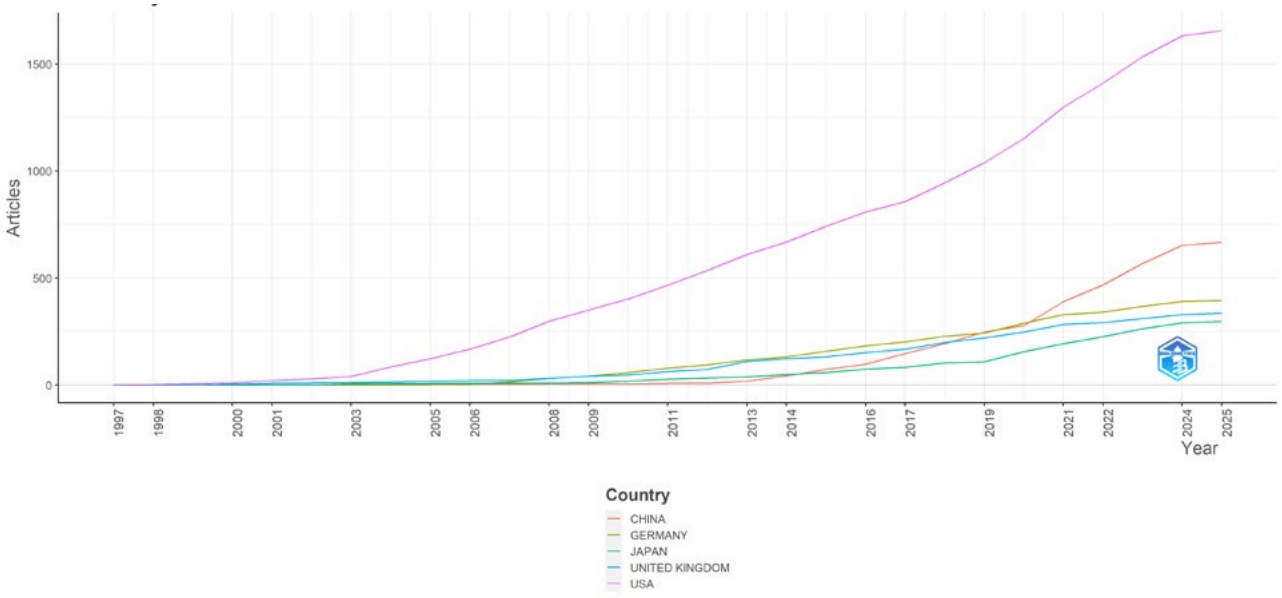

Figure 4 – Top published Countries' Production over Time

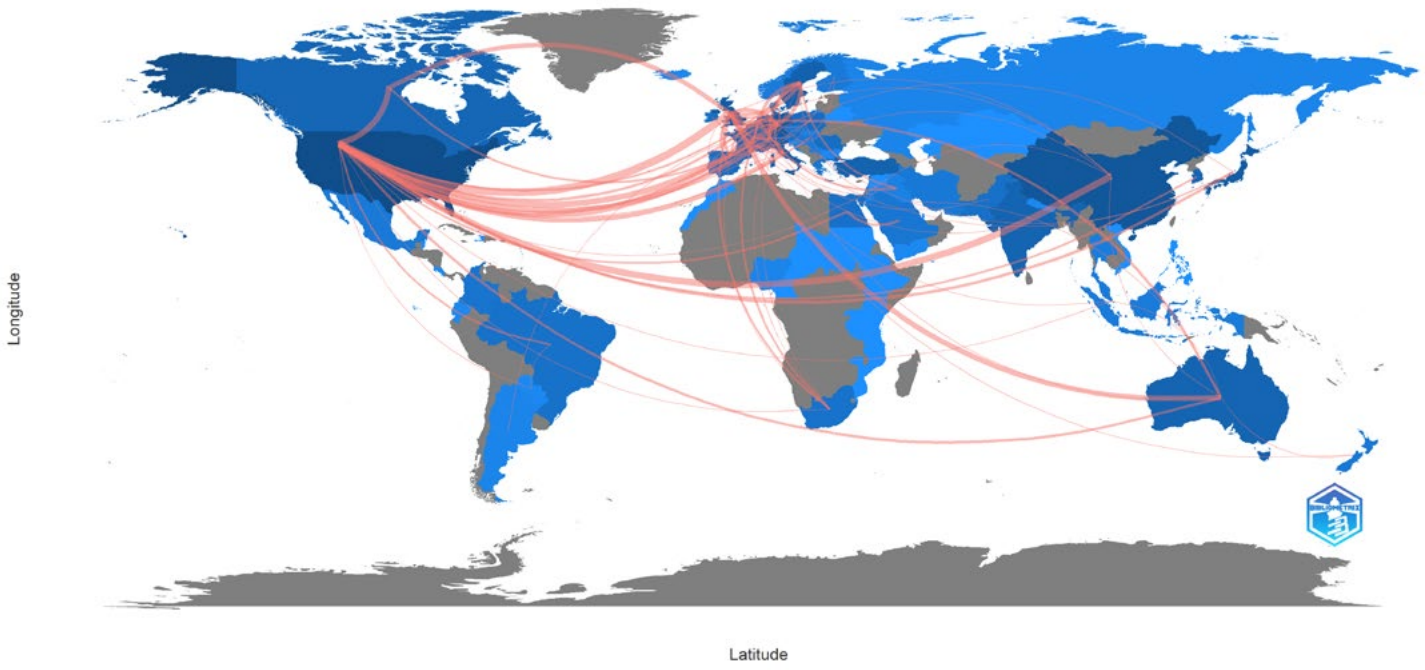

Figure 5 – Countries' Collaboration World Map

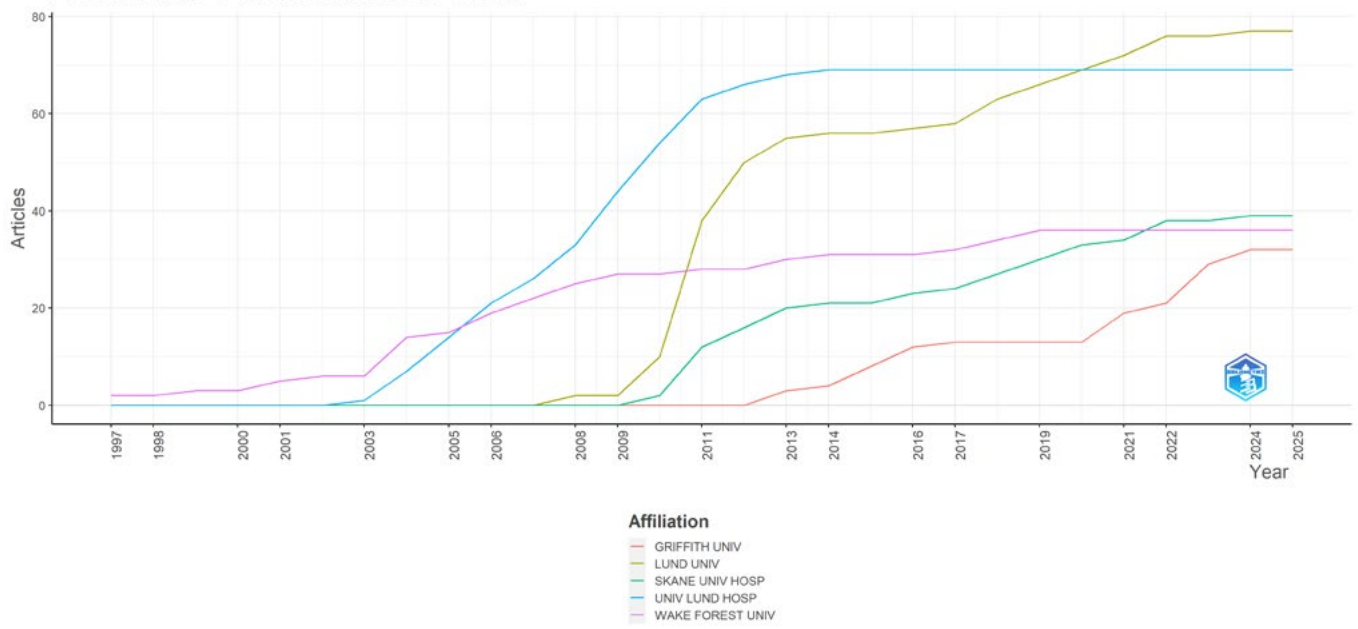

Figure 6 – Affiliations' Production over Time

authors. The USA leads globally in both total of publications (n=1,660) and citations (n=21,068), with a strong citation rate of 30.80 per article. Germany (395 publications, 24.50 average citations), Sweden (255 publications, 32.80 average citations), and the Netherlands (144 publications, 34.10 average citations) are among the high-performing European countries. The United Kingdom, with 336 articles, exhibits a moderate influence (19.80 average citations). In contrast, China exhibits a notable quantity-quality gap, ranking second in total publications (n=668), but with a low number of citations (8.20 average citations). With 153 publications and 671 total citations (8.8 average citations), Turkey maintains to hold a middle-of-the-road position, while more focused, high-impact research helps smaller but more productive producers like Switzerland (23.00 average citations) and Belgium (21.20 average citations). The remarkable number of citationss of the Netherlands (34.1%) and South Africa (39.90 average citations) are especially noteworthy, indicating focused expertise in particular research fields.

The analysis of the number of publications by country is shown in Figure 4. The United States recorded significant growth, rising from two publications in 1997 to a total of 1,656 in 2025, and has been the country with the highest annual output every year. In the United Kingdom, an increase in the number of publications was observed after 2010. Japan's publication count remained at a low level until 2003, then peaked between 2020 and 2023. Germany's first publication activities began in 2004, and an increase in the number of publications was observed in subsequent years. No publications were released from China until 2005, but there has been an exponential increase over the past decade.

