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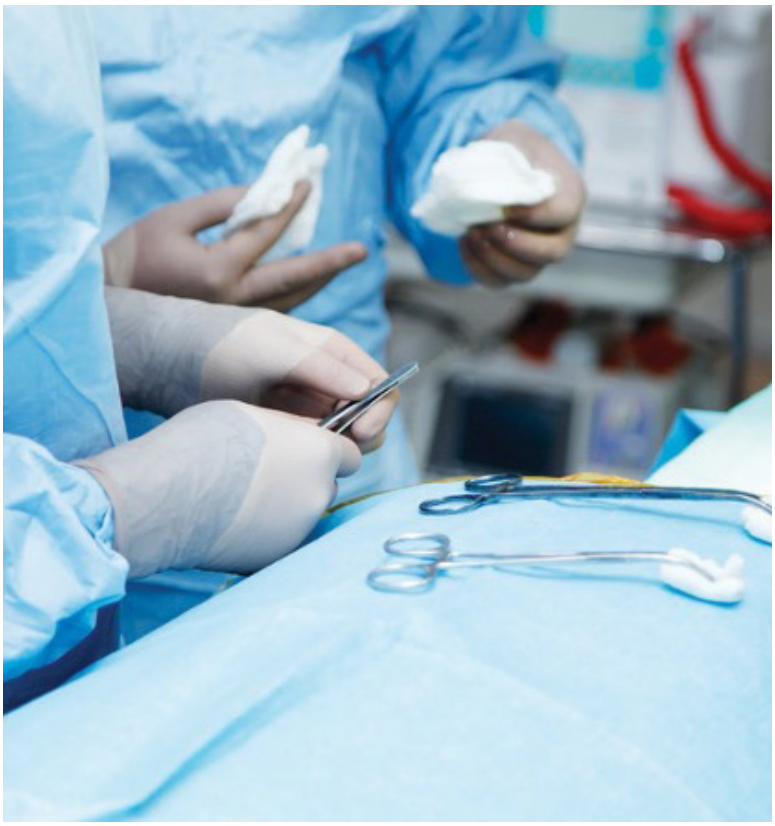

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The Effect of Melatonin Adjuvant on Cognitive Function and Melatonin Levels in Schizophrenia Patients

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Abstract

Background: Schizophrenia is an important and serious mental disorder. It has been studied that serum melatonin levels were significantly decreased in schizophrenia patients.

Objective: To investigate the effect of adjuvant melatonin on cognitive function and serum melatonin levels in schizophrenia patients receiving risperidone therapy.

Methods: This study used a randomized controlled double-blind design. Forty-four male schizophrenic patients were successfully enrolled and randomized into two groups: 22 treatment patients who received risperidone therapy (4-6 mg/day) and melatonin (5 mg/day) and 22 control patients who received risperidone and placebo. The Montreal Cognitive Assessment Indonesian version (MoCA-INA) scale and serum melatonin levels were measured before and after 8 weeks of therapy. Data were analyzed using chi-squared, independent samples t-test or Mann-Whitney and Spearman correlation tests.

Results: Changes in MoCA-Ina scores were significantly different between the treatment and control groups ($p<0.001$), particularly in the visuospatial/executive, language, memory and orientation dimensions ($p<0.05$). The change in melatonin levels was also significantly different between groups ($p<0.05$). The decrease in melatonin levels in the treatment group was about three times lower than in the control group (-4.34 pg/mL vs. -13.61 pg/mL). There was no significant correlation between melatonin levels and MoCA-Ina scores.

Conclusion: Adjuvant melatonin could improve cognitive function and slow the rate of melatonin decline in schizophrenia patients receiving risperidone.

Keywords: Cognitive function; Melatonin; Schizophrenia; Risperidone.

Introduction

Schizophrenia is a serious mental disorder that has positive symptoms (i.e. delusions, hallucinations), negative symptoms (i.e. blunted affect) and also includes cognitive symptoms (i.e. impaired memory and executive function) [1]. According to the World Health Organization (WHO), the prevalence of schizophrenia is estimated to be around 24 million people worldwide, or 0.32% of the population. In Indonesia, the prevalence of schizophrenia was 6.7 per 1000 households, placing South Sulawesi in 5th place nationally [2, 3].

Cognitive impairment in people with schizophrenia could be considered a core symptom, as 98% of people with schizophrenia show cognitive decline, with broad areas of cognitive impairment of varying severity, and with or without antipsychotic medication [4].

Vigano et al. reported that serum melatonin levels were significantly decreased in schizophrenia patients compared to healthy controls [5]. Decreased melatonin may occur due to decreased pineal gland function. This is in line with the findings of Findikli et al. that there was a

significant decrease in pineal volume on MRI in schizophrenia patients [6].

Moreover, reduced melatonin levels could also be caused by antipsychotic medication, but a study by Maiti showed that reduced melatonin also occurred in schizophrenia patients without antipsychotic medication [7]. It concluded that there was no difference in melatonin levels between schizophrenia patients who had received antipsychotic therapy (e.g. haloperidol, risperidone) for 4 weeks and those who had not.

In addition, two studies had mixed results about the effect of melatonin administration in patients with schizophrenia. The first study reported that the addition of 2 mg of prolonged-release melatonin had no significant effect on cognitive and psychosocial function in schizophrenia patients undergoing benzodiazepine withdrawal compared with placebo [8]. Meanwhile, another study reported that cognitive impairment in schizophrenia patients was significantly improved with the administration of melatonin agonists at a dose of 8 mg/day for 6 months [9].

Theoretically, melatonin can improve the sleep-wake cycle, mood disorders, cognition and memory, and is neuroprotective. The cognitive impairment may be due to the disruption of melatonin regulation, as shown by the inhibition of long-term potentiation (LTP) in the hippocampus [10].

As mentioned above, it is important to investigate the effect of adjuvant melatonin on cognitive function and melatonin levels in schizophrenia patients under antipsychotic medication. To the best of our knowledge, this is the first study to investigate this issue in the case of Indonesian schizophrenic patients.

Methods

Study design and Participants

The study was a double-blind randomized controlled trial (RCT) conducted at the Dadi Regional Special Hospital, South Sulawesi, Indonesia. It enrolled 59 hospitalised male schizophrenic patients in the stable phase with the following inclusion criteria: diagnosed with schizophrenia according to the 10th revision of the International Classification of Diseases (ICD-10), aged 20-45 years, with disturbed sleep (Pittsburgh Sleep Quality Index score > 5), receiving risperidone therapy 4-6 mg/day, and able and willing to participate in the study. Participants were excluded if they had organic co-morbidities, a history of drug use in the 6 months prior to hospitalisation, or were taking anti-inflammatory drugs or antibiotics. During the intervention, patients were also excluded if they did not take melatonin or risperidone regularly for 2 consecutive days, refused to continue the study, were discharged from hospital or died. There were eight participants who did not meet the inclusion criteria, leaving a sample of 51 (Figure 1).

Randomisation and Blinding

Participants were randomly assigned to treatment and control groups (25 treatment and 26 control). However, 3 patients in the treatment group and 4 patients in the control group dropped out during follow-up, leaving 22 participants in each group (Figure 1). The treatment group received risperidone 4-6 mg/day and adjunctive melatonin 5 mg/day for 8 consecutive weeks. The melatonin dosage was referred to previous study of Duan and her colleagues [11]. Meanwhile, the control group received risperidone 4-6 mg/day and placebo. The researcher ensured that neither the participants nor the treating physicians were aware of the group allocation of any participant.

Cognitive function assessment

Cognitive function was assessed using the Montreal Cognitive Assessment Indonesian version (MoCA-Ina) at baseline (week 0; pre-intervention) and week 8 (post-intervention). It consisted of eight domains: visuospatial/executive, naming, attention, language, abstraction, memory and orientation. Scores range from 0-30 with a cut-off of 26 (normal ≥26) [12,13]. The procedure was carried out through a face-to-face interview conducted by a professional psychiatrist, lasting approximately 10–15 minutes.

Melatonin measurement

Melatonin was measured from a blood sample taken from the patient's median cubital vein at 21:00 o'clock at baseline and at the end of the intervention (week 8). The sample was immediately centrifuged and stored at -70°C. The procedure was using enzyme-linked immunosorbent assay (ELISA) kits (Humat MT, Elabsience, Catalog No: E-EL-H2016) with the Thermo Scientific Multiskan FC device according to the Hasanuddin University Medical Research Center (HUM-RC) lab protocol. The protocol has been specifically established for use in medical and health-related research settings.

Ethics

All participants were asked to sign a written informed consent to participate in this study. The study has received ethical permission from the Research Ethics Commission of

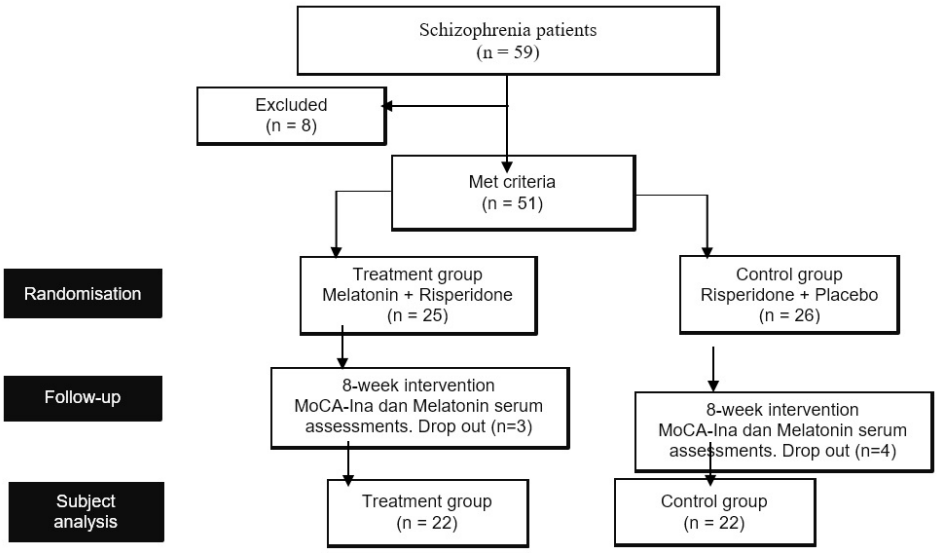

Figure 1 – Participant recruitment flow

the Faculty of Medicine, Hasanuddin University (No:479/UN4.6.4.5.31/PP36/2023).

RCT Registration

This study was clinically registered in the Thai Clinical Trials Registry (TCTR; https://www.thaiclinicaltrials.org/) and received approval with the number TCTR20250222001 (https://www.thaiclinicaltrials.org/show/TCTR20250222001).

Data analysis

Bivariate data were analysed using chi-squared for categorical or proportional data and Mann-Whitney test for numerical data or mean differences. Spearman correlation tests was performed to examine the correlation between melatonin serum level and cognitive function score. Data were tested at the 95% confidence level with α=0.05 using SPSS version 27 and and Microsoft Excel 2013.

Results

All participants in both groups were 100% male and did not differ in age (p=0.410), education (p=0.335) and marital status (p=0.741; Table 1).

Table 2 shows that there was a significant difference in the change (Δ) in total cognitive function according to the MoCA-Ina score between the treatment group (risperidone+melatonin) and the control group (risperidone+placebo). In addition, the change (Δ) was also significant in the cognitive function dimensions of visuospatial/executive, language, memory and orientation.

Table 3 shows that there was a difference in the change in melatonin level between the treatment and control groups.

Table 1 Characteristic of respondents

Variable	Risperidone + Melatonin (n=22)	Risperidone + Placebo (n=22)	p
Age (mean ± SD)	**33.55 ± 6.88**	**35.77 ± 6.85**	**0.410[a]**
Education			0.335[b]
No school	2 (9.0%)	3 (13.6%)	
Elementary school	8 (36.4%)	3 (13.6%)	
Junior high school	6 (27.3%)	5 (22.7%)	
Senior high school	6 (27.3%)	5 (22.7%)	
College	0 (0.0 %)	1 (4.4%)	
Marital status			0.741
Married	7 (31.8%)	6 (27.3%)	
Unmarried	15 (68.2%)	16 (72.7%)	

[a]Mann Whitney test. [b]Chi-Square test

The change in melatonin level in the treatment group was -4.34 ± 3.13 pg/ml, which was lower than the change in melatonin level in the control group (-13.61 ± 17.22 pg/ml). Melatonin levels at baseline were not different between groups (p=0.816). The difference was significant at week 8 after the intervention (p=0.015).

Table 4 shows that there was no significant correlation between the change (Δ) in melatonin levels and the MoCA-Ina score (r = 0.035, p=0.822).

Table 2 Cognitive function between treatment and control groups

MoCA-Ina Score	Risperidone + Melatonin (n = 22)			Risperidone + Placebo (n = 22)		
	Baseline	Week-8	Δ	Baseline	Week-8	Δ
Total	15.68 ± 6.09	19.82 ± 4.66	4.14 ± 3.51	17.73 ± 6.74	18.73 ± 5.51	1.00 ± 2.47***
Visuospatial/executive	2.64 ± 1.59	3.22 ± 1.27	0.59 ± 1.09	3.04 ± 1.43	3.04 ± 1.43	0.00 ± 0.53*
Naming	2.54 ± 0.67	2.68 ± 0.48	0.14 ± 0.35	2.50 ± 0.91	2.59 ± 0.85	0.09 ± 0.29
Attention	3.13 ± 1.81	3.77 ± 1.57	0.63 ± 0.95	3.82 ± 1.87	4.04 ± 1.65	0.23 ± 1.02
Language	1.72 ± 0.94	2.14 ± 0.71	0.41 ± 0.85	1.77 ± 1.51	1.77 ± 1.15	0.00 ± 0.00*
Abstraction	0.78 ± 0.81	0.95 ± 0.79	0.18 ± 0.50	1.27 ± 0.77	1.36 ± 0.90	0.09 ± 0.61
Memory	1.59 ± 1.74	2.77 ± 1.41	1.18 ± 1.22	1.91 ± 1.82	2.36 ± 1.49	0.45 ± 1.01*
Orientation	3.27 ± 1.78	4.27 ± 1.51	1.00 ± 1.19	3.41 ± 1.53	3.54 ± 1.33	0.13 ± 1.67**

*Data in Mean ± Standard Deviation (SD). Mann Whitney test. ***p<0.001, **p<0.01, *p<0.05.*

Table 3 Melatonin levels between treatment and control groups

Melatonin (pg/mL)	Risperidone + Melatonin (n = 22)		Risperidone + Placebo (n = 22)		p-value
	Mean + SD	Δ Change	Mean + SD	Δ Change	
Baseline	63.77 ± 13.19	-4.34 ± 3.13	64.73 ± 13.97	-13.61 ± 17.22*	0.816
Week-8	59.43 ± 12.69		51.12 ± 8.56		0.015

*Data in Mean ± Standard Deviation (SD). Independent sample t-test. *p<0.05.*

Table 4 Correlation of changes (Δ) in melatonin levels and MoCA-Ina scores

Variable	r	p-value
Melatonin (Δ) and MoCA-Ina (Δ)	**0.035**	**0.822**

Spearman correlation test.

Discussion

In this study, the results showed that schizophrenia patients experienced better improvement in cognitive function after adjuvant melatonin therapy. The improvement appears to be greater in the treatment group than in the control group. This suggests that there is an effect of adjuvant melatonin therapy in improving cognitive dysfunction in schizophrenia patients.

This study was conducted among male patients with schizophrenia. Men were reported to experience worse cognitive dysfunction than women with schizophrenia [14]. Gender is thought to influence differences in neurotransmitters and brain function and response in schizophrenia patients. Women may be biologically more advantaged than men with regard to the neuroprotective effects of hormones on cognitive function, such as estrogen [15-17]. Therefore, this study focuses on men to avoid bias related to gender differences in cognitive function and response to treatment.

This finding is consistent with studies investigating the potential of oral melatonin supplements in the treatment of schizophrenia-like behaviour. Melatonin administration was able to improve cognitive functions, including working memory and social interaction [18]. Other studies also report the supportive evidence on the efficacy of melatonin to improve cognitive function [19,20].

Melatonin enhances neuroprotection through its antioxidant, anti-inflammatory and anti-apoptotic properties [21]. Schizophrenic patients are biochemically more oxidised, and to compensate, the body responds by reducing endogenous melatonin production. However, the antioxidant properties of the adjuvant melatonin may help to slow the rate of autoxidation by reducing about 83% of dopamine enzymatic oxidation and about 35.7% of dopamine autoxidation [22]. In addition, melatonin may also improve cognitive function or cortex-related cognition by regulating the tryptophan catabolic pathway that responds to stress or cortisol release [23]. Further, the antioxidant effect of melatonin may also protect the brain from oxidative stress and inflammation caused by sleep deprivation by improving sleep quality, leading to better cognition [20].

Furthermore, melatonin adjuvant may improve visuo-spatial/executive, language, memory and orientation dimensions of cognitive function. It may be due to its ability to enhance neurotransmitter functions such as serotonin, dopamine and acetylcholine, which are involved in attention, memory and mood regulation. It may also increase neuroplasticity to adapt and change in response to new experiences, which can improve cognitive flexibility, problem solving and memory [20].

In schizophrenia patients receiving risperidone therapy, serum melatonin levels decreased in both groups. However, the decrease was smaller in patients receiving adjunctive melatonin therapy. Maiti et al. had reported that first-line antipsychotics such as haloperidol and risperidone significantly reduced melatonin levels [7]. However, Mishra et al. found that melatonin levels increased in patients on antipsychotic drug therapy with adjuvant melatonin therapy compared with controls [24]. This may suggest that adjuvant melatonin may help prevent antipsychotic-induced decreases in melatonin levels.

No significant correlation was found between changes in serum melatonin levels and changes in MoCA-Ina scores. However, the significant of changes in cognitive function showed in group that received risperidone and adjuvant melatonin therapy than control. The possibility may be involved other biological markers for cognitive significance or may be the efficacy of risperidone medication. Some studies have reported the benefit of risperidone medication on cognitive function in schizophrenic patients [25-28]. Another explanation may be that the dose of adjunctive medication given was not enough to show a significant correlation with cognitive improvement [24].

There are several limitations to this study. Firstly, the small sample size may not provide adequate power and therefore generalization should be made with caution. The insignificance of the correlation between melatonin and cognitive function may be influenced by the inappropriate dose of adjuvant prescribed, as the previous study reported the higher dose and showed efficacy [9], thereby limiting the strength of the current evidence. Additionally, concurrent risperidone therapy may have confounded the observed outcomes. Since risperidone is known to improve cognitive symptoms, it may have masked the potential effects of melatonin, resulting in a non-significant correlation. The variation in risperidone dosage (ranging from 4 to 6 mg/day) may have further introduced confounding effects. Moreover, the relatively short intervention period of only 8 weeks, although sufficient to observe initial effects, may limit the interpretation of long-term efficacy. An extended intervention period of 3–4 months would be more ideal for assessing sustained outcomes. The dosage of melatonin used in this study may also have been suboptimal, thereby limiting the strength of the findings. Future research should explore the efficacy of different melatonin doses and include placebo-only groups to more accurately evaluate and validate melatonin's role as an adjuvant therapy. Additionally, potential confounding factors should be carefully considered to enhance the reliability of the findings [29].

Conclusion

The current study concludes that adjuvant melatonin could improve cognitive function and slow the rate of melatonin decline in schizophrenia patients receiving risperidone. However, this study could not prove the significance of the correlation between the change in melatonin levels and the change in cognitive function. In addition, adjuvant melatonin may help prevent antipsychotic-induced decreases in melatonin levels.

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Beyond Survival: A 12-Year Chronicle of Pediatric Kidney Transplantation in Kazakhstan

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Abstract

Pediatric kidney transplantation (KTx) is a life-saving procedure for children with end-stage renal disease (ESRD), yet outcomes and risk factors remain underreported in Central Asia. We aimed to provide valuable insights into the long-term impact of KTx on pediatric patients.

Materials and methods: This article presents a retrospective review of 12-year experience of pediatric KTx at the «University Medical Center» Corporate Fund (Astana, Kazakhstan). The analysis included the records of 146 patients under the age of 18. The characteristics of the patients included causes of chronic kidney disease (CKD), clinical features and transplantation outcomes: overall survival (OS), graft survival (GS), graft loss (GL), transplant rejection (TR) and complications.

Results: Of 146 recipients (mean age 139 months, 56.8% male), the leading cause of ESRD was congenital anomalies of the kidney and urinary tract (CAKUT, 50.7%). Living donor KTx (LDKT) accounted for 73%, while Deceased donor KTx (DDKT). TR occurred in 18.5% of cases and was significantly associated with GL (OR = 7.19, 95% CI: 2.55-20.25, p < 0.001). GL occurred in 13.0% of patients, and mortality was 6.2%. Complications were reported in 43.8% of patients and were significantly associated with mortality (p = 0.034). No association between donor type (LDKT vs. DDKT) and rejection was found. Gender, diagnosis, BMI, age at transplantation, donor type were not significant predictors of GL or patient death.

Conclusion: Pediatric KTx in Kazakhstan demonstrates promising outcomes, with relatively low mortality (6.2%) and GL (13.0%) rates. TR (occurred in 18.5% patients) significantly predicts GL, while post-Tx complications (occurred in43.8% of patients) are associated with decreased patient survival. These findings support the need for improved early monitoring and long-term management strategies to optimize outcomes.

Keywords: kidney transplantation, child, graft loss, rejection, complication, survival analysis.

Introduction

Pediatric KTx is a cornerstone of treatment for children with ESRD, offering a significant improvement in survival rates and quality of life compared to dialysis [1]. Advances in surgical techniques, immunosuppressive therapy (IST), and post-Tx care

have dramatically improved short-term outcomes for pediatric KTx recipients, with graft survival rates and patient survival increasing over the past few decades [2]. However, the pediatric transplant population presents unique challenges, including managing the risks of TR, infections, and the long-term impact of IST on growth and development [3].

The development of organ transplantation in Kazakhstan began not long ago, but it is progressing at a rapid pace. According to studies conducted, despite the annual increase in the number of kidney transplants in Kazakhstan, the number of patients in need of transplantation is also increasing. Notwithstanding, the frequency of DDKT in Kazakhstan is significantly lower compared to other countries. This may be due to poor public awareness as well as ethical and religious beliefs [4, 5, 6].