Figure 5 shows the frequency of international links among the countries with the highest publishing volumes. The USA maintains the most intensive relationships with the UK (24), China (18), Canada and Germany (15 each), while the UK

Figure 7a – TreeMap

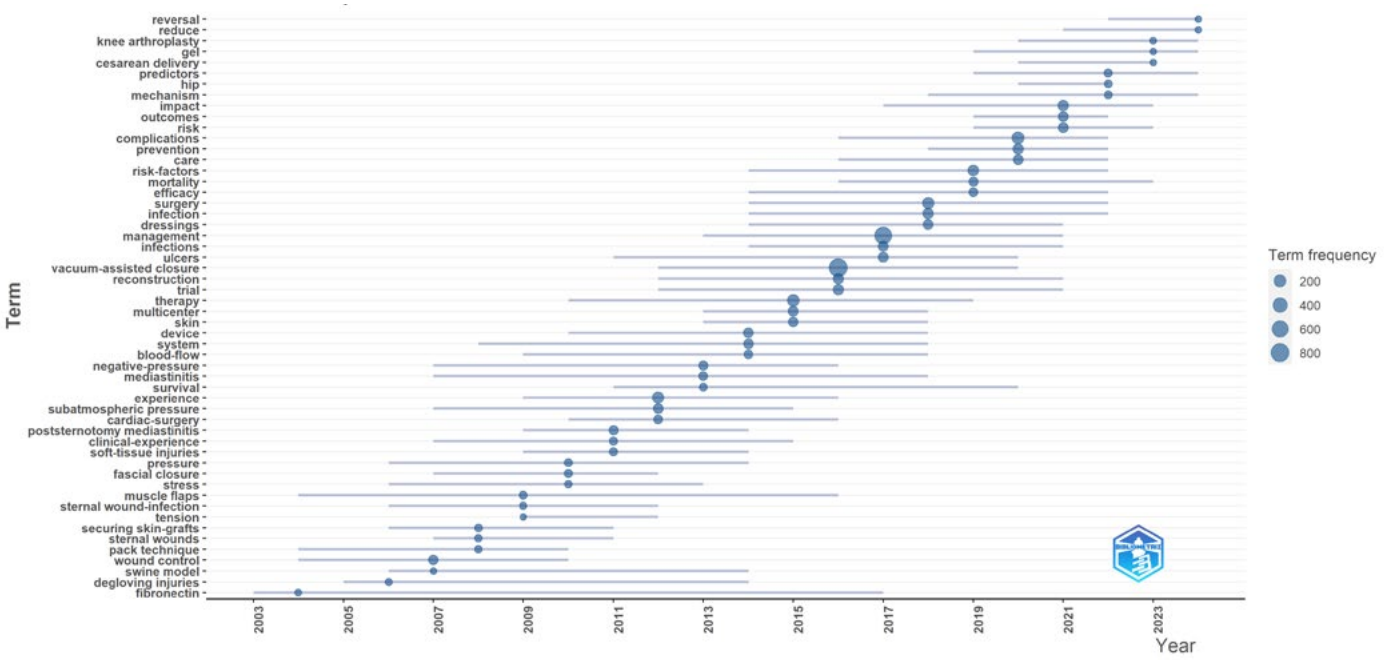

Figure 7b – Trend Topics

interacts most with Australia (11) and Canada (9). Germany has strong ties with Sweden, Switzerland and the United Kingdom (8 each). European countries (France, Italy, the Netherlands, Switzerland) maintain close ties with each other, while Asian and Middle Eastern countries are less frequent. Overall, the USA and Western countries are predominantly in the center.

Most Relevant Affiliations

When examining the cumulative number of publications by institution, certain regional clusters and academic networks stand out. Sweden demonstrates a high research output through the Lund academic network, which primarily consists of Lund University (n = 77 publications), Lund University Hospital (n = 69), and Skåne University Hospital (n = 39). While the bibliometric tool used in this study records these institutions as separate units, it does not allow for manual corrections. Additionally, the United States has made significant publication contributions through Wake Forest University (n = 36), Harvard University (n = 24), and the Mayo Clinic (n = 18). Other major contributing institutions are, in order: from China, Wuhan University (n = 30) and Shanghai Jiao Tong University (n = 24); from Germany, Hannover Medical School (n = 22) and the University of Erlangen-Nuremberg (n = 21); from the United Kingdom: the University of Oxford (n = 23) and the University of Warwick (n = 18); from Australia: Griffith University (n = 22) and the University of Queensland (n = 19); from Brazil: the University of São Paulo (n = 9); and from Canada: the University of Toronto (n = 18). Although automated software analyses present institutional rankings separately based on name variations in metadata, this assessment has identified institutions affiliated with Lund, along with Wake Forest University—recognized as a pioneer of modern NPWT—as the most productive institutions.

Figure 6 shows the distribution of annual publication numbers for the most productive institutions in the field of NPWT over the years. The data indicates that Wake Forest

Table 5 Keyword analysis

Words	Occurrences
vacuum-assisted closure	828
management	643
complications	210
surgery	187
therapy	178
experience	154
risk-factors	119
infection	113
prevention	111
impact	109
reconstruction	109
trial	107
device	95
infections	87
dressings	85
multicenter	83
ulcers	83
system	80
closure	76
subatmospheric pressure	75
outcomes	74
care	71
risk	69
skin	66
trauma	64
wound control	64
pressure wound therapy	55
negative-pressure	54
surgical site infection	54
metaanalysis	53
poststernotomy mediastinitis	52
open abdomen	47
mortality	46
mediastinitis	44
randomized controlled-trial	43
fractures	41
cardiac-surgery	39
efficacy	39
blood-flow	38
mechanisms	38
skin-grafts	38
repair	37
cost	36
diabetic foot ulcers	35
flap	35
state	35
wound therapy	35
instillation	34
amputation	33
laparotomy	32

University, based in the USA has maintained its leadership in the field with a steady increase, while Swedish institutions such as Lund University and its affiliated hospitals, as well as Skåne University Hospital, have made a significant surge, particularly after 2010. Additionally, there has been a rapid rise in the number of publications from Griffith University in Australia in recent years.

Keyword analysis and trend topics

Figure 7a and Table 5 summarize the keyword analysis data, while Figure 7b summarizes the trend topic analysis. The term “vacuum-assisted closure,” which appeared 828 times, is the most frequently used word. Terms such as “therapy” (n = 178), “pressure wound therapy” (n = 55), “negative pressure” (n = 54), and “wound therapy” (n = 35) were also used very frequently. These synonymous or closely related technical terms were not manually merged due to the software’s design, which preserves the repeatability of the raw metadata.

Following these keywords, the term “management” (n = 643) stands out, highlighting the field’s emphasis on developing systematic approaches, particularly for patient care. The prominence of the terms “complications” (n = 210) and “infection” (n = 113) underscores the persistent challenges clinicians face in postoperative care and wound healing. Related terms such as “surgical site infection” (n = 54) and “mediastinitis” (n = 44) reveal the scope of their application in this context. The surgical dimension is strongly represented through multiple keywords, including "surgery" (187), "reconstruction" (109), "closure" (76), and "flap" (35), demonstrating the interdisciplinary nature of wound management that bridges general surgery, plastic surgery, and specialized interventions. Therapeutic approaches constitute another major theme, with "therapy" (178) serving as an umbrella term complemented by more specific modalities like "pressure wound therapy" (55), "negative-pressure" (54), and "wound therapy" (35). The technical aspects of treatment are further emphasized by terms such as "device" (95), "system" (80), and "dressings" (85), reflecting the continuous innovation in wound care technologies. Research methodology emerges as a significant focus area, evidenced by the frequent appearance of "trial" (107), "randomized controlled-trial" (43), and "metaanalysis" (53). These are complemented by "multicenter" (83) and "experience" (154), suggesting a field that values both rigorous evidence-based approaches and collective clinical expertise. Outcome measurement is prominently represented through "outcomes" (74), "efficacy" (39), and "mortality" (46), indicating the growing emphasis on result-oriented care. Specialized wound types receive considerable attention, particularly "ulcers" (83) with its subtype "diabetic foot ulcers" (35), as well as "open abdomen" (47) and "poststernotomy mediastinitis" (52). The anatomical focus extends to "skin" (66), "muscle flaps" (20), and "skin-grafts" (38), demonstrating the multifaceted approach to tissue repair. Emerging trends in the field are visible through terms like "risk-factors" (119), "prevention" (111), and "impact" (109), reflecting a paradigm shift toward proactive care and holistic assessment of treatment effects. The inclusion of "cost" (36) and "care" (71) suggests growing recognition of healthcare economics and patient-centered approaches in wound management (Table 5).

The most frequently used keywords in the articles include "vacuum-assisted closure" (828 occurrences), followed by "management" (643), "complications" (210), "surgery" (187), and "therapy" (178). Other commonly appearing terms are "experience" (154), "risk factors" (119), "infection" (113), "prevention" (111), "impact" (109), "reconstruction" (109), and "trial" (107). Additional notable keywords consist of "device" (95), "infections" (87), "dressings" (85), "multicenter" (83), "ulcers" (83), "system" (80), "closure" (76), "subatmospheric pressure" (75), "outcomes" (74), "care" (71), "risk" (69), "skin" (66), and "trauma" (64) (Figure 7a).

The trend analysis of keywords (Figure 7b) reveals the evolution of research focus over time. Early terms like "fibronectin" (2003–2017, peak 2004) and "degloving injuries" (2005–2014, peak 2006) were gradually replaced by broader concepts such as "wound control" (2004–2010, peak 2007) and

"muscle flaps" (2004–2016, peak 2009). Between 2009–2014, clinical conditions like "poststernotomy mediastinitis" (peak 2011) and techniques like "subatmospheric pressure" (peak 2012) gained prominence. The period 2012–2016 saw dominant keywords like "vacuum-assisted closure" (peak 2016), "therapy" (peak 2015), and "negative-pressure" (peak 2013), reflecting advancements in wound care technology. From 2016 onward, research shifted toward "management" (peak 2017), "surgery" (peak 2018), "complications" (peak 2020), and outcome-focused terms like "risk factors" (peak 2019) and "mortality" (peak 2019). Recent trends (2020–2024) highlight emerging topics such as "predictors", "hip", "knee arthroplasty", and "cesarean delivery", alongside methodological terms like "mechanism" and "gel", indicating a diversification into specialized surgical and mechanistic studies. Notably, "vacuum-assisted closure" remained highly cited until 2020, while "reconstruction" and "trial" persisted through 2021, underscoring sustained interest in evidence-based surgical solutions.

Discussion

NPWT is a widely recognized method for managing chronic wounds, but a comprehensive analysis of research trends is lacking. Despite similar studies on chronic wounds [17-20], no study specifically focusing on NPWT has been found. This gap highlights the need for a systematic evaluation of global research output, collaboration patterns, and emerging trends in NPWT to guide future scientific studies. A bibliometric analysis could provide insights into the most productive countries, leading institutions, key authors, and international collaborations shaping the field. It could identify research disparities, track temporal trends, and foster stronger global partnerships. Addressing this gap would consolidate existing knowledge and direct future research towards understudied areas [17-19,21]. Based on the bibliometric analysis conducted in this study between 1997 and 2025, we can make the following observations: (i) There has been a steady increase in the number of NPWT studies and this topic has attracted attention with close cooperation among various countries. (ii) Some scholars and journals have focused on NPWT research and achieved significant results and impact, but their impact is not fully consistent with the total number of articles published. Research on surgical procedures has evolved over time, with early focus on biomaterials and injuries, middle periods on clinical conditions and technological innovations, and near-term trends focusing on management, complications, and outcome analysis. Current trends (2020-2024) see a diversified focus on specialized surgical fields (“knee arthroplasty”, “cesarean delivery”), mechanistic studies, and intervention optimization, with evidence-based surgical solutions such as vacuum-assisted closure and reconstruction. This demonstrates a continued interest in NPWT studies.

Research topic that has received attention from numerous scholars and journals. These scholars and journals cover a wide range of specialties. For example, the most publications were in the plastic surgery specialty, and the others were in the wound and anesthesia specialties respectively. The bibliometric analysis of NPWT research publication trends shows a dynamic evolution, with the International Wound Journal dominating the field with 168 articles and an 18.2% compound annual growth rate since 2008. The data shows three phases: early (1997-2007) dominance by Plastic and Reconstructive Surgery, exponential growth of specialty journals from 2008-2019, and a maturation phase (2020-2025) with the stabilization of traditional journals and the explosive growth of open-access platforms like Cureus. Although Plastic and Reconstructive Surgery still leads in terms of h-index and total citations, newer Q1 journals, such as the International Wound Journal, appear to have better annual citation performance, indicating a change in spread of research. The rise of open-access publishing and the predominance of wound care in specialized journals over general surgical journals illustrate the increasing maturity and specialisation of the field, with its own classical and digital habits, influenced by recent advances in the technology of wound care and shifts in publishing trends.

The landscape of NPWT research shows specific trends in relation to scientific activity and cooperation. More than half (n = 13 articles) originated from the United States, representing the United States’ established research infrastructure and national presence, compared with other nations based on its publication volume (1,660 articles) and number of citations (21,068 total citations) in the field. European countries including Germany, Sweden, and the Netherlands produced fewer papers, but have relatively high average citation rates (24.50–34.10 per article). In contrast, while China ranks second in terms of output (668 articles), its citation count is lower (an average of 8.20).

Collaboration networks further illustrate the dynamics of NPWT research, with the USA maintaining the strongest ties, particularly with the U.K., China, and Germany. European countries exhibit dense intraregional partnerships, while Asian and Middle Eastern nations remain relatively isolated. Leading institutions such as Sweden’s Lund University and the USA’s Wake Forest University dominate production, with Sweden emerging as a hub post-2010. Wake Forest, a pioneer in NPWT, has sustained steady output, whereas Australian institutions like Griffith University show rapid recent growth, signaling geographic diversification in research. These trends highlight the critical role of international collaboration in driving innovation, with Western nations remaining central to global networks while newer players gradually expand their influence. The rise in the number of publications by institutions in Australia and China points to a changing landscape.

Among the latest innovations in NPWT is application and waiting time (NPWTi-d), which enhances wound healing through debridement that does not require irrigation and excision. Clinical evidence shows that NPWTi-d reduces hospital stay and healing time, especially when using 'passage' perforated mesh open-cell foam (ROCF-CC) to facilitate the removal of necrotic tissue and infected material [9]. Our keyword analysis confirms that applications requiring specialized expertise are increasingly coming to the fore, with “instillation” emerging as a significant keyword (n = 34). These data reflect the growing academic interest in NPWTi-d, highlighting this topic as a new area of focus in the literature.

Limitations

To the best of our knowledge, there has been no bibliometric study about NPWT research-associated literature investigating WoS database globally. Our research, analysis of NPWT research trends has several limitations. It contained only the studies that were indexed in the Web of Science database, which may have been excluded some studies on different databases or published with languages other than English. When evaluating the findings of this study, certain methodological limitations should be taken into account: a title-only search strategy, a lack of affiliation disambiguation, and limited keyword harmonization. We made this choice to ensure direct access to the core NPWT literature. Another limitation of the study concerns the use of

the terms “citable years” or “citability length.” This concept does not represent an independent metric. It reflects the age of the publication or the defined citation window. To avoid any misunderstanding, readers are advised to interpret this concept as an indicator of the time elapsed since the publication date.

Citation metrics may highlight articles published in the past and, as a result, underestimate the contribution of more recently published high-quality research. It should be noted that the decline in the average number of citations observed in recent publications stems from a shorter citation accumulation period rather than a decline in the research’s impact or quality; therefore, readers should evaluate the article from this perspective. This study focuses solely on numerical data related to research productivity; no content analysis was conducted. Differences in institutional names and changes in the institutions to which authors are affiliated may reduce the reliability of institutional productivity rankings. Based on keyword and trending topic analyses, synonyms such as ‘NPWT,’ 'VAC,‘ and 'vacuum-assisted closure’ were treated as separate nodes to preserve the raw repeatability generated by the software; it is acknowledged that this approach leads to a certain degree of thematic fragmentation in semantic mapping. However, the bibliometric method used does not allow for manual revision. Scientific literature is dynamic, and since indexing may be delayed, the most recent reports have not been included. Future studies could be made more efficient by incorporating alternative metrics and utilizing multiple databases.

Conclusion

In our study, we examined only the core NPWT literature with high specificity; that is, we created a dataset by conducting a “title-only” search. According to our findings, the number of publications on NPWT has increased globally, and research focuses have diversified. The United States and Sweden are the countries with the highest share of publications; institutions based in the USA and Sweden have emerged as the most productive and influential centers. According to our data, the current research focus has shifted from technical developments to clinical outcome reports. Additionally, the data indicates that evidence gaps still exist in areas such as local protocols and cost-effectiveness, and that academic interest in instillation therapy is increasing.

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Challenges in Healthcare Access and Social Inclusion of Children with Autism Spectrum Disorder: Evidence from Kazakhstan and International Studies

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ABSTRACT

Autism spectrum disorder (ASD) is a neurodevelopmental condition with a steadily increasing global prevalence. Despite advances in early diagnosis and intervention, children with ASD and their families continue to face significant challenges related to healthcare access, social inclusion, and stigma. These challenges are particularly pronounced in low- and middle-income countries but remain relevant worldwide. This narrative literature review synthesizes evidence on social, medical, and structural barriers affecting children with ASD, with a focus on healthcare access, comorbidity-related medical vulnerability, and public stigma in Kazakhstan and internationally. Databases including PubMed, Scopus, Web of Science, Google Scholar, eLIBRARY.ru, and KazNEB were searched for Russian- and English-language publications. Access to ASD-specific services – such as early intervention, behavioral therapy, and speech and neuropsychological support – remains limited due to shortages of trained specialists, long waiting lists, and high out-of-pocket costs.

Children with ASD frequently have comorbid chronic conditions, increasing their need for general healthcare, yet health systems are often poorly adapted to their sensory, communicative, and behavioral needs. Persistent stigma across social, educational, and medical settings further restricts access to care, while socioeconomic inequalities exacerbate disparities. Overall, children with ASD, particularly those with comorbidities, constitute a highly medically vulnerable population. Addressing these challenges requires integrated policy approaches, workforce development, expansion of publicly funded services, and targeted efforts to improve public awareness and reduce stigma.

Keywords: autism, stigma, early intervention, health service access

Introduction

Autism spectrum disorders (ASD) are a group of neurodevelopmental conditions characterized by impairments in social communication, restricted interests, and repetitive behaviors [1]. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), ASD encompasses diagnoses of autism, Asperger syndrome, and other pervasive developmental disorders [2, 3].

ASD is not associated with a child's social background, the level of education of their parents, or their mentality and culture. However, the diagnosis of ASD in children, as well as the social integration of such children, are highly dependent on the healthcare and education systems in a particular country [4, 5].

According to the World Health Organization, the prevalence of ASD worldwide is approximately 1 in 100 children, but in some countries, such as the United States, this figure reaches 1 in 36. According to the Centers

for Disease Control and Prevention, approximately 1 in 31 8-year-old children (≈3.2%) in the United States was identified as having ASD in 2022 [6].

An analysis of studies conducted between 2000 and 2020 included multiple studies from Europe, North America, and other regions: for Europe, the median prevalence of ASD is ≈59 per 10,000 (i.e., ~0.59%), with a wide range from 8 to 420 per 10,000 [7].

According to a recent Kazakhstani study, "Epidemiological Trends in Autism and Other Neurodevelopmental Disorders in Kazakhstan (2016–2022): A Regional and National Perspective," the registered prevalence of childhood autism (ASD) in Kazakhstan increased from ~14 per 100,000 children in 2016 to ~59 per 100,000 in 2022 [8].

According to a global review conducted in 2022, 0.77% of children globally are diagnosed with autism [9].

Analyzing the available literature, it can be concluded that children with autism spectrum disorders and their families face multiple challenges both at the societal level and when seeking medical care. These are problems like:

1. Social Isolation and Stigma

Children with ASD are particularly vulnerable to isolation and stigma. Society often demonstrates low tolerance for individuals with developmental disabilities. Parents of children with ASD report instances of discrimination and denial of admission to kindergartens and schools. Children and adolescents diagnosed with ASD also experience difficulties that limit their interactions with society, peers, family members, and relatives. This behavior often leads to social isolation and, consequently, alienation. In the case of ASD, stigma is primarily expressed as misunderstanding, fear, rejection of "other" children, and blame directed at their parents [10]. Research shows that children with ASD often exhibit problems with nonverbal communication, understanding social cues, emotional reciprocity, and behavioral flexibility, which makes it difficult to establish friendships with peers [11].

Furthermore, sensory differences (sensitivity to noise, light, and touch) make visiting public places a difficult challenge for these children, which naturally limits their social participation.

Most children with ASD do not display any obvious outward signs of disability, and others sometimes overlook the problem and perceive the child's behavior as "wrong," "strange," or "abnormal." Parents also face so-called "affiliate stigma"—when a child with ASD is perceived as a "reflection" of the family, and in this case, the parents themselves become the target of judgment and alienation [12,13].

2. Limited access to medical and rehabilitation services

In the Republic of Kazakhstan and Central Asian countries, there is a shortage of specialists—child psychiatrists, neuropsychologists, speech therapists, and behavioral therapists (ABA specialists). There are also no uniform protocols for diagnosing and treating such patients. In 2023, the number of psychiatrists per 10,000 population was 0.4, and the number of psychotherapists was 0.01. The incidence of children with ASD increased from 8.6 (2015) to 161.3 per 100,000 children aged 0-17 years (2023), and the primary incidence increased from 4.3 to 33.6 per 100,000 (2015-2022). Research has shown that 81.4% of children receive interventions, but only 4.3% of them have access to ABA therapy. The average age of diagnosis is 2.5 years, and 79.8% of parents have low awareness – the main barriers are the lack of specialists, the high cost of services and, again, stigma [14,15]. In recent years, Kazakhstan has increasingly focused on the diagnosis, care, and support of individuals with autism spectrum disorders (ASD), both within the scientific community and within the healthcare system. However, data from Kazakhstan remains limited. Research reveals the presence of sociocultural barriers: the public perception of autism is often accompanied by low awareness, persistent stereotypes, and stigma, which hinders early access to special and medical care, as well as the full integration of children with ASD into the educational and social environment [15,39].

3. Financial and emotional difficulties for families

A significant portion of services for children with ASD are provided on a fee-based basis. Parents often experience emotional burnout and a lack of psychological support. Due to long wait times for assistance from public healthcare, many parents of "special" children are forced to resort to paid services. For example, parents pay for ABA therapy, neurologist visits, remedial training, and so on [16]. Currently, the parents themselves experience emotional distress and a lack of psychological support [17].

For example, another study, "Disparities in the quality of and access to services in children with autism spectrum disorders: a structural equation modeling," conducted in Iran, found that the quality of and access to services for children with ASD significantly depend on the family's socioeconomic status. Children from low-status families are more likely to experience limited access to medical services, and in these cases, services are often partially covered or not covered at all by insurance [4].

4. Problems of Inclusion in Education

Despite the implementation of inclusive programs, only a small proportion of schools and kindergartens are equipped with the personnel and resources to work with children with ASD [18]. A review of international and domestic inclusive education practices emphasizes the need for specialized teacher training, the development of an "inclusive culture" in schools and society, and support from psychological and pedagogical services. Not all schools and kindergartens in Kazakhstan and globally can provide such qualifications—this is an obstacle to high-quality support for children with special educational needs, including those with ASD. [19,20] Children with ASD primarily require an individualized approach: program adaptation, specialized methods, support, communication, and socialization. In the absence of qualified teachers, insufficient training, and inadequate resources, inclusion often becomes not support, but stress for a child with ASD and their parents [21].

Given the above, the purpose of this literature review is to analyze current research on the difficulties children with autism spectrum disorders face when interacting with society and receiving healthcare services in Kazakhstan and worldwide.

Methods

This study conducted a narrative review of the literature on social adaptation, inclusion, and access to healthcare for children with ASD.