Semenova et al. conducted a comprehensive analysis of the national heart transplantation program in Kazakhstan spanning 12 years (2012-2023) and found that the recipient's pre-surgery blood levels of creatinine and total bilirubin, along with the presence of infection or sepsis and the use of extracorporeal membrane oxygenation post-surgery were identified as key factors influencing the survival of heart transplant patients [7]. The conducted study on a 7-year experience of liver transplantation in 131 adults showed an increase in patient survival and an improvement in transplantation outcomes over time [8]. In another study, an increase in the frequency of organ transplants was noted and key problems were highlighted, such as a shortage of donor organs and large distances between deceased-donor organs-donating hospitals and transplant centers [9]. A retrospective study of pediatric KTx in Kazakhstan revealed that critical shortage of donor organs remains a significant barrier, emphasizing the need for improved organ donation programs and legislative support for post-mortem donations [10]. Although KTx is known to improve patients' quality of life globally, in Kazakhstan, overall well-being - as assessed by the WHO-5 Well-Being Index - remains moderate. This outcome is influenced by factors such as educational attainment, access to healthcare, satisfaction with medical information, and levels of anxiety, highlighting the need for enhanced post-transplant care strategies [11].

Despite notable progress in the field of organ transplantation in Kazakhstan over the past decade, there remains a significant gap in published data specifically addressing clinical outcomes of KTx in the pediatric population. While adult transplantation has received increasing attention in regional studies, pediatric KTx remains underrepresented in the scientific literature. This article presents a retrospective review of the 12-year experience of pediatric KTx at the «University Medical Center». The findings aim to contribute to the regional and global understanding of pediatric KTx outcomes, highlight areas for improvement in clinical practice, and support the development of national guidelines tailored to the needs of pediatric transplant recipients in Kazakhstan.

Methods

The analysis included the treatment outcomes of all patients who underwent KTx at the «University Medical Center» Corporate Fund under the age of 18 years from November 2012 to July 2024. All collected data were retrospective and obtained through the electronic medical records system. No patient was excluded during analysis. Missing data were handled using listwise deletion; only cases with complete data for the variables of interest were included in the final analysis. The characteristics of the patients included sex, diagnosis, blood type, prior dialysis before KTx, age at the time of transplantation, height, weight, body mass index (BMI), presence of TR, complications and GS, as well as OS. The causes of CKD 5 were studied. The immunosuppressive protocol included interleukin-2 receptor antibodies (basiliximab) 10 or 20 mg (based on patient's weight) or rabbit antithymocyte globulin 1,0 – 1,5 mg/kg/day, followed by Methylprednisolone 600 mg/m2, varied depending on the type of donor (LDKT or DDKT). In patients who received transplantation from a LDKT from 2012-2022, IST started 24 hours before transplantation. Since 2023 IST was initiated 72 hours before transplantation according to international guidelines. In patients who received transplantation from a DDKT IST began during the surgery. The frequency of TR was assessed. Frequencies and profiles of complications after transplantation were analyzed. Post-Tx complications were identified through retrospective chart review and categorized based on their clinical nature. Complications were grouped into the following categories: infectious, urological, cardiovascular, thrombotic, central nervous system (CNS)-related, and adverse effects of IST. Complications were further classified based on timing: early (within 30 days post-Tx) and late (beyond 30 days). Although a formal complication severity grading system (e.g., Clavien-Dindo) was not applied due to the retrospective nature of the study, clinical severity was inferred based on the need for medical intervention, hospitalization, or association with graft dysfunction or patient death. Recurrence of primary disease was evaluated separately. Delayed graft function (DGF) was defined as an immediate kidney failure persisting after transplantation for less than 2 weeks. Primary nonfunction (PNF) was defined as failed function of the transplanted kidney that necessitated continued maintenance dialysis. GS and OS investigated from the point of transplantation to the date of the last follow-up examination. GS was defined as eGFR greater than or equal to 30 ml/min while eGFR less than 30 ml/min and/or the need to restart dialysis, undergo nephrectomy, or receive retransplantation was identified as GL. The correlation between OS and GS, as well as the presence of complications in the postoperative period, was evaluated.

Statistical analysis was conducted using IBM SPSS Statistics 26 (USA) and Jamovi software (version 2.6.17). For qualitative data, the chi-square test or Fisher's exact test was used. Due to the exploratory nature of this study and the limited sample size, no formal adjustments for multiple comparisons were applied. Findings with borderline significance were interpreted with caution. Kaplan-Meier survival curves were used to assess patient survival after KTx over time (in month).

The study was approved by the Local Bioethics Commission of the «University Medical Center» Corporate Fund (Protocol No. 3 dated July 14, 2023).

Results

The total number of 146 patients with CKD 5 underwent KTx, among them 63 (43.1%) girls and 83 (56.8%) boys. The mean age at KTx was 139 (35 - 213) month, height - 133 (82 - 173) cm and weight - 32 (11 - 65) kg. The mean BMI was 17,29 kg/m2. Among pediatric recipients, the most common causes of CKD 5 were CAKUT (n=74) with renal hypoplasia the most common one (n=37). The spinal bladder was found in 7 patients. Additional causes included glomerular disorders (n=39) and genetic diseases (n=19). Among genetic causes following syndromes were found: NPHS1 and NPHS2 genes mutations,

coenzym Q6 and Q10 deficiencies, autosomal dominant and autosomal recessive polycystic kidney diseases, HDR gene mutation, Denys-Drash syndrome and Frasier syndrome, ADAMTS13 gene mutation. Another cause was cardiorenal syndrome in 2 patients. In 6 patients the cause of CKD 5 was unknown. All patients received pre-transplant dialysis. 106 (73%) patients underwent LDKT and 40 (27%) underwent DDKT. The overall table with patient characteristics, rejection, GL, and complications rate is presented in Tables 1 and 2.

Overall survival

Among 146 patients, 9 (6.2%) patients died in the mean period of 34 (0 - 101) month after KTx. The causes of death were: acute cardiovascular failure (CVF) (n=3), pulmonary embolism (PE) (n=2), aortic rupture (n=1), sepsis (n=1), acute bowel obstruction (n=1), and COVID-19 infection (n=1). OS showed a steady decline over time, with the majority of patients maintaining survival beyond the initial month post-Tx (Figure 1A). Out of these patients, 7 patients had complications, 4 patients had TR, and 3 patients had GL. A statistically significant difference in survival was observed between groups based on the presence or absence of post-Tx complications (log-rank test: $\chi^2 = 4.14$, $p = 0.042$), indicating that complications are associated with reduced survival (Figure 1B). When comparing survival by TR status, patients without rejection had better survival outcomes compared to those who experienced rejection. However, this difference did not reach statistical significance (log-rank test: $\chi^2 = 2.68$, $p = 0.102$; Figure 1C). Survival comparison based on GL also suggested a trend toward lower survival in those with GL, but the difference was not statistically significant (log-rank test: $\chi^2 = 2.32$, $p = 0.127$; Figure 1D).

Table 1 Patients' characteristics (N=146).

Variable	M (range) / n (%)
Age (month)	139 (35 - 213)
Sex Male Female	 83 (56.8%) 63 (43.1%)
Donor type LDKT DDKT	 106 (72%) 40 (28 %)
Height (cm)	133 (82 - 173)
Weight (kg)	32 (11 - 65)
Causes of CKD 5	
Congenital and developmental anomalies of the kidney and urinary tract (CAKUT) ● Renal hypoplasia (n=37)	74 (50.7%)
Spinal bladder	7 (4.7%)
Glomerular disorders	39 (26.7%)
Genetic conditions	19 (13.0%)
Cardiorenal syndrome	2 (1.4%)
Unknown	5 (3.4%)

AKI – acute kidney injury, CKD – chronic kidney disease, GBM – glomerular basement membrane, HUS – hemolytic uremic syndrome, IST – immunosuppressive therapy.

Table 2 Patients' outcomes and complications

Rejection Acute Chronic	27 (18.5%) 3 (11.1%) 24 (88.8%)
Graft loss	19 (13.0%)
Complications infectious urological side effects of IST renal vessels thrombosis cardiovascular failure CNS disorders pulmonary embolism (PE)	64 (43.8%) 25 (39.0%) 20 (31.2%) 14 (21.9%) 3 (4.6%) 3 (4.7%) 3 (4.7%) 2 (3.1%)
Delayed graft function	16 (0.1%)
Primary non-function	5 (0.03%)

ST – immunosupressive therapy, CNS – central nervous system

Complications

Different complications after KTx occurred in 64 (43.8%) cases: infectious - 25 (39.0%), urological - 20 (31.2%), side effects of IST - 14 (21.9%), renal vessels thrombosis - 3 (4.6%), CVF - 3 (4.7%), CNS disorders - 3 (4.7%), PE - 2 (3.1%). Complications were classified by type and timing (early vs. late). Among the 64 patients with complications, 24 (37.5%) experienced early complications (within 30 days post-KTx), while 40 (62.5%) developed complications later. Out of these 64 patients, GL was observed in 8 (12.5%) cases. Three (2.05%) patients had morphologically confirmed primary disease recurrence in the transplanted kidney, including IgA nephropathy recurrence in 2 (66.6%) patients and nephrotic syndrome recurrence in 1 (33.3%) patient. The treatment included methylprednisolone 600 mg/m2 IV infusion with subsequent switch to prednisone 2 mg/kg/day with slow taper back to low doses [12]. Patients with nephrotic syndrome recurrence were additionally treated with cascade plasmapheresis and Rituximab 375 mg/m2 [13]. These patients are alive and no one had GL.

Among recipients of LDKT, 41.9% experienced complications, compared to 48.8% among those who received DDKT. There was no statistically significant association between donor type and the occurrence of complications ($\chi^2 = 0.566$, $p = 0.452$).

A χ^2-square test revealed a statistically significant association between postoperative complications and mortality ($\chi^2 = 4.49$, $p = 0.034$). The crude odds ratio (OR) for mortality in patients with postoperative complications compared to those without was 4.91 (95% CI 0.98-24.52), suggesting a potentially increased risk of death, though the wide confidence interval reflects uncertainty due to the small number of events. It should be noted that the type and severity of postoperative complications were not specified in the dataset, which limits interpretation of the observed association.

Rejection

Out of 146 cases, 27 (18.5%) experienced rejection after KTx. Acute rejection (AR) was observed in 3 (11.1%) cases, chronic rejection (CR) – in 24 (88.8%) patients. There was no significant association between the type of donor (LDKT vs. DDKT) and the incidence of TR ($\chi^2 = 0.04$, $p = 0.843$). Among the KTx recipients, GL occurred in 37.0% of those who experienced TR (10 out of 27), compared to only 7.6% of those without TR (9 out of 119). A χ^2-square test showed a statistically significant association between TR episodes and GL ($\chi^2 = 16.9$, $p < 0.001$).

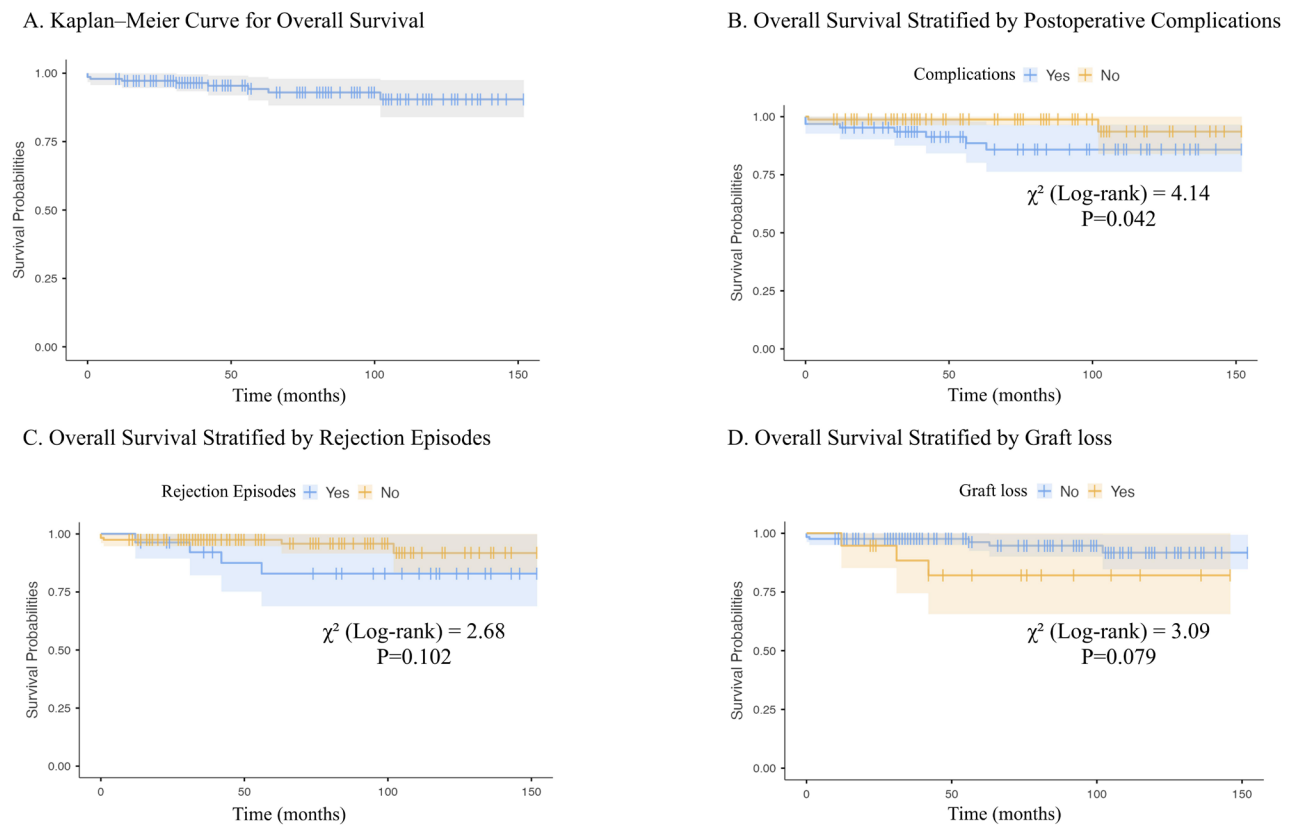

Figure 1 – **Kaplan-Meier Analysis of Overall Survival Among Pediatric Kidney Transplant Recipients.** Kaplan–Meier survival curves illustrating overall survival in the study cohort. (A) Overall survival curve for all patients. (B) Overall survival stratified by the presence of postoperative complications. Patients who experienced complications had significantly lower survival (p = 0.042). (C) Overall survival stratified by occurrence of rejection episodes (p = 0.102). (D) Overall survival stratified by presence of graft loss (p = 0.079). Shaded areas represent 95% confidence intervals. Tick marks indicate censored observations. Survival differences were assessed using the log-rank test.

The crude OR for GL in patients with rejection compared to those without was 7.19 (95% CI 2.55-20.25), indicating that TR was associated with a more than sevenfold increase in the odds of GL.

Mortality was higher in patients who experienced TR episodes (14.8%, 4 out of 27) compared to those without TR (4.2%, 5 out of 119). The association between TR and death was statistically significant ($\chi^2 = 4.29$, $p = 0.038$), though the continuity-corrected result did not reach significance ($p = 0.104$). The crude odds ratio (OR) for mortality in patients with TR compared to those without was 3.97 (95% CI: 0.99-15.90), suggesting that TR may be associated with a nearly four-fold increase in the odds of death. However, the confidence interval is wide and includes values close to 1, indicating uncertainty in the estimate.

Graft loss

GL was observed in 13.0% of patients (19 out of 146). Among those with GL, 15.8% (3/19) died, compared to 4.7% (6/127) among patients without GL. Although the mortality rate appeared higher among patients with GL, the difference was not statistically significant ($\chi^2 = 3.50$, $p = 0.061$), the continuity-corrected test ($p = 0.174$).

Complications were observed in 10 patients with GL: infectious – 4 (40%), renal vessels thrombosis – 2 (20%), IST side effects – 2 (20%), urological – 1 (10%), CVF – 1 (10%), CNS disorders - 1 (10%). In 5 (0.03%) patients PNF occurred. DGF occurred in 16 (0.1%) patients. The occurrence of complications did not show a significant association with GL ($\chi^2 = 0.111$, $p = 0.739$). GL occurred in 14.1% of cases with complications and 12.2% without complications. Other factors, including gender, diagnosis, BMI, age at transplantation, and donor type were not significant GL predictors.

Discussion

The first KTx was performed in 1954 by Joseph Murray and his colleagues from Harvard [14]. This surgery has undergone many changes since that time. Today, the kidney is the most frequently transplanted organ worldwide. Scientists around the world are studying various aspects of KTx, improving it and minimizing the risks. Transplantology in Kazakhstan has only recently started to gain momentum. However, there is still a lack of information on KTx outcomes in children. We decided to collect data on KTx over the last 12 years and analyze our own experience to understand the outcomes and problems.

Overall survival

The results of OS analysis highlight the significant impact of complications occurrence on early survival outcomes in pediatric KTx recipients, showing that complications are associated with reduced OS. These findings are consistent with previous studies indicating that complications such as DGF and urinary tract infections were prevalent and significantly

associated with poorer OS rates [15]. The study of Aneesh Srivastava et al. which included 1945 cases has shown that vascular complications are an important cause of early GL and include predominantly renal thrombosis (3-12%) and arterial stenosis (3-15%) [16]. Recent studies have also focused on investigating the side effects of IST and the potential genetic predisposition to their development [17].

Cause of CKD

Recent studies have highlighted the primary causes of CKD 5 in pediatric populations, providing valuable insights into the epidemiology of this condition. According to a systematic review by Harambat et al., the leading causes of CKD 5 in children include CAKUT, which account for approximately 30-40% of cases. This finding is consistent with earlier reports and recent data emphasize the increasing recognition of CAKUT as a predominant factor in pediatric kidney disease [18]. A multicenter study conducted by Otukesh et al. analyzed data from 907 pediatric patients undergoing KTx and found that the commonest causes of CKD 5 were structural disorders (45.6%) and glomerulonephritis (24.3%) [19]. A recent cohort study by Harada et al. reported that CAKUT is the most common underlying disease in pediatric CKD [20]. In a series of observations by Said et al. (Iraq, 2024), reflux nephropathy was the most frequent cause of terminal CKD among 39 pediatric patients who underwent KTx. [21]. In our observation series the same trend was observed with the CAKUT being the most common cause of CKD 5 and indication to KTx (50.7%), among which the most prevalent was kidney hypoplasia (n=34).

Type of donor

We have examined the association between the type of kidney donor (LDKT vs. DDKT) and the outcomes of GS and TR occurrence. According to the authors at the Radboudumc Amalia Children's Hospital center, median GS after KTx from a LDKT was longer compared to a DDKT: median survival was 20 years (95% CI 16-24) versus 12 years (95% CI 9-15) ($p = 0.01$). However, there was no difference in survival with paternal and maternal donors [22]. Other studies showed the importance to consider that highly sensitized patients face reduced opportunities for DDKT, which can influence both access to and outcomes of KTx, highlighting the need for individualized donor strategies in such cases [23]. In our center no association between donor type and TR was found. Additionally, no difference in GL occurrence was found between LDKT and DDKT. Our single-center experience may reflect consistent quality of care across both donor types, centralized post-transplant care, thereby minimizing the outcome differences traditionally reported in multicenter or heterogeneous populations.

Other donor and recipient characteristics

Previous studies have shown that maternal donation may be preferable to paternal donation because it is associated with a reduced incidence of AR in younger recipients (<4 years of age) [24]. However, other investigators have identified a negative association between GS and maternal donation. These studies demonstrated that paternal transplants lead to better long-term outcomes because of the larger size and number of male kidney nephrons. Trnka et al. showed that increasing age difference between donor and recipient was associated with decreased GS [25]. However, these findings were not supported by other studies [26]. According to our research, gender, diagnosis, BMI, age at transplantation, and donor type were not significant predictors of GL or patient death.

Rejection and GL

In recent years, studies showed that early GL is a key predictor of worse long-term outcomes [27]. It is shown that the AR potentially results in graft dysfunction or loss if not identified and managed in a timely manner [28]. However, our survival comparison based on GL suggested a trend toward lower survival in those with GL, but the difference was not statistically significant. The association between TR and subsequent GL has garnered significant attention in the field of pediatric nephrology. Oomen L et al. compared data from the literature with their own experience of KTx in 411 children between 1968 and 2020 and proved that AR and CR (75%), recurrence of underlying disease (5%) and thrombosis (6%) were the main causes of GL [29]. Our research showed that rejection history was strongly associated with GL ($p < 0.001$). Although it was found that patients without TR had better survival outcomes compared to those who experienced rejection, this difference did not reach statistical significance.

Implications for Clinical Practice and Policy

This 12-year review provides the first comprehensive evaluation of pediatric KTx outcomes in Kazakhstan and offers several important clinical and policy implications. The strong association observed between TR and GL underscores the need for improved early detection strategies and closer post-Tx monitoring. Incorporating emerging biomarkers and tailoring IST to individual risk profiles could significantly enhance graft longevity. Additionally, the link between postoperative complications and mortality highlights the critical importance of structured follow-up care and rapid intervention protocols. Individualized follow-up plans that account for patient-specific risk factors, such as comorbidities, may enhance early detection of adverse outcomes. Strengthening early monitoring practices — particularly within the first year post-Tx — may not only improve outcomes but also help to bridge existing gaps in care continuity, especially in resource-limited settings. Developing multidisciplinary care models that include infectious disease specialists, urologists, and transplant coordinators could mitigate these risks and improve survival rates. Strengthening local capacity for diagnostic testing, including histopathology and molecular analysis, may also enhance post-transplant care and inform therapeutic decision-making.

The findings also suggest broader implications for healthcare policy and donor program development in Kazakhstan. Despite a predominance of LDKT, our results revealed no significant differences in outcomes between LDKT and DDKT, indicating DDKT remains a viable and underutilized resource. One of the major challenges remains the limited availability of deceased donor organs, which continues to constrain transplant activity and delay access to transplantation for many pediatric patients. This shortage is influenced in part by public perception and low levels of societal awareness and acceptance of organ donation, particularly regarding deceased donors. Expanding public education and improving the legislative framework to support post-mortem donation could help address the critical shortage of donor organs in the country.