A literature search was conducted in the scientific databases PubMed, Scopus, Web of Science, Google Scholar, eLIBRARY.ru, and the Kazakhstan National Electronic Library (KazNEB). Publications published between 2016 and 2026 were included in the review, based on the relevance of modern approaches to the problem and the date of the earliest included article. The search strategy was based on the following keywords and their combinations: "autism spectrum disorder," "children with ASD," "access to healthcare," "social inclusion," "barriers," "Kazakhstan," "stigma," "social isolation," and their English-language equivalents. Based on this search and abstract analysis, 3,159 publications were found in the aforementioned

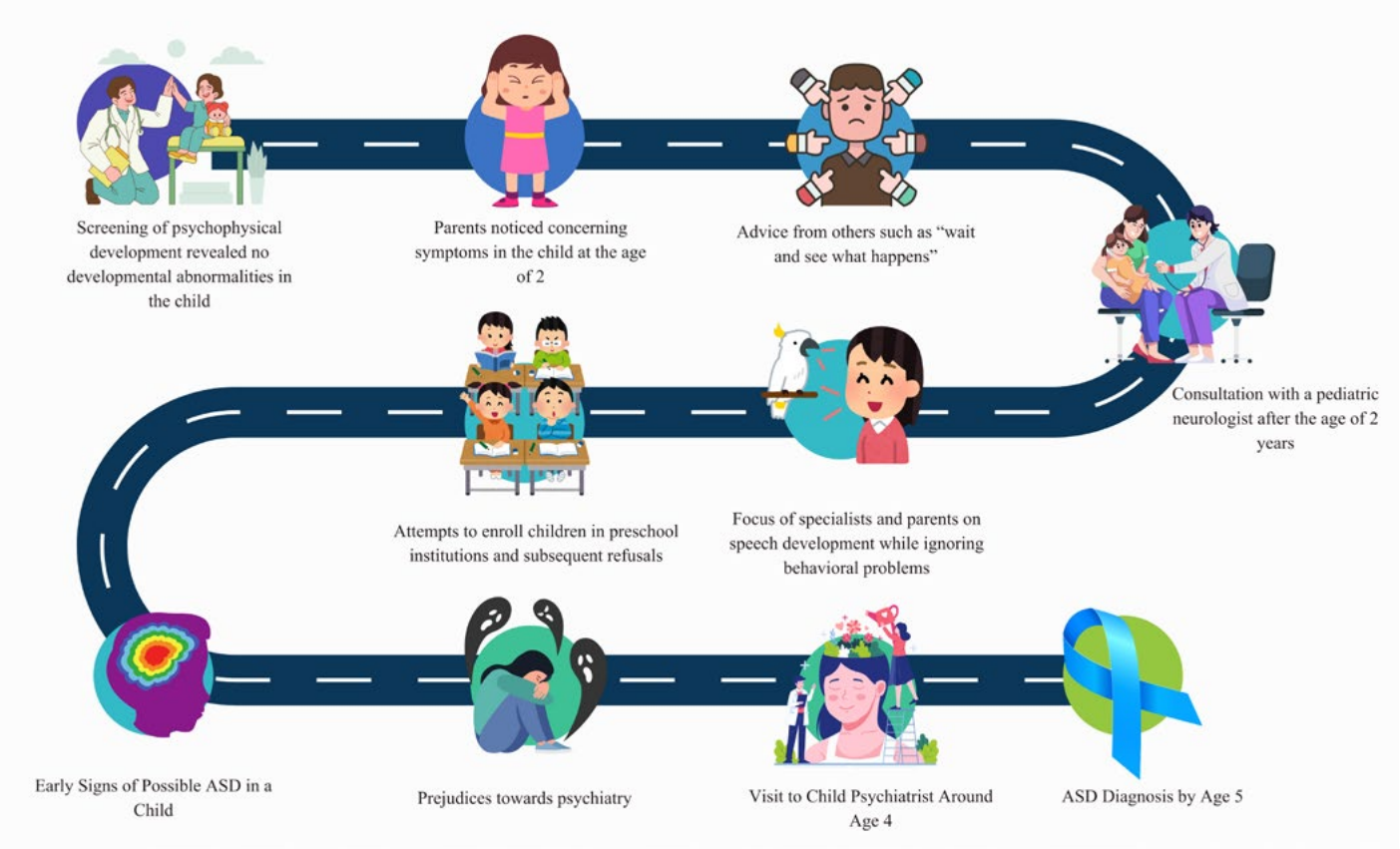

Figure 1 – Family pathways to ASD diagnosis for children in Kazakhstan

scientific search databases. The final review included 56 studies that met the following criteria: studies focused on children with ASD and social adaptation, inclusion, access to health services; cross-sectional, qualitative, review study designs; Russian, English and Kazakh languages of publications. The authors of this review analyzed the abstracts and main conclusions of these publications for compliance with the stated aim, with the subsequent inclusion of relevant publications in the review.

Data synthesis was qualitative and descriptive. Analysis was conducted using a thematic approach, including the identification, grouping, and interpretation of key themes, such as barriers to access to healthcare, manifestations of social stigma, factors of social isolation, and mechanisms for support and inclusion of children with ASD.

Access to ASD-related health service

Access to ASD-Related Healthcare Services. An analysis of existing research shows that limited access to ASD-related services is a widespread and systemic problem observed across all countries, regardless of income level. Early intervention services, including applied behavior analysis (ABA), speech therapy, occupational therapy, and neuropsychological support, remain underavailable compared to demand [22,23,47]. However, the nature and underlying causes of these limitations vary significantly between high-income countries (HICs) and low- and middle-income countries (LMICs). Financial Barriers

In low- and middle-income countries, including Kazakhstan, barriers are primarily structural and financial. Having a disability significantly impacts parents' perceived costs, as children with ASD require a higher level of care. A significant portion of services are provided on a fee-for-service basis, which shifts the financial burden onto families. Empirical evidence shows that parents often have to pay out-of-pocket for ABA therapy, specialist consultations, and other interventions due to limited public services and long waiting times [24, 48–50]. The costs of such services in Central Asia and Eastern Europe can be prohibitive, effectively limiting access to sustainable and intensive interventions. This results in a system in which access to healthcare is highly dependent on socioeconomic status, exacerbating health inequalities from an early age. In contrast, high-income countries tend to have more developed healthcare infrastructure; however, access challenges persist in various forms. Research from Europe shows that even in relatively well-resourced systems, inequalities in access depend on socioeconomic status, regional availability of services, and the presence or absence of coordinated financing mechanisms [25, 27, 51]. Thus, financial barriers are not completely eliminated, but interact with systemic and organizational factors. A key challenge in both low- and middle-income and high-income countries is the shortage of qualified specialists. The availability of child psychiatrists, neuropsychologists, speech therapists, and behavioral therapists remains insufficient to meet the growing demand. In Kazakhstan and other Central Asian countries, this shortage is particularly acute due to limited training opportunities and a small number of specialized centers, leading to delays in both diagnosis and treatment [24]. Notably, similar trends are observed in HICs. Research suggests that additional investment is needed in health services for children with autism, both in terms of the number of specialists and the competencies of those currently working with children with ASD [52,53]. This suggests that workforce shortages represent a global bottleneck, albeit with varying degrees of severity. Delayed Diagnosis.

Delays in diagnosis represent another critical aspect of access. Available evidence suggests that the path from initial parental concern to formal diagnosis is often long and fragmented. For example, research in the Russian Federation highlights systemic issues such as unclear clinical guidelines,

inconsistent diagnostic practices, and reluctance of health care providers to diagnose ASD, which contribute to delayed identification [28]. In low- and middle-income countries, these delays are compounded by low parental awareness and stigma, leading to underrecognition of early symptoms and delayed help-seeking [29]. Cross-country comparisons suggest that delays are determined not only by resource availability but also by cultural, institutional, and professional factors. For example, in Brazil, although parents often notice developmental problems around age two, formal diagnosis may only be made years later due to negative health care experiences and insufficient training of professionals [30].

Similarly, socioeconomic factors play a significant role: higher parental education and income are consistently associated with earlier diagnosis, while socially disadvantaged groups experience delays in disease detection and limited access to services [31]. This highlights the role of social determinants in shaping diagnostic trajectories. Case Study: Kazakhstan

The situation in Kazakhstan illustrates how these factors converge (Figure 1). Despite early parental concern (around 2 years), the average age of diagnosis remains significantly delayed (around 5 years), reflecting systemic inefficiencies in patient referral pathways, specialist availability, and diagnostic capacity [32,33]. Other barriers include limited awareness among both families and healthcare providers, logistical challenges, and dissatisfaction with the quality of services. Importantly, research shows that even healthcare professionals may hold misconceptions about ASD, including its symptoms and diagnostic criteria, further undermining timely and accurate diagnosis [33, 54, 55]. Taken together, these findings suggest that access to ASD-related services is determined by a complex interplay of structural, economic, and sociocultural factors. In low- and middle-income countries, financial constraints and limited infrastructure are prevalent, while in high-income countries, organizational inefficiencies and staff shortages play a significant role. However, across all contexts, delays in diagnosis and inadequate access to early intervention remain persistent challenges. Addressing these challenges requires not only expanding service delivery capacity but also improving care coordination, professional training, and service integration across the health, education, and social sectors.

Access to non-ASD-related health service

ASD present not only with core neurodevelopmental features but also with a substantial burden of comorbid conditions, including somatic, neurological, and psychiatric disorders. This constellation of needs significantly increases their demand for a wide range of health services, from preventive and dental care to emergency and specialized interventions [34]. Importantly, these needs are not merely additive. Comorbid conditions frequently interact, complicating clinical management and requiring coordinated, interdisciplinary care. In practice, however, healthcare systems are often not adequately designed to manage such complexity. Consequently, children with ASD are at increased risk of delayed diagnosis, fragmented care pathways, and insufficient preventive support, which may ultimately contribute to poorer health outcomes [35].

A growing body of evidence consistently demonstrates the high prevalence of comorbidities in this population. For instance, a large case–control study reported significantly higher rates of diverse medical diagnoses across multiple organ systems among children with ASD compared with their neurotypical peers. Similar patterns have been described in retrospective and observational studies, which document a broad spectrum of co-occurring conditions, including neurological, genetic, sensory, and motor disorders, as well as sleep disturbances, gastrointestinal problems, feeding difficulties, obesity, and psychiatric conditions [36, 37]. Taken together, these findings suggest that comorbidity should be regarded as a typical feature of ASD rather than an exception, underscoring the need to move from episodic care toward more continuous and integrated service models.

At the systems level, the capacity to address these complex needs varies considerably across countries. Much of the existing evidence base on ASD-related healthcare originates from high-income settings, where diagnostic pathways tend to be more structured and access to specialized and multidisciplinary services is relatively well established [54,55]. In contrast, in low- and middle-income countries, barriers extend beyond limited resources and include systemic fragmentation. Shortages of trained professionals, restricted access to diagnostic services, and weak continuity of care hinder the implementation of comprehensive, integrated approaches [38,26]. These differences should not be attributed solely to economic disparities. Organizational features—such as the structure of primary care, referral mechanisms, and the integration of health and social services—also play a critical role in shaping both access to and quality of care. Consequently, improving care in these contexts requires not only increased investment but also context-sensitive adaptation of service delivery models [56].

Within this broader context, a substantial portion of the caregiving burden is shifted onto families, making social support systems a key component of the overall response to ASD. Evidence from Kazakhstan illustrates this particularly clearly. A study involving 390 parents of children with ASD identified significant financial and organizational challenges, with more than 70% of respondents reporting insufficient government support, high out-of-pocket costs for treatment and education, and limited access to qualified specialists, especially in rural areas [15]. These findings point to a systemic mismatch between healthcare needs and service provision, with families effectively compensating for structural gaps.

High prevalence of comorbidities among children with ASD not only amplifies the demand for healthcare services but also exposes limitations in how these services are organized and delivered. Addressing these challenges requires a shift toward integrated, interdisciplinary, and context-responsive models of care that can accommodate both the clinical complexity of ASD and the constraints of different healthcare systems.

Public Knowledge and Stigmatization

Stigma against children with autism spectrum disorders (ASD) and their families manifests itself in social, educational, and medical contexts, shaping not only public attitudes but also access to services and long-term outcomes. Importantly, stigma is not an isolated phenomenon but is closely linked to public awareness, cultural interpretations of developmental disabilities, and the structure of healthcare and education systems (Figure 2). Low awareness contributes to misinterpretations of behaviors associated with ASD, which in turn reinforces negative stereotypes and social isolation [39].

Empirical evidence from Kazakhstan demonstrates how limited awareness leads to social distance and emotional reactions. A 2023 study of 410 adults found low willingness to fully accept children with ASD in everyday social settings, regardless of where they lived (urban or rural). When respondents were asked to imagine meeting a child with autism in public,

the most common reactions were fear and curiosity, suggesting a lack of normalization of ASD in society [40]. This pattern is consistent with the results of another national study, which found that only 18.3% of respondents were able to correctly identify key symptoms of ASD and understand appropriate support methods, with the internet serving as the primary source of information [41]. Taken together, these findings suggest that stigma in this context is driven less by overt negative attitudes than by information deficits and uncertainty.

In contrast, data from European countries demonstrate a more nuanced relationship between knowledge and attitudes. For example, in Greece, relatively high levels of knowledge about ASD were associated with moderately positive attitudes [42]. Similarly, in France, although awareness of the term "autism" was nearly universal, deeper understanding remained limited, and some respondents still expressed a preference for social distancing [43]. These data indicate that raising awareness alone does not automatically eliminate stigma; rather, the quality and depth of knowledge, as well as societal perceptions of disability, play a crucial role. Even in countries with generally high levels of awareness, gaps in understanding the causes of ASD and related support mechanisms remain, as shown by recent data from Poland and multi-country European studies [44].

Comparative analyses across countries further highlight the structural and cultural determinants of stigma. A comparative study between the United States and China found that higher levels of knowledge about ASD in the United States were associated with lower levels of stigmatizing attitudes, whereas in China, lower levels of awareness were associated with significantly higher levels of stigma [45]. This suggests a general trend in which knowledge acts as a protective factor against stigma, but only when integrated into supportive social and institutional frameworks.

LMICs, including Central Asia countries, stigma is exacerbated by systemic factors. The legacy of centralized health systems, coupled with limited resources, a shortage of qualified professionals, and limited access to early diagnosis, creates an environment in which ASD remains poorly understood by both professionals and the general population [38]. In such settings, stigma is reinforced not only by cultural beliefs but also by structural invisibility—when children with ASD are underdiagnosed or excluded from formal systems, public awareness and normalization are reduced.

Furthermore, the relationship between stigma and health systems in LMICs is two-way. On the one hand, low awareness and stigma hinder early help-seeking and social integration. On the other hand, weak health systems characterized by limited diagnostic capacity, inadequate workforce training, and competing public health priorities (e.g., a focus on infectious diseases) contribute to persistent low awareness [46]. This creates a self-perpetuating cycle in which stigma, underdiagnosis, and limited-service provision feed off each other.

Thus, differences in stigma between countries should be understood not only as variations in societal attitudes, but also as a reflection of broader healthcare system capabilities, educational efforts, and the sociocultural context. Therefore, combating the stigma of autism spectrum disorders requires comprehensive strategies combining public education, professional training, and systemic improvements in access to diagnosis and treatment, especially in low- and middle-income countries.

Summary

Many countries worldwide face a shortage of specialists in autism diagnosis, late diagnosis, and inadequate access to evidence-based treatments. Economic barriers are particularly pronounced in low- and middle-income countries, including Central Asian states, where a significant portion of services are provided on a fee-for-service basis. Research shows that these children are more likely to suffer from somatic, neurological, genetic, and psychiatric disorders, sleep disorders, gastrointestinal problems, epilepsy, obesity, sensory impairments, and other

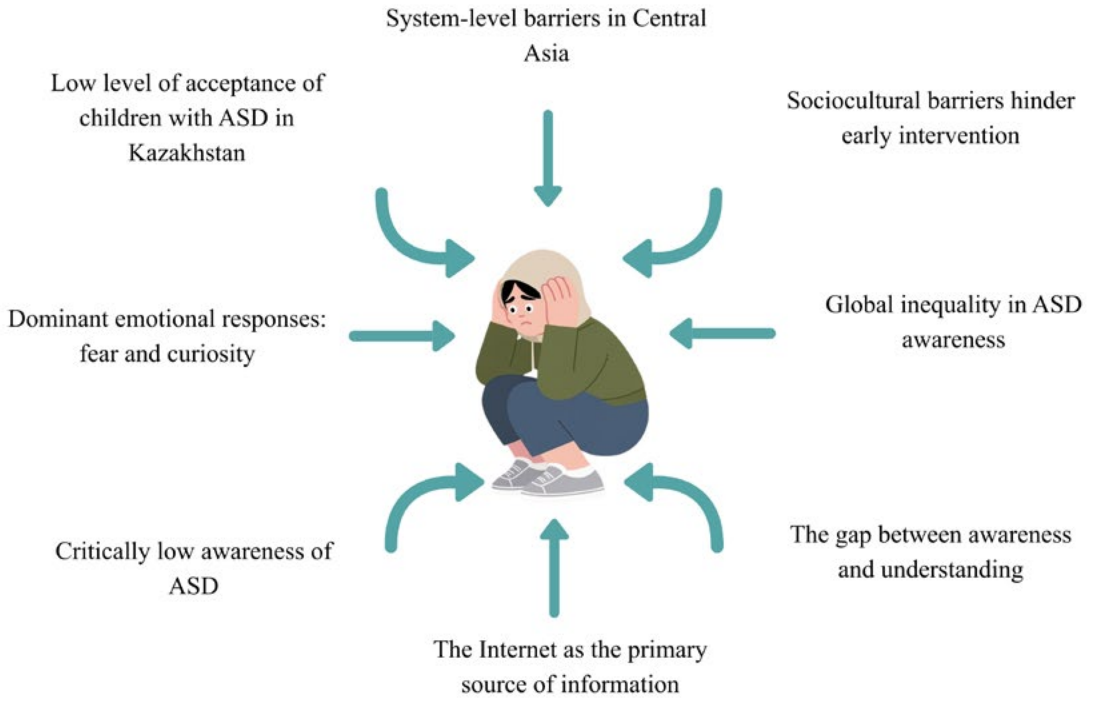

Figure 2 – Key factors limiting the acceptance and social inclusion of children with autism spectrum disorders

conditions. However, healthcare systems are often not adapted to working with children with ASD: doctors are often not trained in communication skills, and facilities are not sensory-sensitive, leading to diagnostic delays, refusals, avoidance, and deteriorating health outcomes. Stigma also remains a key factor hindering access to care.

Even in countries with developed healthcare systems (USA, EU countries), the growth in the number of children with ASD exceeds the rate of specialist training. Rural and remote regions are particularly vulnerable. In Central Asian and Eastern European countries, key barriers include high costs of services, staff shortages, weak interagency coordination, and late diagnosis.

Conclusion

The results of this literature review allow us to draw several important conclusions regarding service access for individuals with autism spectrum disorders. First and foremost, higher awareness of autism is associated with more positive and tolerant attitudes toward individuals with this condition. However, stigma remains a common problem, particularly given the lack of understanding of the characteristics and manifestations of autism. Furthermore, children with ASD, particularly those with comorbid chronic conditions, face increased vulnerability in healthcare due to existing barriers to accessing necessary medical care. These findings highlight the need to further improve support systems for individuals with autism and their families. Raising awareness of autism among the public and healthcare professionals is an important area of focus, as this can contribute to reducing stigma, earlier detection of developmental disorders, and timely assistance. Equally important is the development of human resources by training specialists with the necessary knowledge and skills to work with this patient group. Particular attention should also be paid to strengthening cooperation between healthcare, education, and social protection institutions, since a comprehensive interdepartmental approach allows for more effective meeting both the specific needs of people with ASD and broader needs related to their health and social adaptation.

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Beyond Technical Innovation: Organizational and Economic Evaluation of Minimally Invasive Cardiac Surgery – a Narrative Review and Hospital-Level Framework

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ABSTRACT

Background: Minimally invasive cardiac surgery (MICS) is increasingly recognized as an integrated perioperative care model rather than simply a small-incision surgical technique. Its clinical and economic value depends on patient selection, team experience, recovery pathways, institutional resources, and local cost structures.

Objective: This narrative review summarizes the current evidence on the clinical, organizational, and economic value of MICS, and proposes a practical hospital-level framework for evaluating program performance and maturity.

Methods: A structured narrative search of PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar was conducted between January 2010 and June 2026. The review focused on minimally invasive valve surgery, coronary revascularization, enhanced recovery pathways, programme learning curves, resource utilization, and hospital cost analyses. The conduct and reporting of the review were guided by the Scale for the Assessment of Narrative Review Articles (SANRA). Evidence was synthesized across four domains: clinical safety and effectiveness, programme maturity and learning curves, organizational performance, and economic performance. Since the review was interpretive rather than systematic, a formal duplicate-screening workflow, PRISMA flow diagram, or study-level risk-of-bias instrument was not utilized.

Results: MICS can provide outcomes comparable to conventional sternotomy, with shorter intensive care and hospital stays, lower transfusion requirements, fewer wound complications, and earlier mobilization. However, higher operative costs, specialized equipment, longer cardiopulmonary bypass and cross-clamp times, peripheral cannulation risks, conversion to sternotomy, and learning-curve effects may offset postoperative savings. Economic value therefore depends on whether reduced surgical trauma translates into fewer complications and lower overall resource use.

Conclusion: MICS should be evaluated as a value-based care model. Centres developing MICS programmes should monitor risk-adjusted outcomes, resource utilization, conversions, total costs and learning curve dynamics through a structured institutional registry.

Keywords: minimally invasive cardiac surgery; health economics; resource utilization; recovery; learning-curve; value-based healthcare.

Introduction

Cardiac surgery remains one of the most technically demanding and resource-intensive areas of hospital care [1]. For decades, median sternotomy has been the standard access for most cardiac surgical procedures due its wide exposure and rapid control [2,3]. However, full sternotomy is associated with significant surgical trauma, postoperative pain, wound complications, increased transfusion requirements and delayed recovery [4]. These limitations have led to the development of less invasive surgical strategies [5–8].

Minimally invasive cardiac surgery (MICS) encompasses a spectrum of techniques, ranging from mini-sternotomies and mini-thoracotomies to video-assisted, totally endoscopic, and robotic-assisted approaches, which are now routinely used in valve surgery, coronary revascularisation, and selected combined procedures [2,4,9,10]. Over time, MICS has transformed from a simple incision strategy into a comprehensive perioperative care model that requires highly coordinated workflows across the surgical, anaesthetic, perfusion, intensive care, and nursing domains [6,7,11].