Study Limitations and Future Directions

This study has several limitations. The single-center

design restricts the generalizability of the findings to the national level, and the relatively small sample size limits the statistical power to detect effects for less frequent outcomes such as AR or death. The dataset lacked granularity for several potentially influential variables, such as HLA mismatch, drug adherence and socioeconomic status. Moreover, the study's follow-up period, although spanning 12 years, did not fully capture long-term complications beyond the first decade after transplantation, such as malignancy, chronic graft dysfunction, or psychosocial outcomes in young adulthood. We also recognize that multiple statistical tests were performed without adjustment for multiplicity. While this approach aligns with the exploratory aims of the study, it may increase the risk of Type I error, and thus, significant findings should be interpreted cautiously.

Future research should aim to expand this work through a multicenter, nationwide registry that can capture a broader range of clinical and demographic variability and allow for benchmarking against international standards. Prospective studies that integrate genetic, psychosocial, and environmental data may help identify individualized risk factors and enable more personalized treatment approaches. In addition, assessing long-term health-related quality of life and psychosocial functioning in pediatric transplant survivors is essential for informing holistic care strategies. Finally, evaluating the cost-effectiveness of new interventions, such as early biopsy protocols, educational donor campaigns, or molecular monitoring, can guide efficient allocation of healthcare resources. Cross-border collaboration with international pediatric transplant networks could further enhance capacity building, research innovation, and clinical excellence in Kazakhstan and Central Asia.

Conclusion

In our 12-year experience of pediatric KTx we observed various outcomes and challenges, providing insights into managing pediatric KTx recipients. Recent studies highlight congenital kidney anomalies as the leading cause of CKD 5 in children, and our data confirm that CAKUT, especially kidney hypoplasia, were the main causes of CKD 5. While our analysis showed an association between post-Tx complications and patient death, this finding should be interpreted with caution due to the small number of events and wide confidence intervals. These findings emphasize the importance of early intervention and monitoring to improve outcomes in pediatric KTx recipients. TR was found to be associated with GL, but its association with patient death was inconclusive due to statistical non-significance and wide confidence intervals. We did not observe statistically significant associations between donor type and GL or TR, though the sample size limits definitive conclusions. Future multi-center studies with larger populations are needed to further explore factors affecting graft survival and patient outcomes.

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Comparative Analysis of Regional Mortality Rates and Causes in the Turkistan and Other Regions of Kazakhstan: Data from Unified National Electronic Healthcare System 2018-2023

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Abstract

Background: Turkistan is the most densely populated region of Kazakhstan, with 75% of its population residing in rural areas. The region holds for a rich historical and cultural background as it was recently named as the spiritual capital of the Turkic world. Despite the importance of the place, it has its own challenges in the healthcare field, especially with mortality rates. This study aimed to discover and analyze the structure of the death rates in Turkistan region in order to provide a reliable data for future research projects.

Methods: Mortality data were obtained from the Unified National Electronic Health System of Kazakhstan for 2018-2023 years. The dataset comprised 1,073,382 individual death records. The classification of causes of death followed the WHO Global Health Estimates methodology. All statistical analyses were performed using the Python 3.11.

Results: Turkistan region had 632 deaths per 100,000 population, which was lower than the national average death rate in 2018, however, during the COVID pandemic the numbers spiked to 882 deaths per 100,000 population. Despite high numbers during COVID, the region has managed this problem and by 2023 remained in the top 3 regions with the lowest death rates. Additionally, the following diseases were identified as the leading causes of death by 2023: other neuropsychiatric disorders, cerebrovascular diseases, chronic obstructive pulmonary disease.

Conclusion: Turkistan region needs to develop targeted health programs against the current top death causing diseases, with the emphasis on enhancing the diagnosis, treatment, coding and registration capabilities.

Keywords: mortality, age-standardized mortality rates, disease trends, Turkistan region, Kazakhstan.

Introduction

Turkistan region, located in the south of the Republic of Kazakhstan, covers approximately 4.3% of the country's total territory. The region is characterized by a sharply continental climate, with hot, arid summers and relatively mild winters [1]. In 2018, the former president of Kazakhstan renamed the South Kazakhstan region to the Turkistan region, causing the shift of administrative center from Shymkent to Turkistan [1]. As of June 1, 2025, the population of Turkistan region totaled 2,151,000 people, of whom 543,500 (25.3%) reside in urban areas and 1,607,500 (74.7%)

in rural areas [2]. With an average population density of over 18 people per square kilometer, the region remains one of the most densely populated in the country, placing increased demands on the healthcare system and its resilience.

In addition to its geographical and demographic characteristics, Turkistan holds significant cultural and historical heritage. The region is regarded as the spiritual center of the Turkic world and is known for its religious, architectural, and archaeological monuments. This cultural uniqueness influences the lifestyle of the local population, health-related attitudes, and the perception of preventive and medical interventions [3].

According to official statistics for 2023, the crude mortality rate in Turkistan region was 4.63 per 1,000 population, which is below the national average. The leading causes of death include circulatory system diseases (approximately 23% of all registered cases), neoplasms (9.3%), external causes including accidents, poisoning, and injuries (9%), as well as diseases of the respiratory and digestive systems (7.2%) [4]. These data reflect the predominance of chronic non-communicable diseases, aligning with broader epidemiological trends observed in Central Asian countries and other middle-income nations.

Despite the availability of aggregated statistics, a comprehensive analysis of the mortality structure at the regional level is still lacking. Most existing studies focus on national or interregional demographic indicators, making it difficult to develop strategies that are locally relevant. As a result, mortality patterns specific to Turkistan region, including territorial disparities, age and sex structures, and cultural-medical aspects, remain insufficiently explored.

The aim of this study is to conduct an in-depth analysis of the main causes of mortality in Turkistan region using the available epidemiological data. The findings are intended to fill a gap in the scientific literature and provide an up-to-date understanding of mortality patterns in the region. This, in turn, can serve as a foundation for evidence-based health policy, the identification of priority areas for medical intervention, and the development of targeted prevention programs that take into account the demographic and cultural characteristics of the local population.

Methods

This study is a retrospective descriptive analysis based on national mortality data from Kazakhstan, specifically focusing on the Turkistan region.

Data Sources

Mortality data were obtained from the Unified National Electronic Health System (UNEHS) of Kazakhstan for the period of 2018-2023 years [5, 6]. The dataset comprised 1,073,382 individual death records, of which 70 187 cases were attributed to Turkistan, and included the date of death, age at death, place of death, sex assigned at birth, ICD-10 coded cause of death, and the region. After excluding 39 duplicate entries, the final sample included 1,073,343 death records. This dataset reflects deaths registered within the public healthcare system and does not account for deaths in penitentiary or military systems. Consequently, annual totals are approximately 5-8% lower than those reported by the World Health Organization (WHO) and the Bureau of National Statistics of Kazakhstan (BNSK) [7, 8].

Cause-of-death coding was conducted by certified medical professionals. For deaths outside medical institutions, a physician performed an external examination and determined the cause based on available medical history and, where applicable, autopsy findings. In-hospital deaths were documented by attending physicians.

Population data were sourced from the BNSK and included yearly totals (2018-2023), disaggregated by age (single-year groups), sex, and region.

Data Analysis

All statistical analyses were performed using the Python 3.11. The following libraries were used: pandas (v2.2.2) for data wrangling, statsmodels (v0.14.2) and scipy (v1.13.1) for statistical inference, matplotlib (v3.9.1) and seaborn (v0.13.2) for data visualization, geopandas (v1.0.1) for geospatial analysis, and scikit-learn (v1.5.0) for model implementation.

Mortality rates were computed using standard epidemiological formulas. Age-standardized mortality rates (ASMR) were calculated using the direct method with the total Kazakhstan population as the reference. Age was grouped by single-year intervals from 0 to 84 years, with 85+ as the final category.

Temporal dynamics of mortality were visualized using daily-level calendar heatmaps, where deviations from expected death frequencies were assessed with Chi-squared tests and visualized through standardized residuals. Seasonal decomposition was applied to monthly aggregates to extract trend, seasonal, and residual components.

Linear regression models were fitted for each region to evaluate ASMR trends from 2018 to 2023. The year was used as the independent variable, and ASMR as the dependent variable. Slopes indicated annual changes; significance was evaluated at $\alpha=0.05$. Models were re-run excluding 2020 and 2021 to account for pandemic-related distortions.

Cause of Death Classification

The classification of causes of death followed the WHO Global Health Estimates (GHE) methodology [9]. ICD-10 codes were mapped to standardized cause categories as per WHO guidelines. Two modifications were introduced:

1. ICD-10 codes absent in the WHO GHE mapping were categorized based on clinical review.
2. Codes G93.4, G93.6, and G93.8, which inflated the 'Other neurological diseases' category, were grouped into a new category termed 'Other disorders of the brain'.

More detailed information on the redistributions of the categories can be found in the study published earlier [10].

Results

General ASMR Trends (2018-2023)

The tables below present data on the age-standardized mortality rate (ASMR) in various regions of Kazakhstan from 2018 to 2023.

The table 1 presents raw ASMR values across all regions of Kazakhstan without the trend analysis. However, the data structure allows for the observation of mortality levels and the comparison of Turkistan with other regions for specific years.

In 2020 and 2021, Turkistan region experienced two pronounced mortality peaks, likely associated with the impact of the COVID-19 pandemic. In subsequent years, the indicators decline: by 2023, the ASMR level even falls below that of 2018.

In terms of interregional comparison, Turkistan showed a higher-than-average national mortality rate during the pandemic years but did not fall into the group of regions with the most severe

Table 1 Overall age-standardized mortality rate trends (per 100,000) and statistical significance of the trend over six-year period

Region	2018	2019	2020	2021	2022	2023	slope	p-value
Turkistan	632,01	639,93	860,86	882,23	603,81	596,71	-7,53	0,84
Zhambyl	659,79	658,49	816,29	1015,87	619,99	618,90	-3,44	0,94
North Kazakhstan	751,64	777,65	889,92	990,30	756,13	734,23	-1,46	0,96
West Kazakhstan	718,02	701,99	873,15	991,80	691,60	690,57	-1,42	0,97
Pavlodar	743,11	743,92	880,50	1027,00	753,82	703,40	-0,64	0,99
Akmola	704,99	693,30	846,33	985,19	699,08	677,95	0,60	0,99
Kostanay	677,64	688,93	819,01	961,77	748,36	703,59	12,88	0,67
Mangystau	574,38	575,84	804,13	888,94	633,34	628,74	15,12	0,68
Atyrau	621,71	513,37	866,91	1004,15	688,56	633,49	20,62	0,68
Kazakhstan	580,80	583,48	757,94	937,92	678,38	655,56	23,96	0,52
Shymkent	537,93	534,95	845,08	1035,30	680,34	672,82	37,17	0,48
Kyzylorda	450,89	506,94	765,21	839,23	640,26	638,01	40,28	0,30
Aktobe	459,60	559,26	736,03	923,91	666,23	674,31	45,21	0,28
Astana	430,10	388,18	660,47	897,41	608,22	588,38	48,24	0,32
Almaty Region	382,07	360,89	446,50	519,23	77,45	576,16	48,37	0,00
Almaty	394,49	379,18	526,39	947,97	692,99	643,97	74,58	0,16
East Kazakhstan	416,65	398,68	468,17	588,69	775,96	728,03	80,26	0,01
Karaganda	415,98	454,55	634,76	864,51	796,47	749,06	83,45	0,03
Abai	-	-	-	-	685,20	698,73	-	-
Ulytau	-	-	-	-	800,08	778,42	-	-
Zhetysu	-	-	-	-	640,88	630,32	-	-

situations, such as Karaganda, Pavlodar, and North Kazakhstan regions. In 2022-2023, the ASMR levels in Turkistan became comparable to those in other southern regions – Shymkent, Kyzylorda, and Zhambyl – and by 2023 even dropped below their levels.

In terms of ASMR, the region is positioned between the so-called 'high-burden' areas with persistently elevated mortality and large metropolitan cities such as Almaty and Astana, where the rates are lower.

Thus, even without calculating trend indicators, it can be concluded that Turkistan region demonstrated a high level of post-COVID recovery in terms of mortality rate, which allowed to stay in top 3 regions with the least deaths.

The table 1 also contains the calculated parameters of the linear trend: slope and p-value (statistical significance of the trend). This allows for the assessment of both absolute indicators and their changes over the six-year period.

Mortality in the region increased sharply in 2020-2021, most likely due to the COVID-19 pandemic, and then returned to a level below the pre-COVID baseline (in 2023, it was lower than in 2018) [11]. However, the p-value greater than 0.05 indicates that the overall trend is not statistically significant, meaning these fluctuations may be random and do not reflect a stable increase or decrease in mortality.

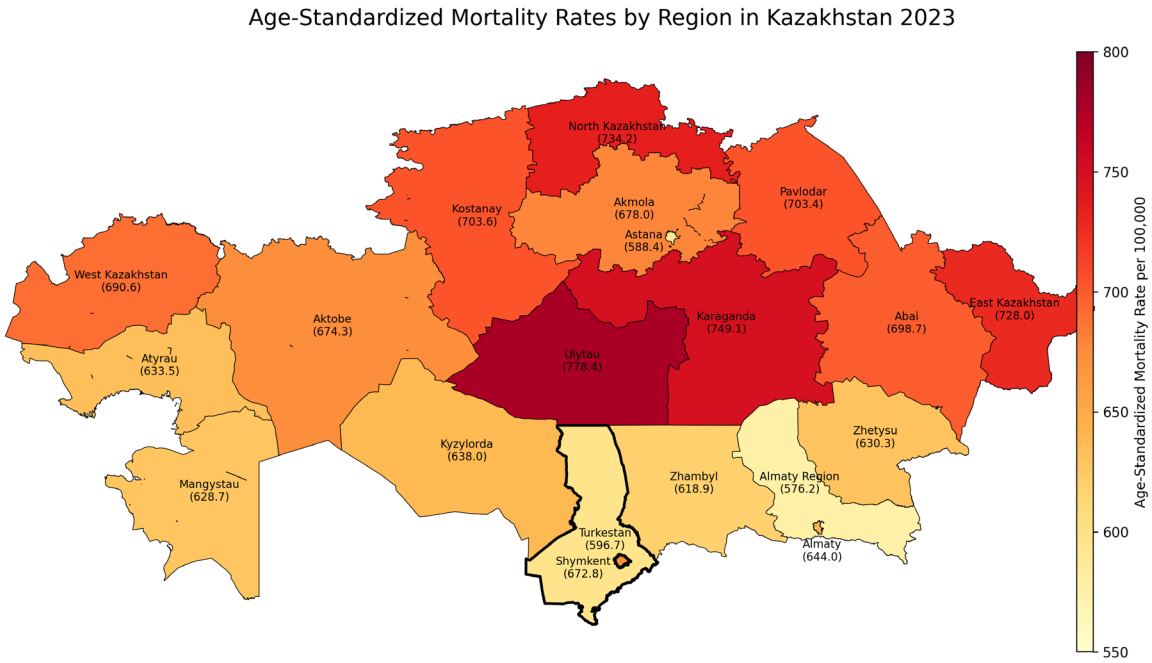
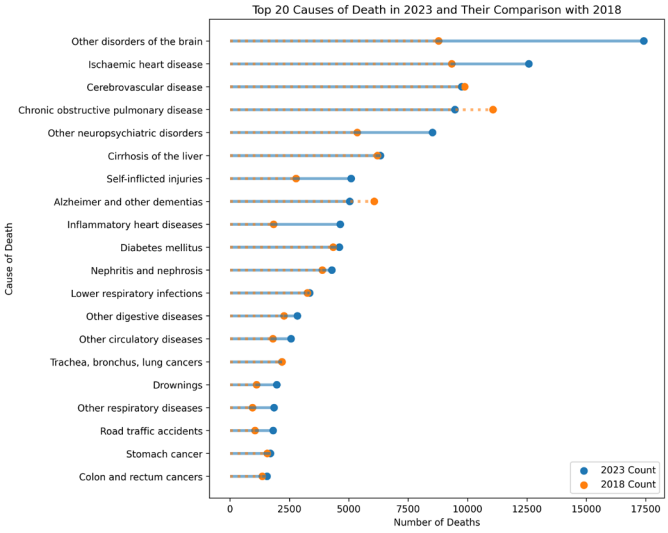
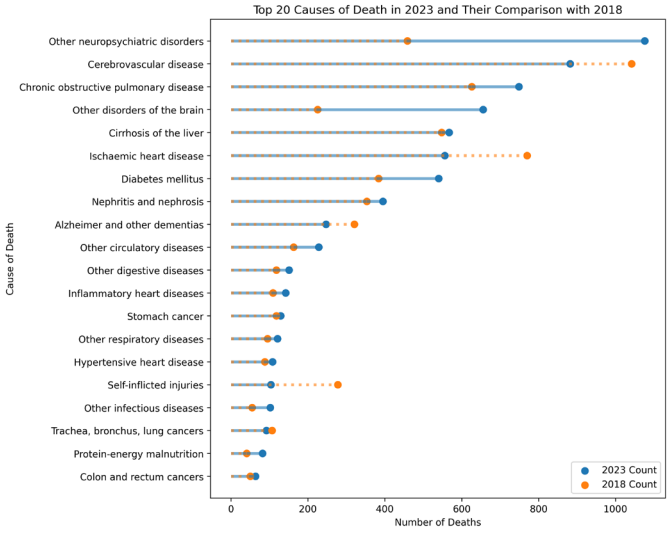


Figure 1 – ASMR by regions in Kazakhstan (2023)



2a



2b

Figure 2 – Comparison of top 20 causes of death in Kazakhstan (2a) and of Turkistan region (2b) for 2018 and 2023 years

Cause of Death	Rank in 2018	Rank in 2019	Rank in 2020	Rank in 2021	Rank in 2022	Rank in 2023
Other disorders of the brain	4	2	1	2	1	1
Ischaemic heart disease	3	4	2	1	2	2
Cerebrovascular disease	2	3	3	4	3	3
Chronic obstructive pulmonary disease	1	1	4	5	4	4
Other neuropsychiatric disorders	7	6	6	6	5	5
Cirrhosis of the liver	5	5	7	7	6	6
Self-inflicted injuries	11	11	12	13	8	7
Alzheimer and other dementias	6	7	8	9	7	8
Inflammatory heart diseases	14	13	13	11	10	9
Diabetes mellitus	8	8	9	8	9	10
Nephritis and nephrosis	9	9	10	12	11	11
Lower respiratory infections	10	10	5	10	12	12
Other digestive diseases	12	12	14	15	13	13
Other circulatory diseases	15	15	15	14	14	14
Trachea, bronchus, lung cancers	13	14	16	17	15	15
Drownings	20	18	21	18	16	16
Other respiratory diseases	22	22	18	16	19	17
Road traffic accidents	21	21	22	19	17	18
Stomach cancer	16	16	19	20	18	19
Colon and rectum cancers	17	17	20	21	20	20

3a

Cause of Death	Rank in 2018	Rank in 2019	Rank in 2020	Rank in 2021	Rank in 2022	Rank in 2023
Other disorders of the brain	4	2	1	2	1	1
Ischaemic heart disease	3	4	2	1	2	2
Cerebrovascular disease	2	3	3	4	3	3
Chronic obstructive pulmonary disease	1	1	4	5	4	4
Other neuropsychiatric disorders	7	6	6	6	5	5
Cirrhosis of the liver	5	5	7	7	6	6
Self-inflicted injuries	11	11	12	13	8	7
Alzheimer and other dementias	6	7	8	9	7	8
Inflammatory heart diseases	14	13	13	11	10	9
Diabetes mellitus	8	8	9	8	9	10
Nephritis and nephrosis	9	9	10	12	11	11
Lower respiratory infections	10	10	5	10	12	12
Other digestive diseases	12	12	14	15	13	13
Other circulatory diseases	15	15	15	14	14	14
Trachea, bronchus, lung cancers	13	14	16	17	15	15
Drownings	20	18	21	18	16	16
Other respiratory diseases	22	22	18	16	19	17
Road traffic accidents	21	21	22	19	17	18
Stomach cancer	16	16	19	20	18	19
Colon and rectum cancers	17	17	20	21	20	20

3b

Figure 3 – Top causes of death for Kazakhstan (3a) and for overall Turkistan region (3b) during the 2018-2023

Turkistan Compared to Other Regions:

- ASMR values in Turkistan were higher than in the urbanized centers such as Almaty, Astana, or Almaty region at the beginning of the period, but lower than in northern and eastern regions like Pavlodar and North Kazakhstan, where mortality is consistently high.
- Some regions (e.g., Karaganda and East Kazakhstan) showed a statistically significant increase in mortality ($p < 0.05$), while no such trend is observed in Turkistan.

The analysis showed no significant trends in the Turkistan region ($p = 0.84$). The overall trend for Kazakhstan was also not statistically significant ($p = 0.518$).

Leading Causes of Mortality in Turkistan Region Compared to Overall Trends in Kazakhstan

For Turkistan region (Figure 2b) the top mortality cause was 'Other neuropsychiatric disorders', which has more than doubled over the 6-year period, together with 'Other disorders of the brain' category. Other diseases were more or less stable over the period, except for 'Cardiovascular diseases', 'Ischemic heart disease', and 'self-inflicted injuries', which showed sharp decline as a cause of death.

By 2023, the top cause of the death in Kazakhstan as a whole (Figure 2a), was the 'Other disorders of the brain'; from 2018 to 2023 the cases of mortality from this disease have doubled. Other deadly diseases that showed continuous growth were 'Ischemic heart disease', 'Other neuropsychiatric disorders', 'self-inflicted injuries' and 'Inflammatory heart disease'. Other diseases showed almost no growth or stability over the period except for 'Chronic obstructive pulmonary disease' and 'Alzheimer and other dementias', which showed slight decline.

At the national level, the structure of causes of death is characterized by greater stability compared to the Turkistan region (Figures 3a and 3b). For Turkistan region, the 'other neuropsychiatric disorders' have held the leading position by 2023, rising from fifth place in 2018. The top-5 cause of death by 2023 were cerebrovascular diseases, chronic obstructive pulmonary disease (COPD), other diseases of the brain, and cirrhosis of the liver. All of the other causes of death in Turkistan region stayed more or less in a stable position through 6-year period, except for other infectious diseases, malnutrition issues, cancers of large intestine, which demonstrated a sharp rise from 2018 to 2023.