While the potential advantages of MICS include reduced trauma, fewer transfusions, and faster recovery, these benefits are neither universal nor guaranteed [12,13]. MICS frequently prolongs cardiopulmonary bypass and cross-clamp times, requires specialised equipment, and demands a substantial institutional learning curve [14,15]. Consequently, a central challenge in evaluating MICS is the financial and operational misalignment across the hospital episode: the intraoperative period is often significantly more expensive due to prolonged operating time and specialised disposables, whereas the postoperative period generates savings through reduced intensive care unit (ICU) and hospital stays [16–19]. This tension is particularly acute for resource-constrained healthcare systems and tertiary centres, where ICU capacity is a major operational bottleneck and investments in technology must be justified by measurable hospital-wide utility. Institutional value also depends on programme maturity; early implementation typically incurs high costs and longer procedures, whereas experienced teams achieve predictable recovery pathways and efficient resource utilisation [3,11].

Although recent state-of-the-art reviews have extensively examined the historical and technical evolution of MICS [4,10], less attention has been paid to how hospitals can determine whether a programme generates measurable institutional value [20]. Current literature often reports clinical outcomes, recovery, costs, or learning curves in isolation. An integrated framework is needed to connect these aspects while maintaining procedure-specific benchmarks.

The aim of this narrative review is to analyze the current evidence regarding to the organizational and economic value of MICS. Additionally, the review proposes a practical framework for evaluating MICS programs at tertiary cardiac centres. The novel aspect of this review is the integration of risk-adjusted clinical outcomes, organizational performance, total episode costs, and program maturity into a unified governance framework. This framework is designed for local implementation and does not suggest that different MICS procedures are clinically interchangeable.

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Methods

Review design

This article is presented as a narrative review and practical framework paper. Its aim is not to perform a formal meta-analysis or to assert that MICS is superior to conventional surgery. Instead, it identifies the clinical, organizational, economic, and programme conditions under which MICS may generate value. The review was conducted according to the principles of transparent narrative synthesis and followed the Scale for the Assessment of Narrative Review Articles (SANRA) [21]. Due to significant differences in study procedures, patient populations, comparators, institutional experiences, healthcare systems, economic methodologies, and outcome definitions, quantitative pooling of the data was not appropriate. As a result, the evidence

was organized into clinically relevant domains and translated into a practical evaluation model for hospital governance.

Search strategy and study selection

PubMed/MEDLINE, Scopus, Web of Science and Google Scholar were searched for English-language publications published from January 2010 to June 2026, with the final search conducted in June 2026. The search terms included combinations of "minimally invasive cardiac surgery," "minimally invasive mitral valve surgery," "minimally invasive aortic valve replacement," "robotic cardiac surgery," "robotic coronary artery bypass grafting," "minimally invasive coronary revascularization," "enhanced recovery after cardiac surgery," "early discharge," "intensive care unit," "length of stay," "transfusion," "cost analysis," "cost-effectiveness," "learning curve," "conversion," "peripheral cannulation," "value-based healthcare" and "middle-income countries." Additionally, the reference lists of major reviews and relevant primary studies were examined.

Eligible types of evidence included randomized trials, prospective and retrospective cohort studies, propensity-matched studies, registry analyses, systematic reviews, meta-analyses, and hospital-based economic evaluations. Narrative reviews were used to provide technical context, describe programme implementation, and highlight additional primary studies. Trial protocols were considered only as contextual information and were not regarded as evidence of clinical or economic outcomes. Studies focused exclusively on paediatric populations, conference abstracts without full text, isolated technical descriptions without outcome data, non-peer-reviewed materials, and publications unrelated to hospital-level value evaluation were excluded. In line with the narrative design, a formal duplicate-screening process, study-count flow diagram, or study-level risk-of-bias scoring was not performed.

Evidence synthesis

Evidence was organized into four predefined domains: (1) clinical safety and effectiveness; (2) organizational performance and recovery; (3) economic performance; and (4) program maturity. The review prioritized several key metrics, including mortality rates, stroke incidence, myocardial infarction, bleeding events, reoperations, acute kidney injury, pacemaker implantation, and wound and vascular complications. It also assessed conversion rates, ventilation time, stays in the ICU and wards, total length of stay, discharge destination, readmission rates, transfusions, operative and postoperative costs, total episode costs, annual patient volume, chronological case numbers, and learning curve dynamics. Each evidence stream was analyzed based on procedure type, access route, comparator, and program maturity, rather than being consolidated into a single effectiveness estimate. The framework is procedure-agnostic at the governance level, but benchmarking must remain specific to each procedure. Programs for mitral, aortic, coronary, mini-sternotomy, mini-thoracotomy, and robotic surgeries should be stratified and not combined, unless there is a clearly defined analytical rationale for doing so.

Development of the hospital-level framework

The proposed framework was developed by mapping recurring variables from the clinical, organizational, economic, and implementation literature to four predefined domains. Candidate indicators were selected based on three practical criteria: clinical relevance, feasibility of measurement using routinely collected hospital data, and usefulness for ongoing program governance. The synthesis process was iterative and pragmatic rather than conducted through a formal Delphi or consensus process. Accordingly, the framework is intended as an adaptable institutional measurement tool rather than a validated universal cost-effectiveness model, because reimbursement systems, staffing costs, device prices, accounting standards, and discharge infrastructure vary across healthcare systems. The governance framework itself is procedure-agnostic; however, clinical benchmarking and economic comparisons must remain procedure-specific. Analyses should therefore be stratified by procedure type (mitral valve surgery, aortic valve surgery, or coronary revascularisation) and by access strategy or technology (mini-sternotomy, mini-thoracotomy, or robotic assistance), unless a prespecified methodological rationale supports pooling.

MICS as an Integrated Care Model

A narrow definition of minimally invasive cardiac surgery (MICS) that focuses only on the length or location of the incision is inadequate. Although reduced access trauma allows quicker recovery, how effectively this opportunity is realized depends on the surrounding perioperative system. Key factors include patient selection, preoperative monitoring, cannulation planning, anaesthesia, perfusion, myocardial protection, pain management, early extubation, timely removal of drains and catheters, mobilization, discharge criteria, and post-discharge follow-up [6–8,13,22,23].

Even if a mini-thoracotomy is technically successful, prolonged hospitalization can occur if pain management is insufficient, extubation is delayed, mobilization is inconsistent, or discharge criteria are unclear. On the other hand, patients who undergo sternotomy within a well-established enhanced recovery pathway may experience rapid recovery [7]. The impact of MICS should therefore be viewed as an interaction between surgical access and pathway performance, rather than as an inherent advantage of having a smaller incision [24].

This distinction has important practical implications. A program that introduces a new access route without standardized patient selection, team training, enhanced recovery protocols, clear outcome definitions, cost tracking, and monitoring of the learning curve may complicate the procedure without demonstrating benefits.

Safety, Outcomes and Patient Selection

The contemporary evidence generally supports the feasibility and safety of MICS for appropriately selected patients treated by experienced teams. The UK Mini Mitral randomized trial demonstrated that minithoracotomy achieved high-quality, durable mitral repair with safety outcomes similar to those of sternotomy. However, it was not superior for the primary physical-function endpoint at 12 weeks [25].

Meta-analyses of mitral surgery suggest broadly comparable mortality and major morbidity rates between the two approaches, with potential benefits in terms of hospital stay and recovery outcomes [26]. For aortic valve replacement, both meta-analytic and Cochrane evidence suggest that limited-access techniques can reduce ICU or total hospital stay in some cases; however, these approaches may lead to longer bypass and cross-clamp times, and the reliability of outcomes varies [12,14].

The strongest value proposition is unlikely to be a universal reduction in mortality rates, as mortality in elective cardiac surgery is already low and heavily influenced by baseline risk factors. More plausible benefits of improved surgical techniques and care include reduced exposure to blood transfusions, fewer sternal wound- related complications, shorter ventilation and

ICU stays, earlier mobilization of patients, and faster overall functional recovery. Although these outcomes are clinically significant, their impact can vary depending on the specific procedure, the patient's profile, the maturity of the surgical program, and the perioperative pathway used [14,23,26].

MICS also has an access-specific risk profile. Peripheral cannulation may be associated with vascular injury, bleeding, groin infection, lymphatic complications, limb ischemia, and concerns related to retrograde perfusion in patients with aortic atherosclerosis. Limited exposure may require conversion to sternotomy because of bleeding, adhesions, haemodynamic instability, difficult anatomy, inadequate visualization, or an inability to achieve a safe, durable repair or revascularization. Conversion should be recorded prospectively as a safety and programme-maturity endpoint rather than concealed as an isolated technical failure [9,13,15].

Patient selection is therefore central to both clinical outcomes and economic interpretation. Mitral surgery requires assessment of pathology, repair complexity, annular or leaflet calcification, ventricular function, pulmonary hypertension, prior surgery, chest anatomy, vascular access, and the anticipated probability of a durable repair [9]. Aortic valve strategy depends on valve morphology, aortic anatomy, vascular access, frailty, comorbidities, and the relative suitability of surgical and transcatheter options. Minimally invasive coronary revascularization requires evaluation of target vessel anatomy, conduit strategy, completeness of revascularization, peripheral vascular status, and the feasibility of hybrid treatment [4,5].

Because MICS cohorts are frequently selected for anatomical suitability and lower procedural complexity, unadjusted comparisons may overestimate benefit. Hospital evaluations should use risk adjustment, propensity methods, stratification, or multivariable modelling when comparing MICS with sternotomy. Comparative analyses should remain specific to each procedure and should not combine mitral, aortic, coronary, mini-sternotomy, mini-thoracotomy, and robotic procedures without a predefined rationale. The heart team should also be treated as an auditable organizational process: the programme should document whether the case was discussed, which options were considered, why MICS was selected or rejected, and whether the decision conformed to predefined criteria.

Organizational Performance: Enhanced Recovery, Pain, and Discharge

Enhanced recovery is one of the main mechanisms through which MICS can generate organizational value. Cardiac ERAS recommendations emphasize preoperative education and optimization, anaemia management, multimodal analgesia, early extubation, prevention of delirium and nausea, blood conservation, early mobilization, and standardized discharge planning [22]. MICS-specific reviews and cohort studies indicate that these elements are most effective when implemented as a coordinated pathway rather than as isolated interventions [7,23].

Pain management requires particular attention. Thoracic access may still produce substantial pain through intercostal nerve irritation, pleural manipulation, retraction, and chest drains. Poor analgesia impairs respiratory exercises, mobilization, physiotherapy, sleep, and readiness for discharge. Multimodal systemic analgesia and selected regional techniques can reduce opioid exposure and support recovery, although protocols vary and evidence is heterogeneous[6,13]. Pain should therefore be measured as a pathway performance variable rather than treated solely as a comfort outcome.

Early discharge is meaningful only when it reflects clinical readiness. In selected, uncomplicated patients after minimally invasive valve surgery, discharge within three postoperative days has been associated with comparable short-term outcomes and lower costs, but strong selection effects must be recognized [8]. Hospitals should define minimum discharge criteria that include stable rhythm and haemodynamics, no oxygen dependency, adequate pain control and mobility, acceptable renal function, no major wound or groin problem, no clinically important pleural or pericardial collection, an appropriate anticoagulation plan, and clear access to follow-up.

Organizational performance should be measured using hours as well as calendar days. Ventilation duration, time to mobilization, ICU hours, time to drain removal, ward days, discharge destination, and 30-day readmission together provide a more informative picture than hospital length of stay alone. A short admission achieved through premature discharge is not a benefit if it increases emergency visits or readmissions. Conversely, reduced ICU use may improve bed availability, reduce cancellations, and increase surgical throughput even when direct accounting savings are modest.

Technical Complexity, Learning Curve, and Programme Maturity

MICS is a team-dependent technical platform. It may require peripheral cannulation, long-shafted instruments, thoracoscopic or robotic visualization, advanced transesophageal echocardiography, alternative myocardial protection strategies, and close coordination among surgeons, anaesthesiologists, perfusionists, nursing staff, imaging specialists, and the ICU team [11,13,15]. During early implementation, operative duration, bypass and cross-clamp times, conversion rates, and resource use may be higher than during the mature phase.

The learning curve should be measured rather than assumed. A recent systematic review and meta-analysis of MICS procedures revealed significant variation in the number of cases needed to achieve consistent performance [16]. This variation reflects procedure complexity, prior surgeon experience, case selection, proctoring, team consistency, and institutional volume. The relevant unit of learning is therefore not only the individual surgeon but the multidisciplinary programme.

Programme maturity should therefore be assessed longitudinally, using chronological case number, annual procedural volume, operative and bypass times, conversion, transfusion, complications, ICU use, hospital stay, readmission, and cost. Moving averages, phase-based comparisons, and cumulative-sum methods can identify improvement, deterioration, or persistent process failures. Early-implementation and mature-programme results should be reported separately; a single average across all cases may obscure both learning-related risk and later efficiency gains.

Low annual volume creates a distinct challenge: a team may complete the initial learning phase yet fail to maintain proficiency. Centres should define a realistic procedural scope, select early cases conservatively, use proctoring and simulation where available, standardize equipment and team composition, and review adverse events through multidisciplinary audit. Expansion of indications should follow demonstrated stability in safety and process metrics rather than technical enthusiasm alone.

Economic Evaluation: Total Episode Cost and Complication Burden

Economic studies of MICS report heterogeneous findings, which is expected because costs depend on procedure type, device prices, operative duration, accounting methods, staffing, reimbursement, ICU practice, discharge culture, complication rates, and programme maturity [17–20]. Results from one health system cannot be transferred directly to another without adjustment for local unit costs and care pathways.

A hospital-level analysis should separate at least three cost layers. The first is procedural cost, including operating-room time, anaesthesia, perfusion, imaging, instruments, disposables, prostheses or rings, robotic acquisition or depreciation, maintenance, sterilization, and training. The second is routine postoperative cost, covering ICU and ward care, medications, laboratory testing, imaging, physiotherapy, and transfusion. The third is complication-related cost, which includes conversion, reoperation for bleeding, stroke, renal replacement therapy, pacemaker implantation, infection, sepsis, prolonged ventilation, readmission, and reintervention.

Available studies illustrate why operative cost alone can be misleading. In minimally invasive mitral surgery, higher procedural expenditure may be offset by lower transfusion use, shorter ICU or ward stay, and reduced downstream resource consumption [19,20]. Economic analyses of minimally invasive aortic valve replacement and robot-assisted coronary surgery likewise show that the balance between higher technology or operating room costs and postoperative savings is highly context dependent [17,18]. The most informative internal endpoint is therefore total hospital episode cost, ideally from admission through discharge, plus 30-day readmission and early complication costs.

Complications should be analyzed simultaneously as clinical and economic events. A rare stroke, reoperation, vascular complication, or prolonged ventilation episode may outweigh savings from many uncomplicated short stays. Programmes should report both complication frequency and cost. When detailed micro-costing is unavailable, a pragmatic model can use local average costs for the operation, ICU days, transfusion, reoperation, major complications, and readmission.

The term "cost-effectiveness" should be reserved for analyses with an explicit effectiveness denominator, such as quality-adjusted life years, validated measures of functional recovery, or other defined patient outcomes. For routine governance, "cost analysis", "resource utilization analysis", and "total episode cost" are usually more accurate. Within a value-based healthcare perspective, value reflects outcomes achieved relative to total cost, not cost reduction alone [27,28].

Proposed Hospital-Level Evaluation Framework

The proposed framework is intended for tertiary cardiac centres introducing, evaluating, or scaling a MICS programme. It links four domains, including clinical outcomes, organizational performance, economic performance, and programme maturity, where distinguishes a mandatory core dataset from optional research-grade variables. The primary dataset should be collected prospectively using predefined, procedure-specific definitions that align, where possible, with recognized national registries and consensus endpoint standards. This framework serves as a governance tool for local measurement and comparison, rather than functioning as a universal cost-effectiveness model.

Table 1 Evidence domains and their implications for hospital-level MICS evaluation

Domain	Representative evidence	Main signal	Implication for hospitals
Clinical safety and effectiveness	Randomized, matched, registry, and meta-analytic evidence [5, 9, 12, 14, 25, 26, 29, 30]	Comparable safety in selected patients; effects vary by procedure and programme maturity	Use risk-adjusted, procedure-specific benchmarks; stratify distinct procedures; record conversion and access-related complications
Organizational performance	ERAS, pain, and early-discharge studies [6–8, 13, 22, 23]	Shorter ICU or hospital stay is pathway-dependent	Measure ventilation, ICU hours, mobilization, discharge readiness, destination, and readmission
Economic performance	Hospital cost analyses [17–20]	Postoperative savings may offset higher procedural cost	Analyze local cost layers and total episode cost rather than operating-room cost alone
Programme maturity	Learning-curve and programme reports [10, 11, 15, 16]	Efficiency, conversion and resource use change with cumulative experience	Track chronological case number, annual volume, temporal trends, mentoring, and audit

The principal evidence domains, their main signals, and their implications for hospital-level MICS evaluation are summarized in Table 1.

The core framework should address five key questions:

1. Were patients appropriately selected?
2. Was the procedure conducted safely?
3. Did recovery and resource utilization improve?
4. Did the total cost of the episode change?
5. Did performance improve with experience?

These questions should be examined collectively and within procedure-specific strata. A shorter length of stay without ensuring adequate safety, or low operative costs accompanied by a high complication rate, do not truly represent value. The minimum indicators required to answer these questions are outlined in Table 2 (see the next page).

The core dataset must remain appropriate for routine clinical use. Centers that have adequate research, informatics, and analytical resources may choose to collect additional variables to study aspects such as functional recovery, components of care pathways, capacity effects, detailed cost analysis, and program evaluation. These extended research-grade and service-development variables are presented in Table 3 (see the next page).

Implementation cycle

To operationalize the proposed framework, institutions should follow a continuous, five-step implementation cycle (see Figure 1). This structured workflow bridges clinical decision-making with continuous quality improvement:

1. Define the specific eligibility criteria and contraindications for the procedure, and document the decisions made by the heart team.
2. Standardize the perioperative pathway, which includes monitoring, anesthesia, perfusion, analgesia, extubation, drain removal, mobilization, and discharge criteria.
3. Collect the core dataset prospectively, using predefined definitions for outcomes and conversions (see Table 2).
4. Calculate the resource utilization and total episode cost,

Table 2 Core dataset for hospital-level value evaluation of MICS

Domain	Core indicators	Timing	Purpose
Case mix and risk	Age, sex, BMI, diabetes, COPD, renal disease, previous cardiac surgery; EuroSCORE II or STS risk; LVEF; NYHA class	Baseline and admission	Risk adjustment and case-mix comparison
Anatomy and selection	Valve or coronary pathology, calcification, chest anatomy, vascular access, heart-team decision and reason for approach	Preoperative	Selection transparency and access planning
Procedure	Operation and access type, cannulation strategy, the time of surgery, CPB and cross-clamp	Intraoperative	Technical complexity and resource use
Safety	Conversion (definition and reason), operative mortality, stroke/TIA, myocardial infarction, reoperation for bleeding, AKI/dialysis, pacemaker implantation, vascular complications, and wound complications	In hospital and 30 days	Risk-adjusted safety and complication-cost analysis
Recovery	Ventilation hours, mobilization, pain control, ICU hours, total hospital stay	Postoperative	Organizational performance
Discharge	Discharge readiness, destination, postoperative day of discharge, emergency visits, 30-day readmission, and cause	Discharge and 30 days	Safety of accelerated pathways
Blood management	PRBC, FFP, platelet, and fibrinogen-product exposure	In hospital	Clinical outcome and cost driver
Economics	Procedural, ICU, ward, transfusion, complication, and total episode costs	Admission to 30 days	Hospital-level economic performance
Programme maturity	Chronological case number, annual volume, surgeon/team experience, conversion and key outcomes over time	Continuous	Learning curve and scaling decisions

Definitions note: Each program should specify outcome and conversion definitions in advance, aligning them where applicable with recognized procedure-specific standards. Definitions based on STS can support outcomes in adult cardiac surgery, MVARC can aid in mitral valve endpoints, and VARC-3 can assist with aortic valve endpoints [31–33].

including major complications and 30-day readmissions where applicable.

5. Analyze performance based on the chronological case number and the phase of the program. Review the results through a multidisciplinary audit and use predefined thresholds to guide decisions regarding expansion or corrective actions (see Table 3).

Application in Tertiary and Resource-Constrained Settings

The transferability of published economic results is limited in settings where staff costs, ICU-day costs, reimbursement rates, device pricing, discharge infrastructure, and rehabilitation pathways differ from those in the original studies. For such centers, the primary focus should not be on a complex cost-utility model, but rather on establishing a reliable institutional registry linked to practical local costing. The core indicators presented in Table 2 provide a feasible starting point for such a registry.

A relatively small core dataset can address management questions that are directly relevant to program governance. For instance, are MICS and sternotomy patients comparable in terms of baseline risk? Does MICS lead to reduced ICU hours, shorter total hospital stays, fewer transfusions, or lower rates of wound complications locally? Does it increase operative time or the need for conversions? Which complications incur the greatest costs? Do total episode costs decrease, increase, or remain similar? Does performance improve as experience accumulates?

Table 3 Extended variables for research-grade and service-development analysis

Area	Extended variables	Analytical value
Frailty and function	Frailty scale, baseline mobility, 6-minute walk distance, return to activity, quality of life	Tests whether benefit is expressed through functional recovery rather than mortality
Pain and analgesia	Serial pain scores, opioid consumption, regional block, nausea, sleep	Links pathway components to mobilization and discharge
Detailed blood management	Cell salvage, coagulation testing, individual blood components	Identifies modifiable transfusion drivers
Imaging and access planning	Computed tomography vascular assessment, echocardiographic anatomy, calcification categories	Supports prediction of conversion and access complications
Readmission detail	Timing, cause, intervention, and cost	Tests whether accelerated discharge shifts cost beyond the index admission
Capacity effect	ICU bed turnover, cancelled operations, waiting-list metrics, throughput	Captures organizational value not visible in direct cost accounting
Cost granularity	Personnel time, disposables, robotic depreciation and maintenance, diagnostics	Identifies modifiable local cost drivers
Learning-curve analysis	CUSUM, moving averages, phase-based analysis, surgeon and team exposure	Distinguishes early implementation from mature performance

Capacity effects are particularly significant when ICU beds are limited. A shorter ICU stay may improve access for other patients, reduce the cancellation of planned operations, and enhance overall throughput. These benefits should be reported separately from direct financial savings, as they represent opportunity value rather than necessarily indicating a reduction in pre-booked expenditures. Suggested indicators for measuring these capacity effects are included in Table 3.

For academic tertiary centers, the registry also serves as a platform for quality improvement and research. The same structured data can support service evaluations, doctoral projects, comparative-effectiveness studies, economic analyses, and publications. Therefore, data collection should ideally be designed collaboratively by clinicians, hospital administration, information technology staff, and health economists. Depending on institutional resources and research objectives, the core dataset in Table 2 may be supplemented with additional variables listed in Table 3.