On the other hand, the data for Kazakhstan as a whole demonstrates a more stable structure of causes of death, dominated by central nervous system and neuropsychiatric diseases. Except for some diseases, there was an absence of sharp fluctuations provided by stable epidemiological environment and a more systematic approach to data recording and diagnosis nationally.

Table 2 presents a quantitative comparison of mortality shares across key disease categories between the Turkistan region and Kazakhstan as a whole. The data allows for an interpretation of differences in the mortality structure at the regional versus national levels based on the presented indicators.

The most pronounced difference is observed in the category of 'other neuropsychiatric disorders' in the Turkistan region, they account for 33.2% of all deaths, whereas the national average was only 17.2%. This nearly twofold gap may result from a combination of factors: differing availability of psychiatric care, possible overdiagnosis in the region, characteristics of an aging population, as well as probable differences in death cause coding practices. It should be noted that such a high share indicates either a structural epidemiological anomaly or a specific or inaccurate diagnostic strategy in the region, warranting further audit.

Table 2 The total share of causes (shown in percentages) for mortality in Turkistan region and Kazakhstan

#	Cause	Turkistan region	Kazakhstan
1	Other neuropsychiatric disorders	33,2%	17,2%
2	COPD	11,9%	7,9%
3	Alzheimer's	10,4%	11,3%
4	IHD	8,4%	9,4%
5	Other disorders of the brain	6,7%	19,5%
6	Cerebrovascular	6,4%	6,1%
7	Nephritis	4,4%	3,9%
8	Cirrhosis	2,8%	1,2%
9	Diabetes	2,8%	2,7%

The COPD and liver cirrhosis are also significantly higher in Turkistan compared to national figures – 11.9% versus 7.9% for COPD and 2.8% versus 1.2% for cirrhosis. These data may reflect deteriorated environmental conditions, high prevalence of risk factors (including smoking and alcohol abuse), as well as possible differences in access to medical care. The high COPD share also correlates with previously presented dynamic data, where this cause periodically rose to the top three causes of death in the region.

At the same time, the category 'Other disorders of the brain' shows a noticeably lower rate in Turkistan – 6.7% versus 19.5% nationwide. This may be due to some conditions coded as neurological disorders in the national statistics being classified as neuropsychiatric disorders in Turkistan, further highlighting potential differences in classification approaches and cause-of-death coding.

Lower rates are also observed for ischemic heart disease (IHD) – 8.4% in Turkistan versus 9.4% nationally. This result confirms the previously noted trend of a declining relative significance of IHD in the region's mortality structure. This may reflect either positive effects from preventive programs and therapies or a shift of death causes toward other categories (e.g., 'other neuropsychiatric disorders').

Alzheimer's disease and diabetes show comparable levels in both regions (approximately 10-11% for Alzheimer's and 2.7-2.8% for diabetes), indicating relative uniformity in the diagnosis of these chronic conditions. However, even here it can be assumed that some dementias may have been included in the broader 'Other neuropsychiatric disorders' category in the regional data, reducing the official Alzheimer's share in Turkistan.

Discussion

This is the first regional retrospective study comparing the mortality trend and top causes of death in Turkistan and other regions of Kazakhstan in large national electronic healthcare cohort. Overall, at the beginning of the 6-year period the Turkistan region showed lower death rates compared to the average national level, however, during the peak of COVID-19 pandemic, it rose up by 40% of the initial number and reached

882 deaths per one hundred thousand population. The analysis of the 6-year mortality trend for Turkistan region has not identified any statistically significant differences.

While Kazakhstan's top leading cause for mortality by far was 'Other disorders of the brain', which can be partially explained by the soaring death rate from stroke [12], the structure of Turkistan region's death rate was predominated by neuropsychiatric disorders, which has shown nearly a twofold excess compared to a national average. Such data may indicate either a true epidemiological burden or differences in coding, diagnostics, and recording practices, which in turn may be associated with a shortage of specialists, especially in psychiatric and neurological services, as well as weak coordination between healthcare institutions and statistical authorities.

Consistently high mortality rates from the COPD and liver cirrhosis also drew attention. Likely causes include a high prevalence of risk factors – smoking, alcohol consumption, environmental pollution [13], combined with limited access to specialized medical care, particularly in rural areas [14, 15]. At the same time, these issues receive insufficient attention in state prevention and treatment programs, worsening the situation. Such conclusions would be impossible without local analysis, as the national data does not reflect these regional peculiarities.

Despite the recorded decline in the ASMR in 2023 compared to peak pandemic values, the results do not indicate a stable positive trend ($p=0.84$). This highlights both the instability of the epidemiological situation and potential limitations of the current death registration and monitoring system. In light of this, it is worth recalling that the 75% of Turkistan region's population reside in rural areas, which in turn makes it significantly harder to change the situation on the ground. To highlight the poor healthcare environment in rural areas of Kazakhstan, in the article done by Syssoyev and colleagues, it is mentioned that countryside residents, with congenital heart disease, had significantly lower chances of survival compared to their counterparts in urban settings [16]. The absence of a full-fledged, systematized regional mortality statistics system verified against primary medical records remains one of the key problems [10]. The establishment of a unified data quality control system, regular retraining of medical and statistical personnel, and expansion of medical expertise infrastructure appear to be necessary steps towards improving the reliability and informativeness of official statistics.

This study is the first to utilize individualized mortality data over a six-year period focusing on the Turkistan region. This allowed for not only to describe the general mortality levels but also to identify the unique regional characteristics of mortality indicators. The application of international methodological standards and modern statistical tools enhanced the reliability of the results.

As for the limitations of the study, the dataset used in this study was obtained from public healthcare facilities, meaning that the deaths within military force systems were omitted. This might explain the difference of up to 8% between the WHO and BNSK reports and the dataset used in this study. Another restriction of this study could be one of the applied cause-of-death coding practices, when physicians had to perform manual searches for possible causes of death in order to fill in the blank spaces. This practice could affect the data accuracy. Last but not least, the general limitations for this kind of articles are the lack of data on socio-economic factors, medical infrastructure, and population behavioral characteristics, as well as possible discrepancies in cause-of-death coding practices between regions and a shortage of qualified personnel in rural areas.

Conclusion

This study represents a first step towards a systematic understanding of the structure and dynamics of mortality in the Turkistan region – one of the most populous and culturally significant regions of Kazakhstan. Unlike national reviews that aggregate data for the entire country, this analysis has, for the first time, identified region-specific patterns that differ in composition and intensity from the national average. The main causes of death for the Turkistan region were neuropsychiatric disorders, the COPD, and liver cirrhosis. These findings underscore the need to reconsider the priorities of regional health policy and to develop locally adapted prevention and care measures.

However, the identified patterns not only reflect epidemiological realities but also result from serious systemic limitations. During the study, it became evident that the Turkistan region suffers from an acute shortage of reliable, comprehensive, and comparable statistical data on causes of death. In many cases, the death classification is made with large assumptions or inaccuracies, which can distort both the actual picture and the decisions based on it. The problem is exacerbated by a lack of qualified personnel in medical examination and statistics, especially in remote and rural areas where the overwhelming majority of the region's population resides.

Given these circumstances, further research should focus not only on deepening epidemiological analyses but also on developing the infrastructure for mortality registration and analysis itself. This includes implementing a unified accounting system, digitizing primary medical documentation, training healthcare workers for accurate ICD-10 coding, and establishing regional centers for monitoring and evaluating statistical quality. Without these changes, any measures to improve population health risk being inadequate or ineffective.

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Chemotherapy-Induced Endothelial Dysfunction: a Bibliometric Analysis of Research Trends from 1994 to 2024

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Abstract

Chemotherapy-induced endothelial damage is a complex phenomenon that contributes to cardiovascular toxicity and cellular injury in cancer patients. This study presents a comprehensive bibliometric analysis of research on chemotherapy-induced endothelial dysfunction, evaluating global publication trends, key contributing countries, institutions, authors, journals, and research themes.

Methods. The research uses a descriptive bibliometric approach to gather and analyze data from 3,430 publications indexed in the Web of Science and Scopus databases, covering the years 1994-2024. The bibliometric analysis includes research articles and review papers written in English that explore endothelial dysfunction in chemotherapy patients. Data analysis was performed using RStudio and the Bibliometrix R package, with VOSviewer employed for visualization.

Results. Annual publication trends showed fluctuations, peaking in 2005, with continued scientific interest in recent years. The United States led in publication volume (1,328 articles), followed by China and Japan. High levels of international collaboration were observed, particularly between the United States and European countries. Harvard Medical School and the National Cancer Institute were among the top contributing institutions. Prominent authors included Wang and Kim, while Cancer Research was identified as the most influential journal, underscoring the interdisciplinary relevance of this topic. The analysis also revealed evolving research trends focusing on angiogenesis, VEGF inhibitors, and international collaboration.

Conclusions. This analysis underscores the need to integrate vascular toxicity monitoring into cardio-oncology care to improve early detection and patient outcomes. Given the recent introduction of new classes and groups of chemotherapeutic drugs into practice and the growing body of knowledge in this area, a renewed surge of interest in this topic can be anticipated. Further research will support evidence-based guidelines for managing endothelial damage in cancer therapy.

Keywords: endothelial dysfunction; vascular toxicity; cancer research; chemotherapy; bibliometric analysis; publication trends.

Introduction

Cardiovascular diseases account for approximately 18 million deaths annually, while cancer causes over 9 million deaths worldwide [1]. Chemotherapy has significantly improved cancer survival but is associated with severe cardiovascular complications [2]. As cancer treatments advance, the number of survivors increases; however, they remain at a heightened risk of cardiovascular morbidity and mortality [3].

The endothelium is NOT merely a passive lining of blood vessels but an active organ with structural and functional roles. It regulates vascular tone, fluid exchange, hemostasis, inflammation, and angiogenesis

[4]. Solid tumors induce new blood vessel formation to sustain growth, and tumor endothelial cells express specific markers targeted by anti-angiogenic therapies [5]. These treatments, including VEGF inhibitors and endothelial proliferation blockers, have expanded oncology options [6,14].

Some conventional cytotoxic agents exhibit unintended anti-angiogenic effects, functioning as "accidental" anti-angiogenic drugs [5]. Miller et al. proposed criteria for identifying such chemotherapies that damage endothelial cells at concentrations below those needed to kill tumor cells [7]. Mechanisms of vascular injury include direct endothelial damage, coagulation activation, autonomic dysfunction, vasculitis, and fibroblast activation [8-12].

Endothelial dysfunction (ED) is an early predictor of cardiovascular events and correlates with traditional risk factors, potentially progressing to severe disease even in low-risk patients [13-15]. Despite treatment advances, ED remains a major mortality contributor, with systemic inflammation from chemotherapy-induced cellular damage playing a key role [16].

ED leads to increased vascular permeability and occurs in conditions such as diabetes, Alzheimer's disease, atherosclerosis, and ischemia-reperfusion injury, with oxidative stress as a central mechanism [17]. Although chemotherapy aims to eliminate cancer cells, many drugs are more toxic to endothelium, contributing to cardiotoxicity and vascular complications [18].

Bibliometric analysis offers insights into research structures and dynamics, identifying challenges and future directions beyond traditional reviews. Despite numerous studies on ED, a comprehensive bibliometric analysis in the context of chemotherapy is lacking.

This study presents a bibliometric analysis of publications from 1994-2024, examining countries, institutions, journals, authors, and keywords related to chemotherapy-induced ED. The overview assesses scientific productivity, research directions, and collaborations, providing references for future investigations.

Methods

Data Sources and Search Strategies

On November 15, 2024, we conducted a systematic search in the Web of Science (WoS) and Scopus databases to identify publications related to endothelial dysfunction and chemotherapy. The search terms included "Endothelium" and "Chemotherapy," along with their synonyms (Table S1 in Supplementary Materials). These were applied across titles, abstracts, keyword plus, and author keywords for comprehensive coverage. The datasets from WoS and Scopus were merged (R-codes provided in Table S2).

The search covered publications from 1994 to 2024. Figure 1 summarizes the detailed search process. Inclusion criteria were English-language original research articles and reviews. Excluded were conference proceedings, book chapters, abstracts, editorials, letters, and notes (Table S3 in Supplementary Materials).

Bibliometric data were retrieved in full-record citation format (WoS) and BibTeX format (Scopus), including publication year, title, authors, countries, institutions, abstracts, keywords, citations, journal titles, impact factors, and H-indices. This dataset allowed analysis of research trends, key contributors, and collaboration networks.

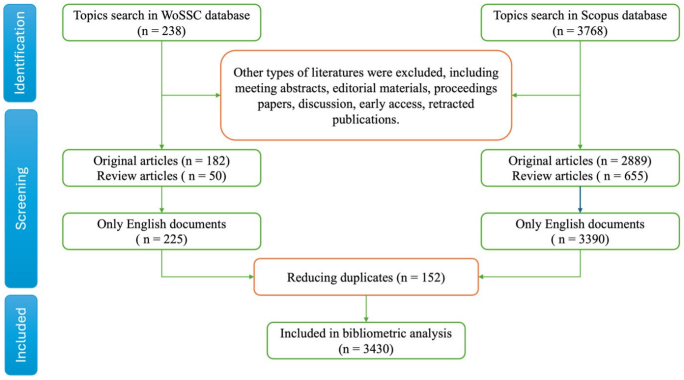


Figure 1 – Flowchart of data filtration processing from 1994 to 2024 at chemotherapy-induced endothelial dysfunction field

Bibliometric Analysis and Visualization

Data from studies selected from the WoS were analyzed using the bibliometric software package RStudio (Version 2024.04.2, PBC, Boston, MA) along with the Biblioshiny web application (http://www.bibliometrix.org; access date: November 15, 2024). The software tool VOSviewer (version 1.6.20) (https://www.vosviewer.com; access date: November 15, 2024) was utilized to build and visualize bibliometric networks based on citations, bibliographic linkage, shared citations, or co-authorship relationships. These applications enable the visualization of accumulated scientific knowledge, revealing the field's structure, distribution, and connections and generating visualization maps that reflect progress and trends in endothelial dysfunction research.

For analyses of countries, institutions, authors, and journals, we set the numerical threshold of each node (item, according to VOSviewer terminology) in the visualizations to three. Consequently, only elements with a value greater than three were shown in the graphs. The nodes' size reflects each element's strength, such as the number of citations or articles, while the distance between nodes indicates the strength of their connection. The wider the communication lines between nodes, the stronger the collaboration.

We employed Bradford's law to present the core journals contributing the most to citations in the field. We visualized bibliographic relationships as maps, including co-authorship, author countries, institutional affiliations, citations, and keywords. We ranked top authors and institutions based on the percentage of articles they authored. We also identified the ten countries contributing the most significantly to the field. We visualized collaboration patterns between authors, institutions, and countries, highlighting the scientific cooperation network.

A temporal frequency analysis of keywords was conducted to identify trends in keyword usage over the specified time interval. The most frequently occurring keywords and their usage trends were presented and visualized. Thematic analysis was carried out to identify temporal trends in the selected publications.

For the bibliometric analysis, data were imported into VOSviewer, version 1.6.20. Term maps were generated using the following options: "Create a map based on bibliographic data," "Read data from bibliographic database files," "Type of analysis: co-occurrence," "Unit of analysis: all keywords," and "Counting method: full counting." A thesaurus file was constructed using standard terms to eliminate non-specific words from the analysis. This file was manually curated to ensure that related terms were identified as the same. The

software analyzes keywords in the titles and abstracts of publications and associates them with documents in which they co-occur (co-occurrence analysis). The co-occurrence frequencies indicate the relatedness of terms.

The parameters for VOSviewer were set to whole counting, with thresholds adjusted based on the observed subjects. R-bibliometrix (version R 4.1.2) was utilized for quantitative analyses, including country collaboration maps, trend topics, and thematic maps of keywords. The H-index was used to quantify researchers' scientific output and influence, and journal impact factors (IF) were obtained from the 2022 Journal Citation Reports (JCR) to evaluate journal influence. The Global research collaboration network, with plots generated by Biblioshiny, was subsequently recreated in Flourish.studio (https://flourish.studio/).

This bibliometric analysis of existing publications does not require ethical approval.

Results

Overall annual publication trends in the field

The chart (Figure 2) illustrates the annual publication trend on chemotherapy-induced endothelial dysfunction from 1994 to 2024. A total of 3,430 articles authored by 14,877 researchers were obtained from the Web of Science Core Collection and Scopus databases. These publications appeared in 1,054 journals, with an average of 82.94 citations per article. The number of publications increased from 40 articles in 1994 to a peak of 250 articles in 2005, followed by a gradual decline to 23 articles in 2024. The linear regression model indicates a slight downward trend in recent years, reflecting fluctuating interest in this research area over time.

We evaluated the completeness of bibliographic metadata for these documents (Figure S1 in Supplementary Materials).

The metadata for Author, Document Type, Journal, Language, Publication Year, Title, and Total Citations was deemed "Excellent," with no missing data. Other categories, including Keywords Plus, Affiliation, and Abstract, showed minor gaps (0.29%, 0.44%, and 1.98% missing data, respectively), resulting in a "Good" rating. DOI and Corresponding Author fields were considered "Acceptable," with missing data rates of 13.06% and 16.44%.

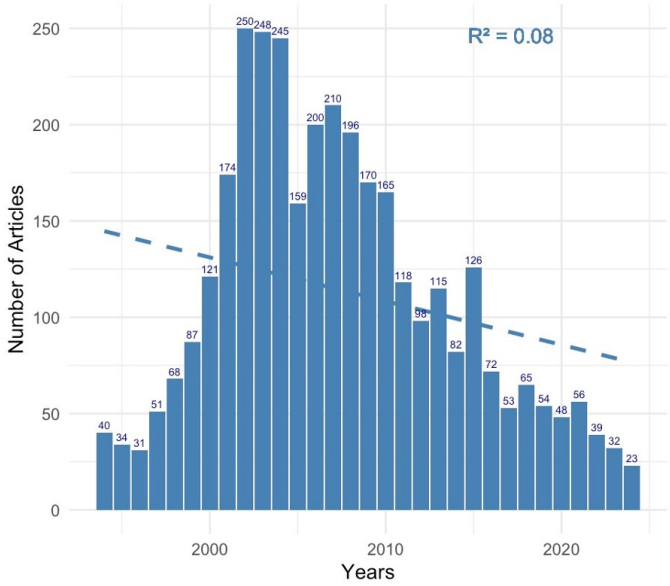


Figure 2 – Dynamics of annual publication trend on chemotherapy-induced endothelial dysfunction (1994–2024)

Distribution of Countries/Regions and Academic Collaboration

Researchers from 58 countries contributed to 3,430 publications. The top 10 most productive countries comprised regions from North America, Europe, and Asia. The United States led with 1,328 publications, followed by China (n = 397) and Japan (n = 385). In terms of citations, the United States ranked first with 106,012 citations, followed by Japan (13,261) and the United Kingdom (13,181). Average citation rates varied, with Belgium (253.80), Finland (191.90), and Israel (126.10) demonstrating strong influence within the field. This indicates that high-income countries, particularly the USA, make substantial contributions both in publication output and citation impact.

Table 1. The top 10 productive countries/regions in the chemotherapy-induced endothelial dysfunction field.

Table 1 The top 10 productive countries/regions in the chemotherapy-induced endothelial dysfunction field.

Rank	Countries/ regions	Publications	Citations	Average Article Citations
1	**USA (North America)**	1,328	106,012	117.80
2	China (Asia)	397	12,693	38.10
3	Japan (Asia)	385	13,261	49.70
4	Germany (Europe)	215	11,587	72.40
5	Italy (Europe)	215	10,459	63.40
6	United Kingdom (Europe)	177	13,181	92.80
7	South Korea (Asia)	128	6,358	52.10
8	France (Europe)	119	6,369	72.40
9	Canada (North America)	95	6,357	97.80
10	Netherlands (Europe)	91	4,857	62.30

Figure 3(A) displays 43 countries with at least five publications in the co-authorship network. Node sizes correspond to publication numbers, while Figure 3(B) visualizes a world map showing publication distribution and collaboration strength. Darker blue symbols indicate higher publication counts, and thicker red lines indicate stronger collaboration. Figure 3(C) shows that the United States, China, and Japan exhibited the most vigorous cooperation during the survey period.

Contribution of Institutions and Academic Collaboration

According to the data provided, 4,243 institutions contributed to this study. Eight of the top 10 institutions were in the United States, while the others were from Taiwan and Japan. As shown in Table 2, Harvard Medical School produced the most papers (n = 186), followed by the University of California (n = 83) and the National Cancer Institute (n = 56).