Discussion

The available evidence indicates that minimally invasive cardiac surgery (MICS) is not inherently superior or cost-saving. However, in carefully selected patients and at experienced facilities, minimally invasive techniques can achieve a level of clinical safety comparable to traditional sternotomy while enhancing certain recovery and resource-use outcomes [12,14,25,26,29,30]. However, these benefits are not produced

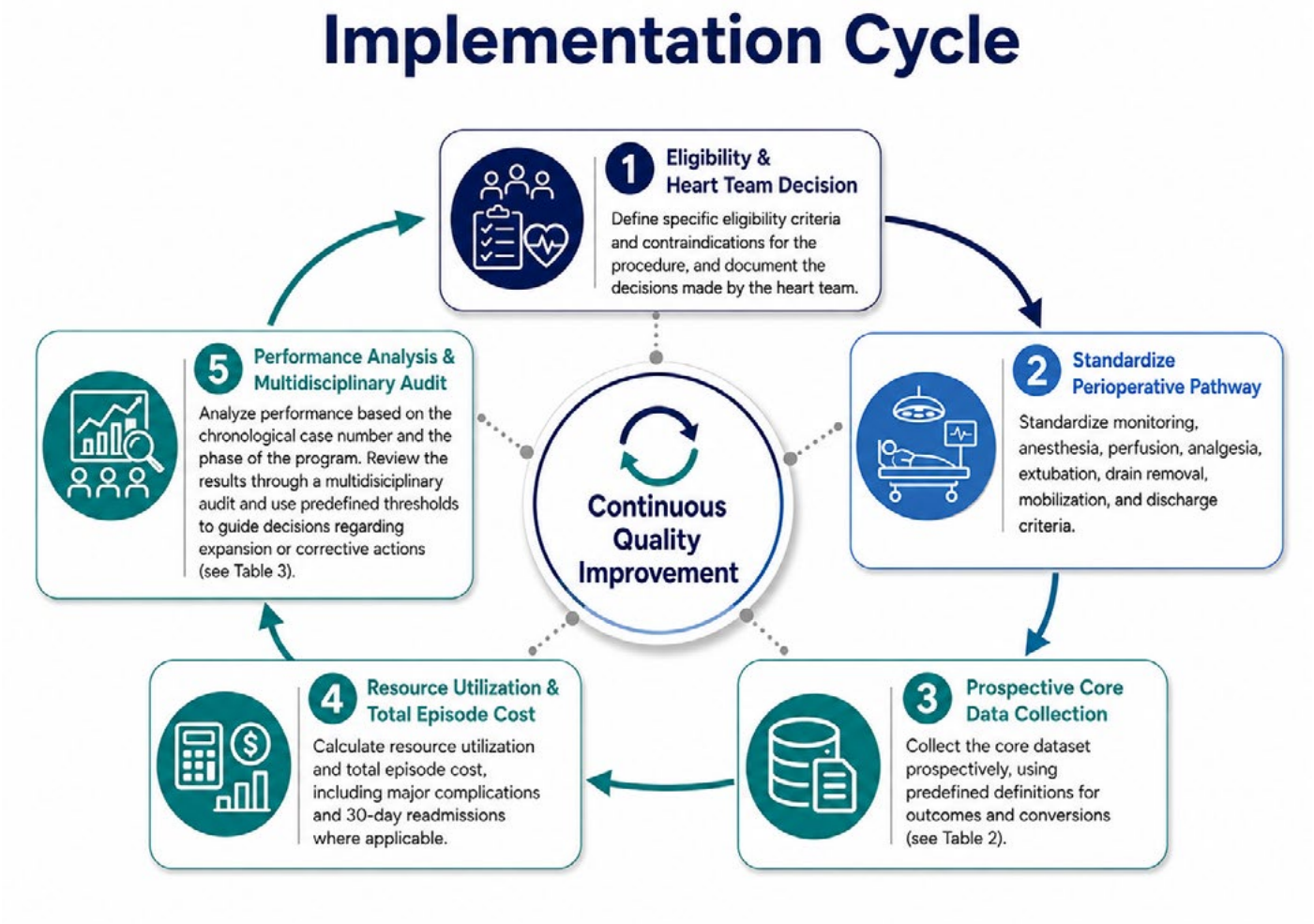

Figure 1 – MICS program implementation and governance cycle

A continuous five-step framework integrating Heart Team–based patient selection, perioperative pathway standardization, prospective data collection, local resource utilization and cost evaluation, and longitudinal learning-curve analysis using CUSUM.

by the incision alone. They arise when reduced surgical trauma is translated into measurable improvements through the entire perioperative care pathway.

This distinction helps explain the heterogeneity of published findings. Established programmes with disciplined patient selection, experienced and stable multidisciplinary teams, standardized anaesthetic and perfusion protocols, effective pain control, early extubation, structured mobilization, and explicit discharge criteria are more likely to convert reduced access trauma into shorter ICU and hospital stays. By contrast, programmes in an early implementation phase may experience longer operating times, higher procedural and device costs, greater variability in postoperative recovery, and additional costs associated with conversion or access-related complications. Combining outcomes from centres at different stages of programme maturity may therefore obscure the conditions under which MICS generates value.

The strongest clinical argument for MICS is not a universal mortality advantage. Mortality after elective cardiac surgery is relatively uncommon and is strongly influenced by baseline risk, procedure type, and case selection. The more plausible benefit is a reduction in the burden of recovery, including shorter ventilation and ICU exposure, lower transfusion requirements, fewer access- or wound-related complications, earlier mobilization, shorter hospitalization, and faster functional recovery. These outcomes are relevant not only to patients but also to hospital operations because they influence ICU capacity, bed turnover, rehabilitation requirements, discharge planning, and readmission.

The economic case must therefore be evaluated across the complete episode of care. If procedural costs increase while the postoperative pathway remains unchanged, the economic advantage of MICS may be limited. Similarly, inappropriate case selection, prolonged operative or cardiopulmonary bypass times, vascular-access complications, frequent conversion, or failure to achieve earlier recovery may weaken the value proposition. Conversely, higher initial operating-room expenditure may be partly or fully offset by reductions in ICU use, ward stay, transfusion, complications, and readmission. The relevant question is therefore not simply whether MICS is less expensive than sternotomy, but under which clinical, organisational, and programme conditions it improves outcomes relative to total hospital resource use.

Risk adjustment is essential when evaluating these outcomes. Observational comparisons are particularly vulnerable to selection bias because patients who are clinically stable and anatomically suitable patients are more likely to undergo minimally invasive procedures. Hospitals should therefore report baseline risk, anatomical eligibility, procedure type, access route, and the rationale for treatment selection. Depending on sample size and data quality, comparative

analyses should use multivariable adjustment, propensity-score methods, matching, weighting, or stratification. Unadjusted comparisons should be interpreted as descriptive rather than causal and distinct procedure classes should be analysed separately.

Programme maturity and the learning curve are additional determinants of clinical and economic performance. Early cases should not be excluded from programme evaluation, but they should be analysed separately as an implementation phase. Chronological monitoring of conversion, operative time, cardiopulmonary bypass and cross-clamp duration, complications, ICU utilisation, length of stay, and total episode cost can demonstrate whether initial investment is followed by stabilisation and improved efficiency. Failure to observe improvement should prompt review of patient selection, training, proctoring, team composition, equipment, and perioperative workflow before further programme expansion. Conversely, favourable mature-programme outcomes should not be assumed to apply immediately to centres at the beginning of implementation.

The proposed framework adopts a value-based perspective by linking patient outcomes to the total resources used across the episode of care [28]. The main contribution of this model is to combine four key dimensions into a single governance framework: risk-adjusted safety and effectiveness, organizational performance, total episode cost, and program maturity. This integration is practical, though not formally validated. Hospitals vary in factors such as reimbursement rates, staffing expenses, ICU capacity, device costs, discharge processes, and rehabilitation pathways. As a result, the economic value of minimally invasive cardiac surgery (MICS) needs to be demonstrated locally and for each specific procedure class. While technology facilitates minimally invasive surgery, achieving sustainable value relies on careful patient selection, standardized pathways, multidisciplinary coordination, transparent measurement, and ongoing program governance.

Practical Recommendations

A strong program evaluation must address situations where MICS may not provide value. Key areas for improvement include:

- Avoid expanding procedures before demonstrating stable safety and performance metrics;
- Ensure thorough vascular and anatomical screening prior to peripheral cannulation or limited-access surgery;
- Standardize definitions and reporting of conversions to sternotomy;
- Prevent unnecessary prolongation of bypass or cross-clamp time without clear recovery benefits;
- Improve pain management, patient mobilization, and standardized criteria for enhanced recovery and discharge;
- Treat early discharge as a clinical safety decision rather than an administrative target;
- Fully account for costs related to robotic systems, including acquisition, maintenance, and training;
- Maintain high team proficiency by avoiding low annual procedure volumes and excessive variation in team composition;
- Adjust comparisons with sternotomy for risk factors, anatomy, and treatment-selection bias.

Recognizing these failure points reinforces the value of MICS and emphasizes the necessary safeguards for effective implementation and quality improvement.

Limitations

This review has several limitations.

First, it is a narrative review rather than a systematic one; consequently, it does not include a PRISMA flow diagram, formal duplicate screening, a quantitative study-selection count, study-level risk-of-bias assessment, or a meta-analysis. The structured search was designed to support transparent interpretive synthesis rather than exhaustive effect estimation, which means that study selection is partly dependent on the authors' judgment.

Second, the evidence varies across different procedures, access routes, comparators, patient risk levels, institutional experience, outcome definitions, and economic methods.

Third, many studies are retrospective and conducted at single centers, which increases the potential for selection bias and limits causal inference.

Fourth, cost data are significantly influenced by local reimbursement policies, staffing levels, device pricing, accounting practices, and length-of-stay policies. Therefore, absolute cost estimates should not be directly transferred between countries. Fifth, the maturity of programs and the experience of operators limit the generalizability of findings from high-volume expert centers to new or low-volume programs.

Finally, the proposed framework has not undergone prospective validation, external benchmarking, Delphi consensus, or formal health-economic validation. It should be viewed as a structured starting point for local measurement and governance rather than a replacement for procedure-specific clinical judgment, validated registry definitions, or formal economic evaluations.

Conclusion

Minimally invasive cardiac surgery represents an important evolution in cardiovascular care, but its value should not be judged by incision size alone. MICS creates value only when appropriate patient selection, technical expertise, multidisciplinary planning, anaesthesia and perfusion protocols, effective pain control, enhanced recovery, early mobilization, safe discharge, complication prevention, and outcome monitoring operate as an integrated system.

In selected patients treated within experienced programmes, MICS can achieve outcomes comparable to conventional approaches and may reduce transfusion exposure, wound complications, ICU use, hospital stay, and time to recovery. Economic benefit is most likely when higher procedural expenditure is offset by lower postoperative resource utilization and fewer high-cost complications.

Tertiary cardiac centres should therefore evaluate MICS through a structured institutional registry that combines risk-adjusted clinical outcomes, organizational performance, total episode costs, readmissions, and learning-curve indicators. Such measurement can support clinical governance, responsible programme expansion, resource optimization, and scientific evaluation. MICS should ultimately be understood not only as a surgical technique, but as a patient-centred, value-oriented, and organization-dependent model of cardiac surgical care.

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