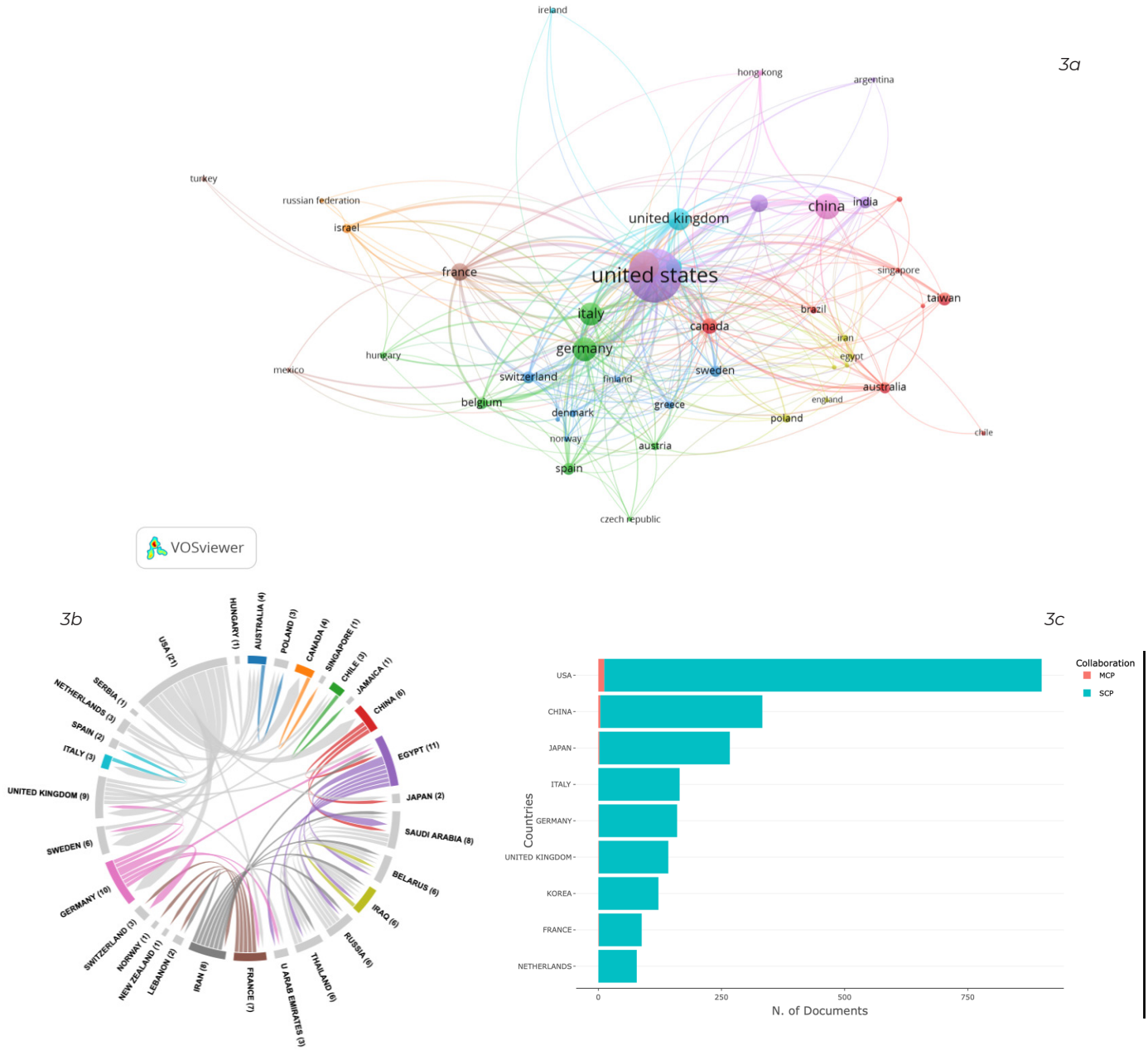


Figure 3 – Coauthorship analysis of countries/regions.

(A) Overlay map of countries/regions with more than five publications. Each node represents a country/region. The size of each node is proportional to the total number of publications. The same color of clusters represents more active cooperation. Lines between two nodes represent the cooperation between two countries/regions.
(B) Global research collaboration network.
(C) Histogram of collaboration status in the top 10 productive countries/ regions.
The x-axis represents the number of publications. The y-axis represents different countries/regions. Publications cooperated with multiple countries/regions (MCP) are plotted in the red part, while the green part means publications with a single country/region (SCP).

Table 2 The top 10 productive institutions in the chemotherapy-induced endothelial dysfunction field.

Rank	Institutions	Countries	Publications
1	**Harvard Medical School**	USA	186
2	University of California	USA	83
3	National Cancer Institute	USA	56
4	Taipei Medical University	Taiwan	52
5	Kyushu University	Japan	41
6	California University Hospital	USA	39
7	University of Tokyo	Japan	37
8	University of Minnesota	USA	36
9	University of Texas	USA	36
10	Duke University Medical Center	USA	35

Contribution of Authors

A total of 14,877 researchers authored the 3,430 publications analyzed. Table 3 presents the publications from the top 10 most productive authors and their H-index. Wang Yan from Tianjin Medical University ranks first with the highest number of articles (n = 41) and an H-index of 31. Kim Soungsoo follows him from SKKU School of Medicine, who has 36 articles and an H-index of 16, and Kim Jeongkon from Asan Medical Center, with 35 articles and the highest H-index among these three (42). Figure 4 illustrates publication counts and citation trends, where node sizes indicate the number of publications, and color gradients reflect increasing citations.

Table 3 The top 10 productive authors and their H-index in the hemotherapy-induced endothelial dysfunction field

Rank	Authors	Articles	H-index	Affiliation
1	**Wang, Yan**	41	31	Tianjin Medical University, Tianjin, China
2	Kim, Soungsoo	36	16	SKKU School of Medicine, Suwon, South Korea
3	Kim, Jeongkon	35	42	Asan Medical Center, Seoul, South Korea
4	Zhang, Yanli	35	21	Southern Medical University, Guangzhou, China
5	Lee, Sung-Jin	34	21	Kangwon National University, Chuncheon, South Korea
6	Lee, Jong-eun	33	45	DNA Link, Inc., Seoul, South Korea
7	Liu, Jianping	33	35	China Pharmaceutical University, Nanjing, China
8	Wang, Jian	33	53	BGI Research, Shenzhen, China
9	Liu, Yang	32	16	Dalian Medical University, Dalian, China
10	Kim, Yung-jin	31	25	Pusan National University, Busan, South Korea

Analysis of Academic Journals

The 3,430 publications were published in 1,054 academic journals. The top 10 academic journals published the most papers in the cancer research field, and their IF (Impact Factor) values for 2023 are displayed in Table 4. Six of these ten journals are from the United States. "Cancer Research" published the most papers (n = 194), followed by "Clinical Cancer Research" (n = 93) and the "International Journal of Cancer" (n = 69). In terms of impact, "Cancer Research" holds the highest IF (IF 2023 = 12.5), followed by "Clinical Cancer Research" (IF 2023 = 10.0) and "Cancer Letters" (IF 2023 = 9.1). These journals play a significant role in disseminating cutting-edge findings in chemotherapy-induced endothelial dysfunction research. Figure 5 displays an increasing growth of publications concerning research in the top 10 journals over the past decades.

The analysis of academic journals reflects the growing interest in chemotherapy-induced endothelial dysfunction across various research fields. This field has gained increased attention from journals focusing on cancer therapy and blood cancers. Furthermore, clinical oncology journals are particularly interested in this field, with endothelial dysfunction emerging as an increasingly prominent topic in cardiovascular, chemotherapy, and pharmacology research. This trend underscores the expanding interdisciplinary relevance of endothelial dysfunction in cancer treatment and beyond.

Table 4 The top 10 academic journals and their IF values

Journal	Number of publications	IF (2023)	Country
Cancer Research	**194**	12.5	USA
Clinical Cancer Research	93	10.0	USA
International Journal of Cancer	69	5.7	Switzerland
Journal of Biological Chemistry	65	4.0	USA
Anticancer Research	52	1.6	Greece
British Journal of Cancer	44	6.4	England
Molecular Cancer Therapeutics	39	5.3	USA
Biochemical and Biophysical Research Communications	38	2.5	USA
Cancer Letters	37	9.1	Netherlands
Plos One	34	2.9	USA

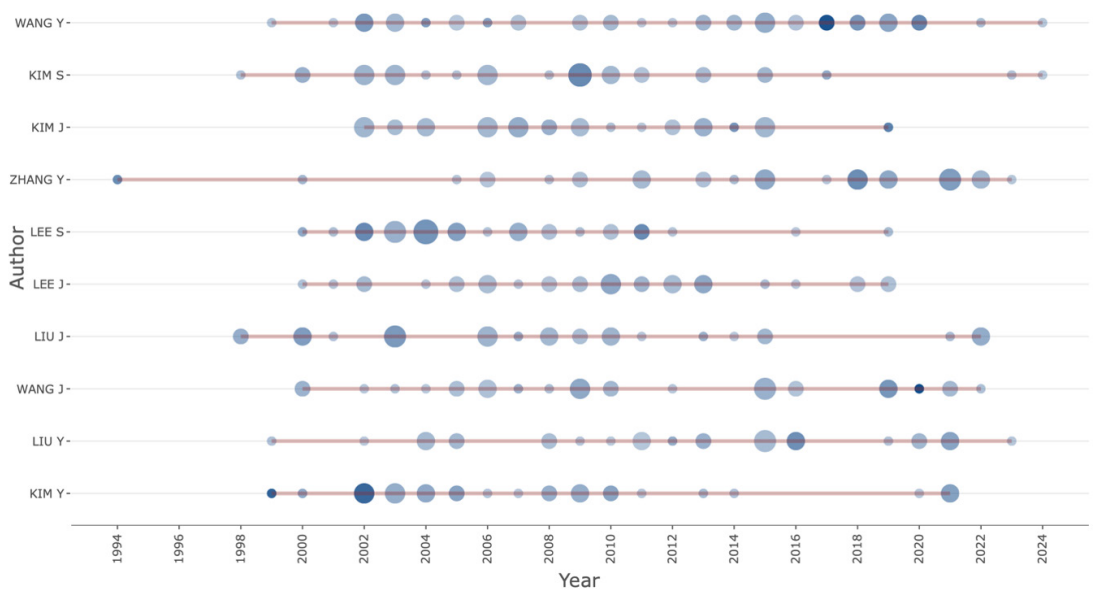


Figure 4 – The annual production number of the top 10 productive authors.

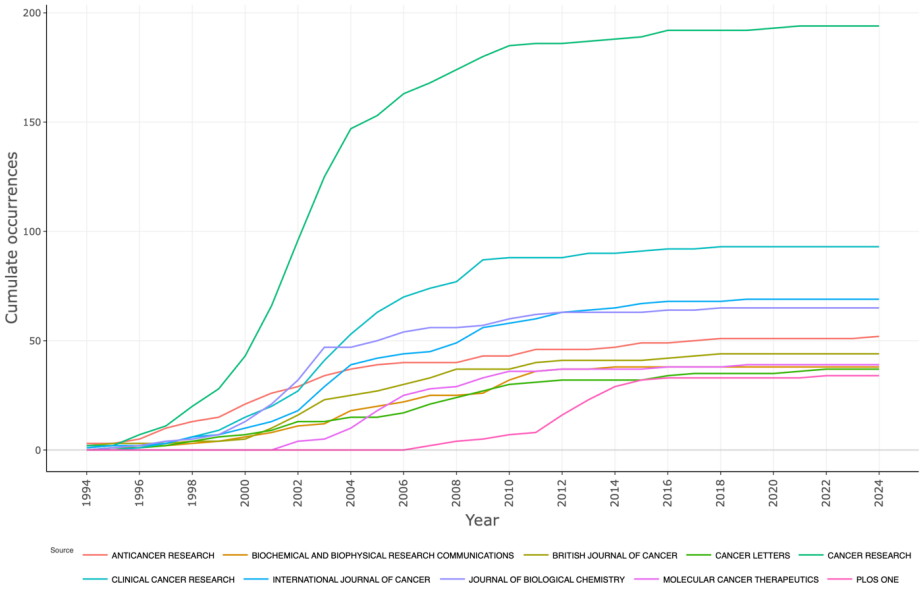
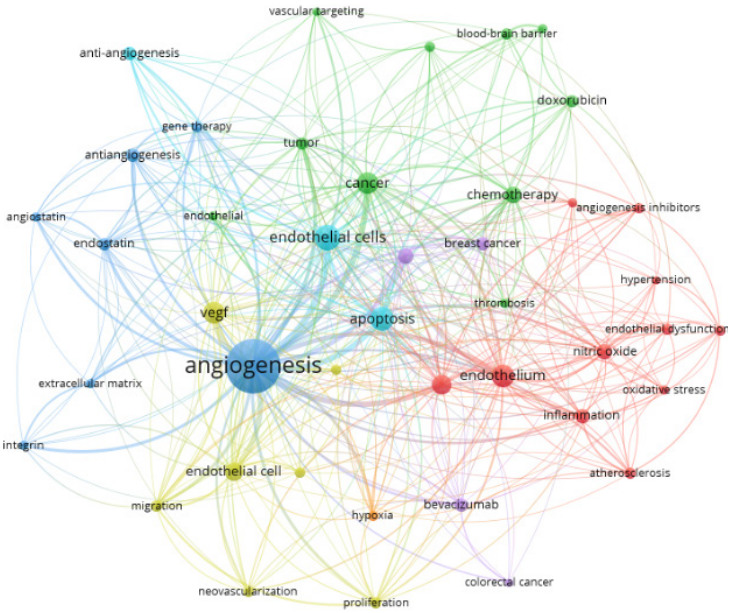


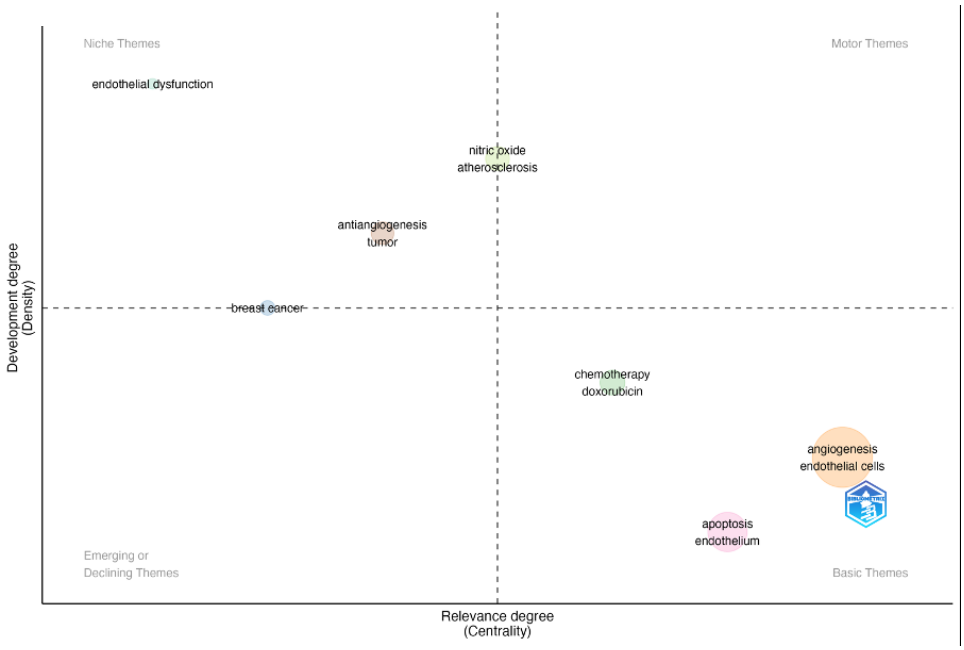
Figure 5 – The annual production of the top 10 academic journals

The x-axis represents years. The y-axis means the number of publications. Different color lines stand for various scholarly journals.



A. Co-occurrence analysis of keywords with a threshold over 10. Each node represents a keyword.

The size of each node is proportional to the occurrence frequency of a keyword. The same color of nodes represents the same cluster. Lines between two nodes represent the relevance of two keywords.



B. Thematic map in the chemotherapy-induced endothelial dysfunction field.

The four quadrants of the two-dimensional diagram are the motor themes (Q1), the highly developed and isolated themes (Q2), the emerging or declining themes (Q3), and the basic and transversal themes (Q4). Each colored bubble represents a cluster of correlative keywords. The bubble size is proportional to the occurrence frequency of associated keywords. The horizontal axis represents the links from one cluster to others, called centrality, and the vertical axis demonstrates the strength of these links, also named density.

Figure 6 – Analysis of keywords

Detection and Analysis of Keywords

Keywords can most accurately reflect the topic of an article and mirror the research frontiers of a given field. In total, 19,392 author keywords were analyzed using VOSviewer. Figure 6(A) shows 43 keywords with more than 20 occurrences. The most frequent were: angiogenesis (714), endothelial cells (168), apoptosis (134), endothelium (124), cancer (116), VEGF (112), vascular endothelial growth factor (99), endothelial cell (89), chemotherapy (65), and metastasis (63).

The cluster analysis grouped the keywords into seven distinct clusters, each focusing on different biomedical themes. Cluster 1 (Red) contains terms associated with endothelial dysfunction and related conditions such as *atherosclerosis*, *inflammation*, and *oxidative stress*, highlighting the involvement of the *endothelium* and *nitric oxide* in vascular health, including *hypertension*. Cluster 2 (Green) focuses on cancer and drug delivery mechanisms. Keywords such as *cancer*, *chemotherapy*, *blood-brain barrier*, and *thrombosis* emphasize the involvement of the endothelium in cancer treatments, particularly with drugs like *doxorubicin*. Cluster 3 (Blue) centers around angiogenesis and inhibitors of this process, incorporating terms like *endostatin*, *extracellular matrix*, *gene therapy*, and *integrin*. These terms suggest the regulation of angiogenesis in cancer and gene therapies. Cluster 4 (Yellow) focuses on *tumor angiogenesis* and vascular changes. Terms like *VEGF*, *proliferation*, and *migration* are closely connected to the processes that drive cancer-related angiogenesis. Cluster 5 (Purple) groups items related to specific cancer treatments and targeted therapies, such as *bevacizumab*, *breast cancer*, and *colorectal cancer*, focusing on how angiogenesis is managed in cancer therapy. Cluster 6 (Cyan) comprises terms like *apoptosis* and *anti-angiogenesis*, emphasizing mechanisms related to programmed cell death and the inhibition of new blood vessel formation. Cluster 7 (Orange) contains only one item: *hypoxia*, critical in regulating angiogenesis and tumor growth, indicating its central role in vascular dynamics during cancer progression. This analysis highlights the interactions between cancer, endothelial function, and angiogenesis, focusing on how these processes intersect in both normal physiological functions and disease states such as cancer.

The thematic map of chemotherapy-induced endothelial dysfunction was further visualized. This map categorizes themes based on their degree of development (density) and relevance (centrality) into four quadrants. Motor Themes (Q1) in the upper right quadrant represent well-developed and essential research areas. Here, central themes like angiogenesis and endothelial cells highlight their crucial roles in vascular biology and cancer research. Niche Themes (Q2) in the upper left quadrant are highly specialized and advanced, though somewhat isolated from mainstream research. Endothelial dysfunction is situated here, indicating its significance but limited connectivity to broader topics. Emerging or Declining Themes (Q3) in the lower left quadrant include research areas either in decline or early stages. Themes such as breast cancer and antiangiogenesis are found here, suggesting they are emerging or potentially losing prominence. Basic Themes (Q4) in the lower right quadrant are fundamental yet not extensively explored or developed. Keywords like apoptosis, chemotherapy, and doxorubicin are placed here, reflecting their essential yet central roles in studying endothelial function and cancer t reatment.

The thematic map illustrates that angiogenesis and endothelial cells are well-established fields with significant relevance, while niche topics such as endothelial dysfunction are highly developed but remain more isolated. Emerging areas like antiangiogenesis and tumor-related processes are still gaining traction. Understanding the distribution of these themes could guide future research and development, particularly in cancer therapy and vascular health.

Discussion

This bibliometric analysis of publications on chemotherapy-induced vasculotoxicity from 1994 to 2024 highlights the growing interest in vascular toxicity mechanisms and endothelial dysfunction. While reviewing the literature, we identified a previous bibliometric analysis examining global research trends on endothelial cells in sepsis over two decades (2002–2022) [19]. That study focused mainly on sepsis-related endothelial mechanisms. In contrast, our analysis is the first to specifically investigate chemotherapy-induced endothelial dysfunction, an area at the intersection of oncology, cardiology, and vascular biology. Unlike previous reviews, it provides an integrated overview of publication dynamics, collaboration networks, and thematic evolution within the context of cardio-oncology vascular toxicity, which has not been comprehensively analyzed before.

By examining publication metrics, scientific output dynamics, international collaboration, institutional contributions, journal preferences, author productivity, and keyword trends, we provided a comprehensive overview of the scientific advancements in the study of chemotherapy-induced endothelial dysfunction. The significant rise in publications in recent years reflects the critical need to understand the mechanisms of vasculotoxicity associated with chemotherapy. This growth emphasizes the urgency of tackling the diagnostic, therapeutic, and management challenges faced by patients affected by endothelial dysfunction due to chemotherapy. Solutions hinge on technological advancements, including the identification of molecular markers and genetic predispositions to cardiovascular complications associated with chemotherapy.

The practical significance of our findings lies in highlighting the importance of systematic vascular toxicity monitoring in oncology protocols. Early detection of endothelial dysfunction through methods such as flow-mediated dilation or circulating biomarkers can enable timely preventive interventions, thereby reducing the risk of cardiovascular complications, improving patient quality of life, and optimizing oncological treatment adherence.

Among the leading countries in publication volume, the United States ranks first, followed by China and Japan. The top positions of high-income countries indicate a correlation between economic development and active research output in chemotherapy-induced vasculotoxicity. International collaboration, particularly involving the United States, European countries, and Japan, is crucial for advancing knowledge and technology in this field. This trend toward active global collaboration reflects the widespread importance of studying chemotherapy-related cardiovascular complications. It underscores the considerable financial and technological resources required for conducting research. The minimal contributions from low-income countries can be linked to funding limitations, inadequate institutional support, and limited educational opportunities, which impede active participation in

research. Furthermore, language barriers and restricted access to technical resources hinder involvement in international research initiatives, resulting in negligible contributions from these countries in this field.

Journals with the maximal influence of Endothelial Dysfunction research on clinical practice

The core journals in chemotherapy-induced endothelial dysfunction include those specializing in oncology and cardiovascular research, reflecting the interdisciplinary nature of the topic. Most articles are published in high-impact journals such as “Cancer Research”, “Clinical Cancer Research”, and “International Journal of Cancer”, emphasizing the significant influence this research has on clinical practice and highlighting cardiovascular health issues in oncology studies. The rapid development of the interdisciplinary field of vasculotoxicity underscores the need for trusted, high-quality, peer-reviewed publications, which are crucial for maintaining the reliability of scientific data. Policymakers and healthcare professionals depend on authoritative sources for decision-making, and reputable journals enhance the credibility of research outcomes, ensuring the integration of evidence-based data into practical medicine.

Key mechanisms of vascular toxicity identified in recent studies

Over the past decades, research has dramatically advanced the understanding of chemotherapy-induced endothelial dysfunction. Recent studies have identified key mechanisms of vasculotoxicity, including the suppression of nitric oxide synthase and the disruption of the balance between vasoconstrictive and vasodilatory factors, leading to impaired regulation of vascular tone [20,21]. Fluoropyrimidines and platinum-based drugs, such as cisplatin, suppress endothelial nitric oxide synthase (eNOS) activity, causing vasoconstriction and direct endothelial damage, as well as the excessive release of endothelin-1 (ET-1) and other factors [22]. Anthracyclines disrupt mitochondrial function in endothelial cells, reducing their energy potential, intensifying apoptosis, and consequently leading to the loss of vasorelaxant effects, suppression of antioxidant functions, and protection against inflammatory reactions, thus accelerating atherosclerosis [23].

Key studies have demonstrated that inhibiting vascular endothelial growth factor (VEGF) with monoclonal antibodies and blocking VEGF receptors, used for treating various cancers, including renal cell and thyroid carcinoma, leads to a range of vascular complications, such as arterial hypertension (AH), myocardial infarction, and thrombosis. VEGF inhibitors suppress the synthesis of nitric oxide and prostacyclin (PGI2), resulting in increased peripheral vascular resistance and the progression of AH [24]. Studies show that elevated blood pressure is detected in 80% of cancer patients undergoing this therapy. Certain tyrosine kinase inhibitors, such as nilotinib, activate perivascular fibrosis processes, exert a proatherogenic effect, and contribute to the development of peripheral arterial diseases [25].

The keyword analysis demonstrates how the research focus on chemotherapy-induced vascular toxicity has shifted and expanded over time. Initially, attention was directed toward general aspects, such as "endothelial cells" and "chemotherapy." However, over time, the focus shifted to more specific mechanisms, such as "VEGF inhibitors," "apoptosis," and "anti-angiogenesis." This evolution highlights a significant deepening in understanding chemotherapy's impact on endothelial function and the development of targeted therapies to mitigate vasculotoxic effects. The changing research landscape reflects the growing importance of studying endothelial dysfunction in the context of cancer. It underscores the need for a comprehensive approach to cardiovascular support for patients undergoing treatment.

Overall, this bibliometric analysis underscores the interdisciplinary nature of chemotherapy-induced endothelial dysfunction research and its clinical relevance. Implementing vascular toxicity surveillance and integrating endothelial health assessments into routine cardio-oncology care will be essential for optimizing treatment safety and efficacy.

Limitations

Apart from this undoubtful strength, the study had inevitable limitations:

1. We searched for relevant sources using only the core Web of Science (WoS) and Scopus collections. The current study did not account for other databases, such as MEDLINE. WoS is the most widely used database in scientometrics, and Biblioshiny and VOSviewer have established a format for recording metadata from WoS.
2. We included only articles in English and excluded papers in other languages, as well as proceeding materials, book chapters, meeting abstracts, editorials, early access articles, letters, and notes.
3. The bibliometric analysis itself has a methodological limitation, such as a lower total citation rates for newer articles [26].
4. The bibliometric and scientometric analysis of articles indexed in the WoS database focused solely on metadata, not their content. Furthermore, analyzing the textual content of the abstracts was not the aim of our study. Article metadata served as sources of information about authors and their countries/ institutions to evaluate their productivity, collaboration, and keyword trends.

Conclusion

Recent studies indicate that endothelial dysfunction is an early marker of cardiovascular changes that can progress to severe complications, even in patients without pre-existing risk factors. This bibliometric analysis uncovered evolving trends in chemotherapy-induced endothelial dysfunction research, particularly in areas such as angiogenesis, VEGF inhibitors, and international collaboration. Our findings highlight the practical importance of integrating endothelial toxicity monitoring into routine cardio-oncology protocols, which could improve early detection, enable timely interventions, and reduce cardiovascular morbidity among cancer patients.

This study also identified the key journals, authors, and institutions contributing to this interdisciplinary field, underscoring its relevance for both oncology and cardiology. The dominance of high-income countries in research output indicates a need to bridge global disparities through enhanced collaboration and resource sharing, ensuring equitable access to emerging knowledge and treatment strategies.

Future studies should focus on developing cardioprotective interventions to mitigate chemotherapy-induced endothelial damage and refining vascular toxicity screening tools for clinical use. Implementing systematic vascular health assessments into oncology care pathways will be critical to optimize treatment safety and long-term outcomes for cancer survivors.

Over the past three decades, research interest in chemotherapy-induced endothelial dysfunction has fluctuated. However, with the introduction of novel chemotherapeutic agents and the growing recognition of vascular toxicity, a renewed surge of interest is expected, potentially leading to evidence-based updates in cardio-oncology guidelines and improved multidisciplinary patient care.

Abbreviations

This bibliometric analysis of publications on chemotherapy-induce:

AH – Arterial hypertension;
ED – Endothelial dysfunction;
eNOS – Endothelial nitric oxide synthase;
ET-1 – Endothelin-1;
H-index – Hirsch index;
IF – Impact factor;
JCR – Journal Citation Reports;
NLRP3 – Nucleotide oligomerization domain-like receptor family, pyrin domain containing 3 (inflammasome);
PGI2 – Prostacyclin;
SCP – Single Country Publication
VEGF – Vascular endothelial growth factor

Supplementary materials

The Supplementary information includes tables:

- Supplementary Table S1.Queries for Web of Science and Scopus databases;
- Supplementary Table S2. R-code for merging data;
- Supplementary Table S3. Summary of analyzed publications,

as well as figures:

- Supplementary Figure S1. Completeness of bibliographic metadata;
- Supplementary Figure S2. Graphical Illustration.

The file can be accessed using: https://www.editorialpark.com/download/article-supp/729/Supplementary-Materials.docx.

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Providing High-Quality Inpatient Care as a Key Criterion for Patient Satisfaction

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Abstract

The introduction of innovative therapeutic technologies into the clinical practice of the inpatient service of the multidisciplinary hospital has demonstrated high effectiveness. The analysis presents data on the application of innovative, highly effective medical technologies, such as a Robotic Surgery Center, a hybrid operating room with a biplane angiographic system, and centers for Cardiac Care, Interventional Radiology, Photodynamic Therapy, and Men's Health. The implemented innovative medical technologies showed high clinical efficacy, contributing to an improved quality of inpatient medical services and a higher level of patient satisfaction. A methodology for assessing the quality of treatment has been developed, which constitutes an innovative technology for the expert evaluation of service quality, aimed at the timely detection of deficiencies in service delivery and forming an integrated policy for healthcare quality management.

Objective: To analyze the effectiveness of innovative medical technologies in providing high-quality inpatient care, to assess patient satisfaction, and to develop an expert evaluation process and a policy for service quality management.

Methods: The study utilized data on the implementation and application of innovative medical technologies in the inpatient services of a multi-profile hospital. It included an analysis of their effectiveness in improving the quality of medical care, the development of a service evaluation method and an integrated quality management policy, and an assessment of patient satisfaction with inpatient care.

Results: The use of innovative medical technologies, including a Robotic Surgery Center, a hybrid operating room with a biplane angiographic system, and centers for Cardiac Care, Interventional Radiology, Photodynamic Therapy, and Men's Health, led to shorter hospital stays, improved quality of inpatient medical care, and greater patient satisfaction. To evaluate the quality of treatment and ensure the timely identification and correction of deficiencies, an innovative assessment technology was developed, and an integrated policy for healthcare quality management in a multi-profile hospital setting was proposed.

Conclusion: The innovative medical technologies introduced into the clinical practice of the multi-profile hospital's inpatient services proved to be highly effective, contributing to an expanded range of services, enhanced quality of care, and increased patient satisfaction. To improve the outcomes of inpatient care, a method for expert service evaluation was developed to identify and promptly address deficiencies, along with an integrated policy for healthcare quality management.

Keywords: inpatient services, innovative medical technologies, effectiveness, quality expertise and management, patient satisfaction.

Introduction

In the contemporary context, the healthcare system represents a field of activity whose primary objectives include maintaining and enhancing public health and ensuring accessible medical care [1]. These measures are aimed at improving the quality of medical care, which is continuously enhanced by reliable and high-quality medical services [2, 3]. Furthermore, it appears that the most crucial factors are to be found in the market attractiveness, innovative nature, and quality of the medical services provided [4].

Presently, many new developments in healthcare are categorized as innovative medical technologies, the main purpose of which is to improve medical services for the public. Medical professionals in all countries are engaged in the development of innovative technologies. The USA, Great Britain, Switzerland, and Sweden are considered the world leaders in innovative medical development. China and India have followed in this ranking [5, 6].

In this context, the situation in Kazakhstan's healthcare sector is characterized, on one hand, by a high degree of innovation across all types of medical activities [7,8]. On the other hand, there is a growing public demand for high-quality medical care, which underscores the need to develop theoretical and methodological frameworks for enhancing the effectiveness of both core and innovative clinical activities aimed at improving service quality [9].

According to the WHO definition, the quality of medical care is the degree to which health services increase the likelihood of desired health outcomes and are consistent with current evidence-based professional knowledge [10].

According to a Report by the WHO, OECD, and the World Bank, quality of care involves services that are patient-centered and practiced by every healthcare worker, where the level of safety, effectiveness, and timeliness determines the likelihood of achieving the desired outcome [11].

In the opinion of WHO experts [12], ensuring the quality of medical services is the result of several important components: the integrity of the healthcare system; the adequacy of actions by service providers; proper governance; skilled human resources; adequate funding; the availability of information systems that allow for continuous monitoring of the quality of care; and equipping organizations with modern technologies.

In line with the WHO's declaration on universal health coverage, adopted in 2019, a commitment was reaffirmed to progressively cover an additional one billion people with quality services by 2023, with the goal of covering the entire global population by 2030 [13]. The same document states that when establishing principles for ensuring the quality of medical care, four systemic components must be considered: 1) the competence of the specialist; 2) the optimal use of resources; 3) patient risk and safety; and 4) patient satisfaction with their interaction with the healthcare system.

Meanwhile, the literature indicates that in the course of receiving medical care, one in every ten patients is affected by some form of error or substandard medical service. These issues are most common in low- and middle-income countries, where adherence to clinical guidelines is below 50% (ranging from 22% to 43.8%), and the accuracy of diagnosis ranges from 3% to 72.2% [10].

Scientific research on improving the quality of medical care substantiates the need for its modernization to ensure the high-quality delivery of services [14].

The literature shows that increasing attention is being paid to assessing the quality of medical care, both worldwide and in post-Soviet countries, and it is one of the most critical issues in modern public health and its development. This situation is also observed in Kazakhstan, where various challenges in ensuring the quality of medical care persist [15].

Thus, an analysis of the contemporary literature highlights the priority of research into the quality of medical care for the population. However, this topic still contains unresolved issues, confirming the existence of certain gaps in the literature.

Objective. To analyze the effectiveness of innovative medical technologies in providing high-quality inpatient care, to assess patient satisfaction, and to develop a framework for expert evaluation and a policy for service quality management.

Methods

This study was conducted in the inpatient services of a multidisciplinary hospital and consisted of several key stages:

1. Selection and Implementation of Innovative Medical Technologies

A range of innovative therapeutic technologies was identified and introduced into clinical practice across various inpatient departments. These included both diagnostic and treatment innovations tailored to different patient populations.

2. Data Collection and Analysis

Quantitative and qualitative data were collected over a defined observation period following the implementation of the new technologies. This included:

– Patient clinical outcomes (e.g., length of hospital stay, complication rates, recovery time)

– Service performance indicators (e.g., readmission rates, treatment delays)

– Patient-reported satisfaction metrics, gathered through standardized post-discharge surveys.

3. Development of a Quality Assessment Methodology

A structured methodology was developed to assess the quality of care provided. It included:

– Expert evaluation criteria

– A scoring system for clinical outcomes and service delivery indicators

– Feedback mechanisms for healthcare providers

4. Creation of an Integrated Quality Management Policy

Based on the analysis, a comprehensive policy for healthcare quality management was formulated. It integrated:

– Continuous monitoring of clinical performance

– Regular evaluation cycles

– Corrective action protocols

– Staff training programs to address identified deficiencies

5. Statistical Analysis

The collected data were subjected to statistical analysis to assess the impact of innovative technologies on treatment outcomes and patient satisfaction. Descriptive and inferential statistics were used to evaluate effectiveness and significance.

Results and Discussion

To achieve the stated objectives, an analysis of the implementation and use of innovative, highly effective medical technologies in the inpatient setting of a multi-profile hospital was undertaken.

In line with this, and taking into account the specified provisions, we studied the overall quantitative and structural

characteristics of the innovative technologies introduced into the hospital's practice. Between 2019 and 2024, a total of 127 innovative technologies were implemented, of which 45 (35.4%) were diagnostic, 66 (52.0%) therapeutic, and 16 (12.6%) organizational (hospital management) in nature. These data indicate that the majority of the implemented innovations were therapeutic.

In this regard, the most high-tech therapeutic methods, introduced into Kazakhstan's healthcare practice for the first time, were analyzed. The most significant of these are: the international Robotic Surgery Center, a hybrid operating room with a biplane angiographic system, the Cardiac Center, the Interventional Radiology Center, the Photodynamic Therapy Center, and the Men's Health Center.

It should be noted that in 2020, the Kazakhstan International Robotic Surgery Center was officially opened at the hospital, a first for the country. The establishment of this center facilitated the development of an International Reference and Training Center, allowing it to become a unique institution and to introduce and expand the volume and types of laparoscopic robot-assisted technologies.

From 2019 to 2024, 953 robot-assisted surgeries were performed, the majority of which were in general surgery (69.1%), followed by surgical gynecology (25.0%), urological surgery (4.4%), and cardiovascular surgery (1.5%).

It is important to mention that while retaining the benefits of laparoscopic methods, robot-assisted surgery offers significant advantages. The results of using this technology have demonstrated its high clinical effectiveness.

In 2019, the hospital also commissioned the only hybrid operating room in the Republic of Kazakhstan equipped with a Toshiba Infinix-i biplane angiographic system, which meets all modern requirements for diagnostics and surgical treatment. The implementation of this technology has made it possible to safely perform interventional procedures, including those on the brain with three-dimensional imaging, and to improve the quality of care, which has spurred the development of new neurosurgical technologies. From 2019 to 2024, 427 procedures were performed in this operating room (encompassing 18 different types of cardiac and neurosurgical operations).

In addition to the innovative services mentioned above, the hospital also operates other units established for the first time in the Republic of Kazakhstan. These include the Cardiac Center, the Interventional Radiology Center, the Photodynamic Therapy Center, and the Men's Health Center.

The main idea behind the creation of the Cardiac Center was to implement a multidisciplinary approach (Heart Team) to preparing patients for surgery, providing diagnostic and therapeutic care for patients with cardiological, cardiac surgery, arrhythmological, and vascular conditions, and managing their post-operative care (Heart-Im). The most frequently performed surgical methods at the Cardiac Center are minimally invasive heart surgeries and combined valve and coronary artery bypass graft procedures, which together accounted for 80.6% of all 274 operations.

Another innovative service is the establishment and operation of the Interventional Radiology Department, where a total of 4,273 surgical procedures were performed from 2019 to 2024. The absolute majority (71.9%) of these were for coronarography and cardiac catheterization, percutaneous coronary interventions, stenting, and balloon angioplasty of the coronary arteries.

Collectively, all surgical interventions performed at the Cardiac Center and the Interventional Radiology Center yielded highly effective results.

A further innovative service is the Photodynamic Therapy (PDT) Center, which has been operational since 2016. The center provides PDT, intravenous laser blood photomodification, intravenous laser blood irradiation (ILBI), and laser destruction of skin neoplasms.

From 2020 to 2024, PDT was performed in 1,101 cases. The method was most frequently used in the treatment of malignant neoplasms and tumors of the digestive system (esophageal and gastric neoplasms), female reproductive organs (tumors, most often malignant), and skin cancers.

Among the innovative technologies introduced into practice is the Men's Health Center, opened in 2019. It provides diagnostics and treatment for diseases of the male reproductive system, erectile dysfunction, fertility issues, penile surgery, benign prostatic hyperplasia, and prostate cancer. It was found that all analyzed parameters show a steady upward trend over time.

Given the importance of the topic under study, a methodology for assessing the quality of treatment was developed. This represents an innovative technology for the expert evaluation of service quality, aimed at the timely detection of deficiencies in their delivery.

The scoring scale for each indicator ranges from 0 to 1 point, with an incremental step of 0.25 points. The overall quality of treatment level is assessed based on the total score: a score from 0 to over 5 points corresponds to a respective level from high to low (Table 1).

An important aspect of the study was the analysis of patient satisfaction. To this end, 3,637 patients who received inpatient treatment between 2019 and 2024 were surveyed. The proportion of patients satisfied with the quality of medical services was

Table 1 Numerical Parameters for the Assessment of Treatment Quality

High Level	Average Level	Below Average Level	Low Level
0–0.24 points	0.25–3.0 points	3.1–5.0 points	Over 5.0 points
Medical care is provided appropriately and meets all quality standards.	Minor deficiencies exist in the provision of medical care.	Significant deficiencies and shortcomings exist in the provision of medical care.	Medical care is inadequate in scope and quality and does not fully meet standards.

Table 2 Key Elements of the Quality Management Policy System in a Multi-Profile Hospital and Their Significance

System Element	Significance of System Elements
Integrated Management Policy	Acts as an internal regulatory document to integrate international standards into the hospital's operational practices.
Planning	Defines a comprehensive framework for planning hospital activities based on critical aspects, emerging issues, and risks.
Implementation and Operation	Executes the established plans, supported by the structured organization of responsibilities, resources, knowledge, information, and documentation.
Monitoring and Corrective Action	Establishes a continuous system of internal hospital audits, corrective actions, and preventive measures.

97.1%, which is noticeably higher than the threshold level of 90% established by the Medical Center of the President's Affairs Administration of the Republic of Kazakhstan. These data demonstrate a sufficiently high degree of patient satisfaction with the quality of inpatient medical care provided.

It should be noted that the implementation of innovative technologies is often carried out without proper scientific support and justification. In light of this, we have substantiated the scientific principles for developing and implementing an organizational policy for quality management in the medical care of the assigned patient contingent within a departmental multi-profile hospital (Table 2).

To implement the main provisions of the proposed system, the following integrated policy for healthcare quality management in a multi-profile hospital setting was developed. The following principles are proposed to address its key objectives:

- Carry out work to ensure the quality of services based on prioritizing patient requirements, optimizing technological processes, correctly substantiating the applied therapeutic and diagnostic procedures, employing an individual approach, and monitoring effectiveness.
- Strive for continuous technological progress, ensure effective innovation management, and implement new therapeutic and diagnostic technologies.
- Clearly define the responsibility of each employee in the structural departments for ensuring quality, and adhere to the principle of continuous improvement and patient safety.
- Conduct continuous staff training and improve employee qualifications, including through the use of modern innovative telemedicine technologies.
- Instill a culture of safety-conscious behavior, enhance approaches to ensuring safety, and systematically improve the health of the organization's personnel.

Conclusions

The innovative medical technologies introduced into the clinical practice of the multi-profile hospital's inpatient services proved to be highly effective, contributing to an expanded range of services, enhanced quality of care, and increased patient satisfaction. To improve the outcomes of inpatient care, a method for expert service evaluation was developed to identify and promptly address deficiencies, along with an integrated policy for healthcare quality management.

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Comparison of Outcomes after Holmium Laser Enucleation of the Prostate and Transurethral Resection of the Prostate

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Abstract

Introduction: Benign prostatic hyperplasia (BPH) is the most common benign tumor in men. Holmium laser enucleation of the prostate (HoLEP), is considered a strong competitor to Transurethral resection of the prostate (TURP).

Objective: To determine the differences in short-term and medium-term outcomes of TURP and HoLEP in treatment of bladder outlet obstruction caused by BPH.

Methods: This was a prospective observational study, carried out on BPH patients who underwent HoLEP or Monopolar TURP from April 2023 to September 2024. A total of 72 patients with prostate volume of less than 100 cc were assigned to two groups. Group A (n = 36) underwent surgical treatment with HoLEP, while Group B (n = 36) underwent monopolar TURP. All the procedures were performed by a single surgeon.

Results: Patients in the HoLEP group experienced shorter catheterization times and hospital stays than those in the TURP group, with both differences being statistically significant ($P < 0.001$). Additionally, the operating time differed significantly between the two groups, with HoLEP averaging 87.53 ± 15.93 minutes and TURP averaging 67.47 ± 9.98 minutes ($P < 0.001$). The mean hemoglobin loss was also lower in the HoLEP group (0.76 ± 0.36 g/dL) compared to the TURP group (1.57 ± 0.69 g/dL). At follow-up, both groups showed significant improvements from baseline in AUA symptom scores, Qmax, and PVR urine volumes

Conclusion: HoLEP offers less perioperative morbidity and similar efficacy at three months. Postoperative complications and improvements in symptoms, after HoLEP are comparable to those seen after TURP.

Keywords: Benign prostatic hyperplasia (BPH), Holmium laser enucleation of the prostate (HoLEP), Transurethral resection of the prostate (TURP), maximum urinary flow rate (Qmax), post-void residual (PVR).

Introduction

Benign prostatic hyperplasia (BPH) is the most common benign tumour in men, with prevalence estimates ranging from 50% for men in their 50s to 90% for men in their 90s. While not all men suffer from this condition, approximately 50% of those with histological hyperplasia will eventually develop moderate-to-severe and bothersome storage and voiding symptoms known as lower urinary tract symptoms (LUTS) [1]. Transurethral resection of the prostate (TURP) is regarded as the gold standard for surgically addressing bladder outlet obstruction caused by BPH. However, TURP is associated with relatively high morbidity, partly because of substantial blood loss (with a blood transfusion rate ranging from 5% to 11%) and partly due to the

occurrence of TUR syndrome in the treatment of larger prostates [2].

Holmium laser enucleation of the prostate (HoLEP) was introduced in 1996 by Peter Gilling and his team as an alternative method to reduce blood loss during prostate enucleation. This technique utilizes a laser combined with mechanical soft tissue morcellation to achieve effective hemostasis and thorough obstruction removal. The holmium YAG laser (Ho:YAG) is a pulse solid-state laser with a wavelength of 2,140 nm, it can be efficiently absorbed by tissue water, resulting in the rapid vaporization of exposed tissue at a depth of around 0.4 mm and leading to tissue coagulation at depths of 3 mm to 4 mm below the surface. This capability is beneficial as it facilitates precise, bloodless procedures, preventing systemic fluid absorption [3].

Other studies have shown that, compared to TURP, HoLEP significantly reduces blood loss and other perioperative complications, shortens urethral catheterization time and hospital stays, while also delivering similar short-term urinary function outcomes [4]. A prospective study was carried out over 17 months at KIMSHEALTH Trivandrum to assess the efficacy of HoLEP as a potential new gold standard for treating bladder outlet obstruction. The study focused on patients with BPH and prostates less than 100 g, comparing the perioperative and postoperative outcomes of HoLEP versus TURP.

Methods

This prospective observational study was carried out in the Department of Urology at KIMS Health hospital, Thiruvananthapuram, India, after obtaining institutional ethics committee approval from April 2023 to September 2024. Informed written consent was taken from all included patients. A total of 72 patients with BPH underwent either HoLEP or TURP based on their preference. The first 36 patients undergoing HoLEP were enrolled in Group A, while the first 36 patients undergoing TURP were enrolled in Group B. Inclusion criteria were age more than 18 years and less than 85 years, prostate volume greater than 30 g but less than 100 g (as determined by abdominal ultrasound), American Urological Association (AUA) symptom score of 12 or higher, and peak urinary flow rate of 15 mL/sec or lower. Exclusion criteria were neurogenic bladder, age <18 years and ≥ 85 years; previous urethral, bladder neck, or prostate surgery; suspected prostatic cancer by abnormal digital rectal examination (DRE); total serum PSA > 4 ng/mL; abnormal ultrasound finding towards carcinoma prostate. American Urological Association (AUA) symptom score were recorded using questionnaire. All men underwent blood investigations, including a complete blood count and serum prostate-specific antigen tests, as well as other assessments such as ultrasonography and uroflowmetry, to record peak urinary flow rates (Qmax) and post-void residual (PVR) urine volume. Intraoperative parameters, including total operating time and resected tissue weight, were documented in the proforma from the operative sheet and electronic medical record (EMR). Postoperatively, early parameters such as hemoglobin drop, presence or absence of blood transfusion, time of catheter removal, and duration of hospital stay were recorded.

Patients were followed up at three months, during which their AUA symptom scores, peak urinary flow rates (Qmax), and post-void residual (PVR) urine volume were recorded. These outcomes of both groups were compared, constituting the primary outcome. Secondary outcomes included differences in hemoglobin drop, catheterization time, hospital stay duration, intraoperative complications, and early postoperative complications in the HoLEP and TURP groups. The presence of any complications (including blood transfusion, transient dysuria, recatheterization, hematuria, and urinary incontinence) was also recorded postoperatively, contributing to the secondary outcomes.

Surgical procedure

All procedures were carried out under either spinal or general anesthesia by the same surgeon. Patients were positioned in the dorsal lithotomy position, and HoLEP was performed using a 26 French resectoscope (Karl Storz, Germany). A laser working element was utilized to stabilize the laser fiber, specifically a 550 μm end-fire optical fiber (Quanta System optical fiber, reusable 10x). The procedure was conducted with a high-power 100 W Ho:YAG laser device (Cyber Ho 100 Quanta system), and a standard 30° telescope was employed with 0.9% saline as the irrigating solution. Resection was initiated at a power of 40 W, with an energy setting of 1.0 J and a frequency of 40 Hz. The en bloc technique was employed in all cases, beginning the resection on either side at a level proximal to the verumontanum. Enucleation of the adenoma was performed in a top-down fashion from 12 o'clock down, releasing the adenoma all around in a symmetrical manner. A morcellator (Bioradmedisys Tomi morcellator) paired with an offset long 26 Fr nephroscope (Karl Storz) was introduced to morcellate the prostate adenoma using suction and cutting blades. TURP was performed with 26 French resectoscope (Karl Storz, Germany) with monopolar current (utilizing a tungsten cutting wire loop with settings of 120 W for cutting and 80 W for coagulation) was employed for the resection. The resection of the prostate adenoma followed a systematic approach using the Mauermayer technique, with glycine as irrigating fluid. Final hemostasis was achieved through careful coagulation of any bleeding points. A 22 Fr 3-way Foley catheter was placed with 30-50 cc in the balloon, allowing for slow continuous bladder irrigation while applying catheter traction to tamponade prostatic bleeding, which continued for 6-8 hours. The urethral catheter was removed on the second or third postoperative day.

Statistical analysis

All the data collected were analyzed using SPSS statistical software version 25. Quantitative variables were summarized using mean and standard deviation (SD) or using median and interquartile range (IQR). Independent sample t test and Mann Whitney test were used to test statistical significance of difference between means of variables among different independent groups depending upon the normality of distribution. Paired t test was used to compare different parameters at pre-op period and at 3 months follow-up period. A p value of <0.05 was considered statistically significant.

Sample size

Assuming a pooled standard deviation of 15 units, the study sample size of: 36 for each group (i.e. a total sample size of 72), to achieve a power of 80% and a level of significance of 5% (two sided), for detecting a true difference in means between the test and the reference group of 10 (i.e. 35 - 25) units to declare that the two groups have significantly different means, i.e. a two sided p-value of less than 0.05.

Results

A total of 72 patients with lower urinary tract symptoms (LUTs) caused by BPH and a prostate volume of less than 100 cc were enrolled. Among the enrolled patients, 36 underwent HoLEP (Group A) and 36 underwent TURP (Group B). Baseline characteristics were comparable between the groups (Table 1). The mean age of patients in the HoLEP group and TURP group was 66.78 and 67.33 years respectively; mean prostate volume in the HoLEP and TURP were 68.51 and 55.52 cc respectively; mean PSA in the HoLEP and TURP were 2.58 and 2.26 ng/dL respectively. Mean AUA symptom scores preoperatively was 22.64 ± 2.88 in HoLEP group and 22.89 ± 2.72 in the TURP group. Baseline Qmax was similar in both groups, 7.76 ml/sec in HoLEP group and 7.22 ml/sec in TURP group. Mean PVR in both the groups is comparable, with 174.97 ml in HoLEP and 166.86 ml in TURP.

The operative time was significantly longer for the HoLEP group compared to TURP group, with mean operative times of 87.53 ± 15.93 minutes for HoLEP and 67.47 ± 9.98 minutes for TURP ($p < 0.001$). Prostate tissue retrieved was greater in the HoLEP group (62.50 gms) compared to TURP group (48.61 gms), ($p = 0.003$). Catheterization time was comparatively less in the HoLEP arm vs TURP arm (38.39 hours vs 66.06 hours), ($p < 0.001$). Additionally, the hospital stay was shorter in the HoLEP arm (59.50 hours) compared to TURP (87.86 hours), ($p < 0.001$). Hemoglobin loss was more in TURP than HoLEP (1.57 vs 0.76 g/dL), (Table 2), (Figure 1).

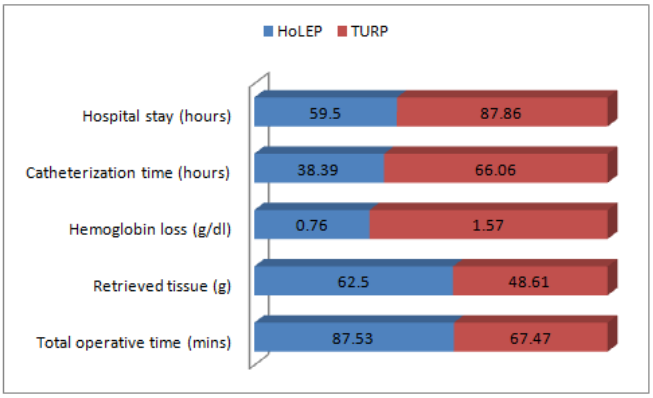

Figure 1 – Comparison of operative and post-operative parameters

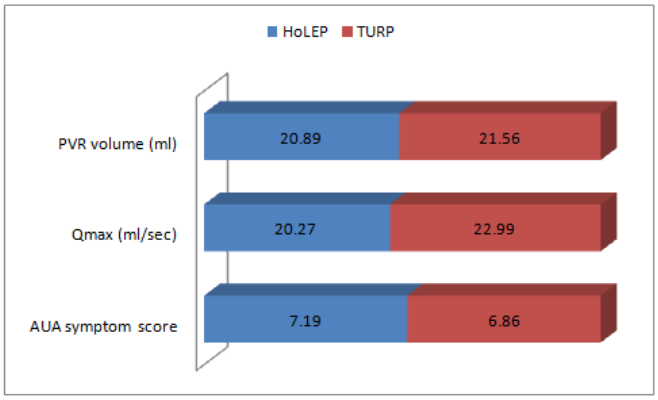

Figure 2 – Comparison of operative and post-operative parameters

Table 1 Baseline preoperative patient characteristics.

Variable	HoLEP (mean ± SD) (n=36)	TURP (mean ± SD) (n=36)	P value
Age (years)	66.78 ± 6.16	67.33 ± 7.60	0.734
Prostate volume (g)	68.51 ± 21.34	55.52 ± 20.94	0.015
Qmax (ml/sec)	7.76 ± 2.17	7.22 ± 2.20	0.295
PVR volume (ml)	174.97 ± 43.18	166.86 ± 55.79	0.493
PSA (ng/ml)	2.58 ± 0.96	2.26 ± 1.08	0.186
AUA symptom score	22.64 ± 2.88	22.89 ± 2.72	0.695

Table 2 Perioperative data

Variable	HoLEP (mean ± SD) (n=36)	TURP (mean ± SD) (n=36)	P value
Total operative time (mins)	87.53 ± 15.93	67.47 ± 9.98	<0.001
Retrieved tissue (g)	62.50 ± 20.76	48.61 ± 18.20	0.003
Hemoglobin loss (g/dl)	0.76 ± 0.36	1.57 ± 0.69	<0.001
Catheterization time (hours)	38.39 ± 15.54	66.06 ± 14.19	<0.001
Hospital stay (hours)	59.50 ± 18.53	87.86 ± 17.83	<0.001

Table 3 Postoperative complications

Complications	HoLEP (mean ± SD) (n=36)	TURP (mean ± SD) (n=36)	P value
Re-catheterization-no(%)	4(11.1)	6(16.7)	0.735
Transient dysuria-no(%)	8(22.2)	10(27.8)	0.586
Transient urinary incontinence-no(%)	6(16.7)	8(22.2)	0.551
Hematuria-no(%)	2(5.6)	5(13.9)	0.429
Blood transfusion	0(0)	2(5.6)	0.493

Based on the Clavien-Dindo classification, this study found that the HoLEP group experienced 20 grade I complications, all of which were managed conservatively. In the TURP group, there were 29 grade I complications and 2 grade II complications, all treated conservatively and with blood transfusions. Neither group required any endoscopic interventions. Transient dysuria in 22.2%, transient urinary incontinence in 16.7%, hematuria in 5.6%, no stricture, and recatheterization in 11.1% of the HoLEP group. In the TURP group, transient dysuria was 27.8%, transient urinary incontinence was 22.2%, hematuria was 13.9%, there were no strictures, and recatheterization was 16.7%. The rate of blood transfusions was 5.6% in the TURP group compared to 0% in the HoLEP group, (Table 3). The difference in the proportion of complications between the two groups was not statistically significant.

At 3-month postoperative follow-up findings for AUA symptom scores, Qmax, and PVR urine volume, in both groups showed statistically significant improvements from baseline in these measures, $p < 0.001$. In the HoLEP group, AUA symptom scores decreased from 22.64 to 7.19, while in the TURP group, they decreased from 22.89 to 6.86. Qmax increased from 7.76 to 20.27 ml/sec in the HoLEP group and from 7.22 to 22.99 ml/sec in the TURP group. PVR decreased from 174.97 to 20.89 ml in the HoLEP group and from 166.86 to 21.56 ml in the TURP group, (Table 4), (Figure 2). There was a statistically significant

Table 4 Postoperative follow up at 3 months

Parameter	HoLEP (mean ± SD) (n=36)	TURP (mean ± SD) (n=36)	P value
AUA symptom score	7.19 ± 1.21	6.86 ± 1.19	0.215
Qmax (ml/sec)	20.27 ± 3.21	22.99 ± 3.11	0.001
PVR volume (ml)	20.89 ± 6.41	21.56 ± 6.86	0.968

difference in Qmax (percentage increase in Qmax) between the two procedures (p = 0.007). However, no significant differences were observed in AUA symptom scores or PVR urine volume at the 3-month follow-up.

Discussion

HoLEP with morcellation is becoming an increasingly popular choice for treating symptomatic BPH. Advances in endourological and laser techniques have led to a shift from more invasive procedures to minimally invasive treatments, which offer high efficacy (by removing a significant amount of prostatic adenoma) with reduced perioperative morbidity. TURP remains the gold standard for BPH management, but its main complications include bleeding requiring transfusions and irrigant absorption, which can lead to TUR syndrome [2]. HoLEP offers several advantages over TURP, including shallower penetration, lower morbidity, reduced need for blood transfusions, shorter hospital stays, suitability for patients on anticoagulants, and effectiveness across a wide range of prostate sizes [5].

Both surgical techniques demonstrated statistically significant improvements from baseline in AUA symptom scores, Qmax, and PVR urine volume at the 3-month postoperative follow-up. There was a statistically significant difference in Qmax between the two procedures (p = 0.001). However, no significant differences were observed in AUA symptom scores or PVR urine volume at the 3-month follow-up. HoLEP, similar to TURP, creates an open prostatic cavity. This could account for the immediate improvement in micturition observed in this study, as well as the fact that mean AUA symptom scores, peak urinary flow rates, and post-void residual urine volumes normalized within three months postoperatively in each group. However, there is no explanation for the statistically significant difference in Qmax between the two groups at the three-month follow-up.

Our study findings align with those of Hamouda et al. [6], who reported similar results at the 3-month follow-up. In their study, AUA symptom scores decreased from 22.33 to 7.57 in the HoLEP group, and from 22.10 to 8.10 in the TURP group. Additionally, Qmax increased from 5.90 to 19.12 ml/sec in the HoLEP group, and from 6.87 to 20.67 ml/sec in the TURP group. Post void residual (PVR) decreased from 160.00 to 17.33 ml in the HoLEP group and from 212.17 to 13.65 ml in the TURP group. They also observed that there was no statistically significant difference between the two groups up to 12 months after surgery, and the results were comparable. In a similar study by Jhanwar et al. [7], a notable difference in IPSS scores was observed for both modalities compared to baseline, although no statistically significant difference was found in IPSS scores until 24 months of follow-up. They also noted a difference in Qmax between TURP and HoLEP at all follow-up points, with a significant difference observed at 12 months. PVR decreased significantly from baseline in both groups, but HoLEP showed a slight advantage, though the difference was not statistically significant. Eltabey et al. [8] found that the AUA symptom score and PVR volumes were significantly better in the HoLEP group compared to the TURP group at all postoperative assessments up to 12 months, while Qmax did not show significant differences between the two groups during the same period.

HoLEP has been demonstrated as a viable alternative for treating small (<40 g) [9], large (>40 g), [10,11] and very large (>100 g) [12] prostates compared to TURP or open prostatectomy. Gilling et al. [13] found similar results when comparing HoLEP to TURP for smaller glands. There seems to be no size limitation for prostate treatment with HoLEP; the largest prostate in our study was 96 g in the HoLEP group and 98 g in the TURP group (higher-sized glands were excluded from the study to maintain uniformity for comparision). The mean prostate volume (g) was 68.51 ± 21.34 (range: 32-96) in the HoLEP group and 55.52 ± 20.94 (range: 31-98) in the TURP group (p = 0.015). While there was no statistically significant difference in PSA between the two groups, the baseline prostate volume showed a significant difference.

In the TURP group, the average hemoglobin loss was 1.57 ± 0.69 g/dL, significantly higher than the 0.76 ± 0.36 g/dL observed in the HoLEP group (p < 0.001). Blood transfusion rates were 5.6% in the TURP group compared to 0% in the HoLEP group; however, this difference was not statistically significant (p = 0.493). Eltabey et al. [8] found HoLEP superior in terms of perioperative morbidity, with significantly less blood loss (p < 0.05). Hamouda et al. [6] recorded mean hemoglobin losses of 1.157 ± 0.918 g/dL for TURP and 0.9 ± 0.419 g/dL for HoLEP, with no statistically significant difference. They also reported transfusion rates of 6.6% in the TURP group versus 0% in the HoLEP group, which was also not statistically significant. HoLEP was noted for its excellent hemostasis, resulting in significantly less bladder irrigation, shorter catheterization times, and shorter hospital stays.

Kanchi et al. [14] found that postoperative catheterization durations were shorter for HoLEP compared to TURP (2.14 vs 3.17 days). Jhanwar et al. [7] reported similar results, noting that catheterization times were significantly reduced in the HoLEP group compared to the TURP group (30.94 hours vs. 48.06 hours, p = 0.0001). They also observed a statistically significant reduction in hospital stay for the HoLEP group compared to the TURP group (41.81 hours vs. 54.58 hours, p = 0.0001). Our study found a mean catheterization time of 38.39 hours for the HoLEP group versus 66.06 hours for the TURP group (p < 0.001), and a hospital stay of 59.50 hours for the HoLEP group compared to 87.86 hours for the TURP group (p < 0.001). A shorter hospital stay in the HoLEP group may be linked to reduced blood loss, as catheterization duration could indicate blood loss.

In this study, the amount of prostate tissue retrieved (in grams) was greater in the HoLEP group compared to the TURP group, with averages of 62.50 vs 48.61 gms, respectively (p = 0.003). Similar studies [6-8] also reported a statistically significant difference in prostate tissue retrieval with the HoLEP group showing greater amount of tissue resected compared to the TURP group. Additionally, HoLEP has been demonstrated to be as effective as open surgery in creating a cavity, as the fiber tip closely resembles the surgeon's index finger during an operation.

In this study, the operative time was significantly longer for the HoLEP group compared to the standard TURP group. This difference is attributed to the procedure's completeness and the morcellation required for tissue retrieval, with mean times

of 87.53 minutes for HoLEP and 67.47 minutes for TURP ($p < 0.001$). Similar studies [6,7,14] also found a longer operative time for the HoLEP vs TURP, and a statistically significant difference in operative time between the two groups. In contrast, Eltabey et al. [8] found that the total operating time for HoLEP was similar to that of TURP. They attributed this result to the use of a mechanical morcellator with new blades for fragmenting the enucleated prostatic lobes within the bladder, which reduces the total operating time to a level similar to TURP. The extended operative time for HoLEP compared to TURP is mainly due to the procedure's complexity, the meticulous nature of tissue removal, and the level of skill and experience required. According to Kim et al. [15], in their retrospective analysis of HoLEP performed at two different centers, the primary significant difference was the operative time, which was attributed to surgeon performance rather than the technique itself. Estimating the number of cases needed to master the technique is challenging.

Bladder mucosal injury, a specific complication of HoLEP, typically results from the unintentional suction of mucosa into the morcellator blades. Despite this risk, our study did not record any instances of such injury. In a one-year follow-up study by Gupta et al. [10] the recatheterization rate was 6% for HoLEP and 4% for TURP. Transient dysuria occurred in 10% of HoLEP cases compared to 2% in TURP ($p < 0.03$), with stricture rates at 2% for HoLEP and 3% for TURP. Incontinence rates were the same for both procedures at 2%, and capsular perforation was seen in 2% of HoLEP cases. Additionally, bladder mucosal injury was noted in 4% of HoLEP cases. Our study showed a recatheterization rate of 11.1% for HoLEP and 16.7% for TURP, with all recatheterizations performed due to failed initial voiding trials. We found transient dysuria in 22.2%, transient urinary incontinence in 16.7%, hematuria in 5.6%, no stricture, and recatheterization in 11.1% of the HoLEP group. In the TURP group, transient dysuria was 27.8%, transient urinary incontinence was 22.2%, hematuria was 13.9%, there were no strictures, and recatheterization was 16.7%. Although these complaints in our study were generally not severe and resolved with treatment, we lack a clear explanation for these outcomes since UTIs were ruled out. At the three-month follow-up, there were no significant differences between the groups regarding the incidence of complications. Based on the Clavien-Dindo classification, this study found that the HoLEP group experienced 20 grade I complications, all of which were managed conservatively. In the TURP group, there were 29 grade I complications and 2 grade II complications, all treated conservatively and with blood transfusions. Neither group required any endoscopic interventions. In our study, no patients developed urethral strictures or required re-operation in either group.

In our study, the patient distribution across groups was 1:1, making the analysis comparable. Additionally, to prevent performer bias, both procedures were performed by the same surgeon. Monopolar TURP exclusively employed to ensure uniformity and facilitate comparison with the HoLEP technique, rather than using both bipolar and monopolar approaches. The study's limitations included a non randomized design, a short follow-up time and a small sample size. The findings of this study warrant further validation through a randomized controlled trial and a prospective multicentric evaluation with a longer follow-up period. This would allow for a more comprehensive comparison of the two techniques regarding functional outcomes and complications, helping to establish the validity of HoLEP as comparable to TURP.

Conclusion

HoLEP appears to be a safe and effective alternative to TURP for treating LUTs due to BPH. It offers less perioperative morbidity and similar efficacy at three months. Compared to TURP, HoLEP is associated with reduced blood loss, shorter catheterization times, and shorter hospital stays. However, HoLEP does have the disadvantage of requiring a longer surgical duration. Postoperative complications and improvements in symptoms, along with micturition parameters, after HoLEP are comparable to those seen after TURP.

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Relationship Between Delta CO_2 and Mortality in Cardiac Arrest Patients

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Abstract

Introduction: Cardiac arrest is a sudden and unexpected condition that occurs for various reasons. Early identification and resuscitation efforts are crucial for both restoring spontaneous circulation and ensuring neurological survival. This study investigated the relationship between arterial partial pressure of carbon dioxide ($PaCO_2$) and end-tidal carbon dioxide ($ETCO_2$) difference stated as the Delta CO_2 and the return of spontaneous circulation (ROSC) in cardiac arrest patients.

Methods: The study was conducted prospectively on patients who had experienced cardiac arrest and were subsequently followed up in the emergency clinic of a tertiary education and research hospital or transported by ambulance. The resuscitation efforts times, outcomes, demographic data, chronic diseases, $ETCO_2$, and $PaCO_2$ levels of the patients were monitored and the collected data were subjected to analysis. The patients who achieved ROSC were classified as No ROSC group1, the patients who did not achieve ROSC were classified as No ROSC group2, and the two groups were subsequently analyzed.

Results: The study cohort comprised 57 patients who had been admitted to the emergency department. Among the arrest patients, 25 (43.9%) achieved ROSC, while 32 (56.1%) did not. A comparison was conducted between the $PaCO_2$ and $ETCO_2$ values of the patients, revealing a significantly higher mean $PaCO_2$ at the 20th minute in No ROSC group2 compared to ROSC group(p=0.000). Mean $PaCO_2$ values at 0 and 10 minutes were significantly higher in ROSC group(ROSC) compared to No ROSC group2 (p=0.036). Conversely, mean $ETCO_2$ values at 0 and 10 minutes were significantly higher in ROSC group(p=0.006 and 0.027, respectively). The deltaCO2 value of the patients was significantly higher in No ROSC group2 compared to ROSC groupat the 0th, 10th, and 20th minutes (0.019, 0.028, and 0.007, respectively).

Conclusion: Our findings suggest that, $ETCO_2$ levels during CPR and a smaller Delta CO_2 may predict a higher likelihood of ROSC.

Keywords: Cardiopulmonary resuscitation, End-tidal Carbon Dioxide, Delta Carbon Dioxide

Introduction

Cardiac arrest (CA) is a critical medical emergency involving the abrupt cessation of cardiac function, leading to a loss of blood flow to vital organs. Cardiac arrest is a leading cause of global mortality with high prevalence in both in and out of hospital settings. The survival rate is typically low; however, various factors and resuscitation effortss can influence outcomes (1, 2).

The term "end-tidal carbon dioxide" ($ETCO_2$) is used to describe the level of carbon dioxide released during the final stage of expiration. $ETCO_2$ levels indicate a multifactorial variable dependant on a ventilatory and perfusion correlation and dependant on ist membrane diffusion. The extant evidence indicates that $ETCO_2$ measurement may serve as an indicator of cardiac output and pulmonary blood flow (1). According

to the American Heart Association's (AHA) 2020 update, cardiac arrest is a frequent occurrence in US emergency departments, with over 347,000 out-of-hospital and approximately 292,000 in-hospital cases reported annually. These statistics underscore the considerable prevalence of cardiac arrest management in the emergency department.

The objective of this study is to evaluate the relationship between the difference (delta CO_2) between simultaneously measured arterial $PaCO_2$ and $ETCO_2$ levels in patients who have experienced cardiac arrest and the outcome of resuscitation. Arterial $PaCO_2$ is useful for assessing ventilation, whereas $ETCO_2$ primarily provides insight into perfusion. This paper postulated that impaired ventilation and perfusion during cardiac arrest negatively affect patient's resuscitation outcomes. This study suggests that delta CO_2 may predict unfavorable resuscitation outcomes and provide potential prognostic value for patients in cardiac arrest (2-5). The objective of our study is to investigate the association between delta CO_2 and the return of spontaneous circulation in cases of cardiac arrest.

Methods

This study was conducted prospectively in the emergency medicine department of a tertiary education and research hospital. The study population included patients above the age of 19 years who experienced a non-traumatic cardiac arrest, either prior to arrival in or during follow-up admission in the emergency department. Exclusion criteria were based on missing data, age under 18 years, traumatic cardiac arrest, and emphysematous disease that could affect CO_2 levels, Patients with conditions such as carbon monoxide poisoning, drug intoxication. Informed consent was obtained on admission from all patients or their next of kin. All measurements were made at room temperature under appropriate atmospheric pressure. The zero starting point is the minute of successful intubation. Guidelines published by international organizations emphasize the use of capnographs. The following statements are made regarding this situation: Cuffed endotracheal tubes: Intubation with a cuffed endotracheal tube can improve capnography and ventilation in patients with poor pulmonary compliance and reduce the need for endotracheal tube changes. It is logical to prefer cuffed endotracheal tubes to uncuffed endotracheal tubes for intubating infants and children. Some studies have found waveform capnography to be 100% specific for confirming endotracheal tube position during cardiac arrest. The sensitivity of waveform capnography decreases after prolonged cardiac arrest. The use of waveform capnography to assess the placement of other advanced airways (e.g., Combitube, laryngeal mask airway) has not yet been studied (1,5).

A standardized patient record form was designed prior to patient recruitment. The standardised data collection form obtained demographic information (age, gender), comorbidities (diabetes mellitus, hypertension, cardiovascular disease, cerebrovascular disease, chronic obstructive pulmonary disease, chronic kidney disease), laboratory findings (arterial $PaCO_2$, $ETCO_2$, ECG), location of arrest, witnessed status, time to CPR initiation, and provider and duration of resuscitation. Intubation was performed during CPR and successful intubation was achieved in all patients, and routine tube verification was performed in each patient.

Once the primary emergency physician established advanced airway management without interrupting standard CPR, simultaneous arterial blood gas and $ETCO_2$ measurements were promptly recorded. Arterial blood gases were obtained at 0 minutes (immediately following advanced airway management without disrupting CPR), at 10, and at 20 minutes. The pre-prepared study form was used to record the $PaCO_2$ and $ETCO_2$ values. In the event that the patient achieved a return of spontaneous circulation (ROSC), arterial blood gas and $ETCO_2$ measurements were repeated and recorded in the study form. Delta CO_2 was calculated by subtracting the $ETCO_2$ value from the $PaCO_2$ value and measured at each time point (0, 10 and 20 minutes).

Statistical Analysis

Data were recorded on a standardized form and analyzed using SPSS version 27 (IBM). Categorical data are presented as percentages and frequencies. All numerical data underwent distribution analyses. Normally distributed data are presented as mean plus standard deviation (SD), while non-normally distributed data are presented as median including interquartile range (IQR).

To compare categorical variables, the chi-square test was employed, and Monte Carlo inference analysis was conducted. For normally distributed numerical data, t-tests were used for comparisons between two groups, and ANOVA was used for comparisons between more than two groups. The Mann-Whitney U test was applied for non-normally distributed data. Statistical significance was defined as $p < 0.05$. Accordingly, the sample size was calculated as 25 for each group with a Type 1 error of 5%, a Type 2 error of 20% (80% power), and provided that a two-way analysis was performed.

Results

The study included 57 patients entering to the emergency department following cardiac arrest. Return of spontaneous circulation (ROSC) was achieved in 25 patients (43.9%) and these were designated as No ROSC group1. In 32 patients (56.1%), ROSC was not achieved and these were designated

Table 1 General demographic data of the patients participating in the study

Parameters	ROSC (n=25)	No ROSC (n=32)	P value
Sex, Female (n, %)	8 (%32)	8 (%25)	0.196
Age (mean±SD)	64.64±15.10	66.31±14.42	0.672
Diabetes mellitus (n, %)	11 (%44)	10 (%31.3)	0.135
Coronary artery disease (n, %)	7 (%28)	13 (%40)	0.138
Hypertension(n, %)	10 (%40)	13 (%40.6)	0.214
Cerebrovascular accident(n, %)	3 (%12)	0	0.079
Chronic kidney disease(n, %)	1 (%4)	2 (%6.3)	0.424
Malignancy(n, %)	2 (%8)	6 (%18.8)	0.165
Place of arrest			
House(n, %)	5 (%20)	14 (%43.8)	0.016
Social area (n, %)	1 (%4)	4 (%12.5)	
Ambulance (n, %)	1 (%4)	0	
Hospital (n, %)	18 (%72)	14 (%43.8)	
Bystander			
Non-healthcare worker (n, %)	8 (%24)	15 (%46.9)	0.047
Healthcare worker (n, %)	19 (%76)	17 (%53.1)	
Underlying Cardiac Rhythm (n, %)			
NEA	9 (%36)	2 (%6.3)	0.013
Asystoles	11 (%44)	26 (%81.3)	
VF	4 (%16)	4 (%12.5)	
nVT	1 (%4)	0	

as No ROSC group2. Cardiac arrests patients in ROSC group(ROSC) occurred more frequently in the hospital setting compared to patients from No ROSC group2 (p=0.016). The frequency of asystole as the initial rhythm was significantly higher in No ROSC group2 than in ROSC group(p = 0.013). General demographic data of the patients participating in the study is given in Table 1.

A comparison was conducted between the $PaCO_2$ and $ETCO_2$ values of the patients. At 20 minutes, the mean $PaCO_2$ was significantly higher in No ROSC group2 (p=0.036). The mean $ETCO_2$ was significantly higher in ROSC groupat both 0 and 10 minutes compared to values detected in No ROSC group2 (p = 0.006 and p = 0.027, respectively). The duration of cardiopulmonary resuscitation (CPR) was significantly shorter in ROSC groupcompared to No ROSC group2 (12 vs. 43.50; p < 0.001) (Table 2-3).

Table 2 Comparison of PaCO2 and ETCO2 over time

PaCO2 Time	ROSC (n=25)	No ROSC (n=32)	p-value
0. minute (n)	57.42±20.30 (25)	57.70±22.72 (32)	0.962
10. minute (n)	63.71±24.93 (13)	62.38±21.30 (32)	0.857
20. minute (n)	38.1±17.45 (4)	63.41±21.9 (27)	0.036
End of CPR (n)	51.41±21.89 (25)	-	-
ETCO2			
0. minute (n)	30.48±14.14 (25)	20.03±13.39 (32)	0.006
10. minute (n)	31.92±19.63 (13)	17.68±12.02 (32)	0.027
20. minute (n)	19.5±5.91 (4)	13.92±11.59 (27)	0.358
End of CPR	31.84±6.91 (25)	-	-

Table 3 Comparison of PACO2 and ETCO2 differences over time

Difference	ROSC (n=25)	No ROSC (n=32)	P value
0th minute	26.94±13.50	37.67±18.78	0.019
10th minute	31.79±12.26	44.69±18.88	0.028
20th minute	18.60±15.54	49.48±20.32	0.007
SDGD time	20.05±12.09	43.1	-

Discussion

In resuscitation research, patient outcome including neurological outcome are the critical endpoints. Resuscitation practices have been guided by established guidelines. Guidelines from the American Heart Association (AHA) and the European Resuscitation Council emphasize the importance of $ETCO_2$ levels and other prognostic data for optimizing CPR. The International Liaison Committee on Resuscitation (ILCOR) plays a pivotal role in shaping resuscitation practices worldwide through its evidence-based guidelines. The development of these guidelines is meticulously conducted through a thorough evaluation of scientific evidence, and they undergo a comprehensive update every five years to align with the most recent advancements in resuscitation science. The influence of ILCOR's guidelines on resuscitation practices, both in-hospital and out-of-hospital, has been substantial, contributing to enhanced outcomes in cases of cardiac arrest on a global scale. The guidelines are adapted by regional resuscitation councils to suit local contexts, ensuring their applicability across diverse healthcare settings. This adaptability is crucial for addressing the varying resource

The $PaCO_2$ - $ETCO_2$ difference was significantly higher in No ROSC group2 compared to ROSC groupat 0 minutes (p = 0.019), 10 minutes (p = 0.028), and 20 minutes (p = 0.007). Additionally, the change in $ETCO_2$ from time intervals of 0 to 10 minutes post cardiac arrest (Delta CO_2) were analyzed. The median Delta CO_2 was significantly higher in ROSC groupcompared to No ROSC group2 (1 vs. -3; p=0.012). ROC analysis revealed that the $ETCO_2$-$PaCO_2$ difference (Delta CO_2) at 0 and 10 minutes were significant predictors of ROSC (Table 4). A Delta CO_2 threshold of 0.5 mmHg yielded a sensitivity of 61.5% and a specificity of 81.2% (Figure 1-2).

Table 4 ROC analysis of delta CO2 according to groups

Variable	Area	p-Value	Boundary	Sensitivity	Specificity
Delta CO_2	0,737	0,005	0,5	61,5	81,2
0. min Difference	0,766	0,001	24,4	76,9	68,7
10. min Difference	0,697	0,015	43,75	84,6	50

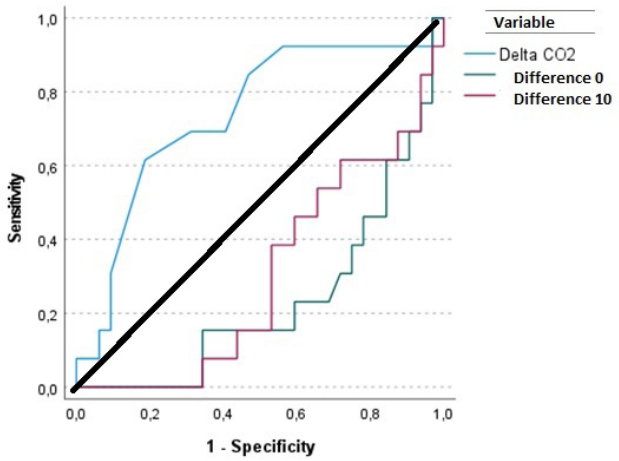

Figure 1 – ROC analysis of delta CO_2 according to groups.

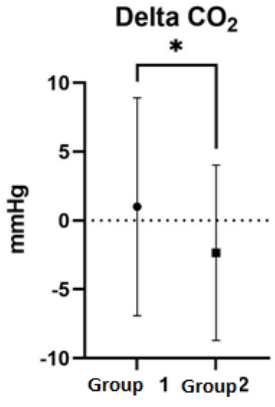

Figure 2 – Delta CO_2 comparison by groups

levels and healthcare infrastructures worldwide. [8,9]. Our study revealed that the $ETCO_2$-$PaCO_2$ difference (Delta CO_2) at 0 and 10 minutes are important predictors of ROSC. A Delta CO_2 threshold of 0.5 mmHg provided a sensitivity of 61.5% and a specificity of 81.2%.

In a study by Baldi et al., the predictive impact of the carbon dioxide difference on resuscitation was discussed. The study

cohort comprised 688 patients, of whom 39 were discharged from the hospital and 649 were observed to have died during the follow-up period (11). In a study conducted by Ruis et al., the impact of carbon dioxide variation on the likelihood of return of spontaneous circulation (ROSC) was assessed. The mean age of the 102 patients included in the study was 69 years, with 35.5% of them being female.

The present study included a total of 57 patients who had experienced cardiac arrest. No ROSC group1, in which 43.9% achieved ROSC, and No ROSC group2, in which 56.1% did not achieve ROSC and subsequently died following resuscitation efforts. The proportion of female patients was 32% in ROSC groupand 25% in No ROSC group2, with no significant difference in sex and age distribution. A review of similar studies in the literature revealed that our findings regarding age and sex distribution are consistent with the reported results.

The distinction between arterial partial pressure of carbon dioxide ($PaCO_2$) and end-tidal carbon dioxide ($ETCO_2$) is of paramount importance in clinical settings, particularly for patients on mechanical ventilation or those with respiratory conditions. The measurement of $PaCO_2$ is conducted directly from arterial blood, thereby providing an accurate indication of the body's CO_2 levels. In contrast, $ETCO_2$ is measured non-invasively from exhaled air and is frequently employed as a surrogate for $PaCO_2$ in clinical contexts. However, the correlation between these two measures can vary considerably depending on the specific clinical scenario and the condition of the patient in question. In patients with traumatic brain injury (TBI), the $PaCO_2$–$ETCO_2$ gradient has been observed to be larger than previously reported, particularly in the presence of concomitant injuries. This gradient was associated with increased mortality, indicating that end-tidal carbon dioxide ($ETCO_2$) may not be a reliable predictor of arterial carbon dioxide ($PaCO_2$) in these patients during the early stages of treatment. In patients with chronic obstructive pulmonary disease (COPD) exacerbations, a positive correlation was observed between $PaCO_2$ and $ETCO_2$ levels. Nevertheless, the mean differences were sufficiently significant to limit the clinical utility of $ETCO_2$ as a standalone measure for guiding treatment. In pediatric patients undergoing cardiac surgery, end-tidal carbon dioxide ($ETCO_2$) provided a close prediction of preoperative partial pressure of carbon dioxide ($PaCO_2$) levels, but the difference became clinically significant postoperatively, necessitating direct arterial sampling for accurate $PaCO_2$ measurement (21).

The $PaCO_2$–$ETCO_2$ gradient can be utilized as an index of physiological dead space, and may prove beneficial in predicting the success of weaning from mechanical ventilation. A threshold of ≤10 mmHg has been proposed as a marker for successful weaning and extubation. In a study conducted by Baldi and colleagues, patients with an $ETCO_2$ pressure above 20 mmHg exhibited significantly lower mortality rates than those with lower levels. In this study, a lower delta CO_2 value was observed in patients who experienced fatal outcomes, and a change above 1 mmHg was associated with an odds ratio of 1.92 (12). As evidenced in the literature, $ETCO_2$ is of paramount importance in determining patient mortality and analyzing 30-day survival rates. In a study conducted by Islam et al., the researchers found that delta CO_2 was not a significant predictor of 30-day survival in patients who achieved ROSC. In their analysis evaluating the effect of delta CO_2 on ROSC, delta CO_2 demonstrated 78.19% sensitivity and 76% specificity at the established threshold. Delta CO_2 measurements play a crucial role in predicting the ROSC in patients experiencing cardiac arrest. Continuous monitoring of the partial pressure of carbon dioxide and the partial CO_2 can provide real-time feedback on the effectiveness of CPR, thus guiding clinicians in their resuscitation efforts. The predictive value of these measurements suggests that they could be integrated into standard protocols for managing cardiac arrest patients. However, it is important to note that while Delta CO_2 and $ETCO_2$ measurements are valuable, they are not infallible indicators of patient outcomes (19).

In our study, the CO_2 difference at 0 and 10 minutes was significantly higher in the ROSC group. ROC analysis demonstrated that this difference is a reliable predictor, with a sensitivity of 61.5% and a specificity of 81.2% at the optimal threshold value of 0.5 mmHg. Our findings suggest that, similar to its use in various diseases and populations, delta CO_2 may serve as a mortality-related marker.

This study was conducted at a single center. Conducting the study at multiple centers and initiating patient follow-up at the onset of CPR would facilitate data expansion and more comprehensive effectiveness analysis. While ROSC is a clear and measurable outcome, we acknowledge that its informativeness regarding patient relevance and long-term prognosis is limited. One of our most important limitations is that patients with pulmonary embolism, pneumonia and lung pathology that may affect the parameters are not included in the scope of the study. Another limitation of study is unsealed airway circuits are critical components in anesthesia and ventilation, designed to facilitate gas exchange while ensuring patient safety. These circuits can be equipped with various technologies, such as artificial airways and re-breathing valves, to enhance their functionality. More detailed neurological recovery and follow-up of patients is needed. In this study, the estimated arrest time and time spent without CPR in out-of-hospital arrest cases were evaluated based on information from 112 teams, raising questions about the reliability of this approach.

Conclusion

During the CPR period, the mean ETCO2 value was significantly higher in patients without mortality compared to those with mortality, while the difference between PaCO2 and ETCO2 was significantly lower. It was observed that high ETCO2 levels during cardiopulmonary resuscitation, as well as a decrease in the difference between partial carbon dioxide amount and ETCO2, are predictive of survival.

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Diagnostic Algorithm Application in Pediatric Patients with Complex Neurological Phenotypes in South Kazakhstan

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Abstract

Introduction: Complex neurological phenotypes (CNPs) in pediatric patients often involve multiple coexisting syndromes that challenge conventional diagnostic pathways. In the present study, we evaluated the diagnostic algorithm developed by our team—"Deep Phenotyping–Based Diagnostic Algorithm for Complex Neurological Phenotypes"—which integrates deep phenotyping and genomics.

Methods: In this single-center retrospective study, 28 children (0–18 years) with CNPs were assessed at the Diagnostic Center Clinic of Khoja Akhmet Yassawi International Kazakh-Turkish University between January and December 2023. Clinical features were encoded as standardized Human Phenotype Ontology (HPO) terms, which were entered into the Phenomizer platform to generate ranked candidate genes. Proband whole exome sequencing (WES) was performed for all participants. Reverse phenotyping re-examined clinical data in light of genetic findings. The algorithm's diagnostic yield and concordance between prioritization and confirmed diagnoses were analyzed descriptively.

Results: A molecular diagnosis was established in 14 of 28 patients (50%). Confirmed etiologies included monogenic neurodevelopmental syndromes, metabolic disorders, mitochondrial encephalopathy, craniosynostosis, and chromosomal aberrations. In all single-gene cases, the causal gene ranked within Phenomizer's top five candidates. Reverse phenotyping corroborated genotype–phenotype correlations and revealed additional clinical features in select patients.

Conclusions: This multi-step algorithm achieved a 50 % diagnostic yield—exceeding typical WES-only approaches—and demonstrated robust concordance between phenotype-driven prioritization and molecular results. The framework is feasible in resource-limited settings and may streamline the genetic workup of complex pediatric neurological disorders.

Keywords: complex neurological phenotypes; Human Phenotype Ontology; gene prioritization; whole exome sequencing; diagnostic algorithm.

Introduction

In recent years, pediatric neurology has faced a growing number of patients whose clinical presentations do not fit into traditional diagnostic categories. These individuals often exhibit multiple co-occurring and persistent neurological syndromes—including epileptic, motor, cognitive, behavioral, and regressive manifestations—which together constitute a complex diagnostic challenge [1–4].

To address this issue, the term complex neurological phenotype (CNP) has been increasingly utilized in the literature. This term is applied to clinical

scenarios involving multiple, phenotypically distinct neurological syndromes that cannot be attributed to a single anatomical lesion or classical nosology [3,4]. According to the working definition proposed in our earlier work, CNPs are characterized by two or more clinically significant and pathophysiologically diverse neurological syndromes that resist classification within a single diagnostic framework.

CNPs are particularly significant in the context of rare genetic disorders, where pleiotropy, expanding phenotypic spectra, and multilocus variation frequently lead to blended phenotypes and diagnostic uncertainty [5–8]. Recent studies have demonstrated that whole exome sequencing (WES) achieves diagnostic yields of over 50% in patients with e phenotypes, which is markedly higher than in cases with isolated symptoms [7–9].

This highlights the growing relevance of phenotype-driven approaches, including deep phenotyping based on the Human Phenotype Ontology (HPO), clinical prioritization tools such as Phenomizer, and structured diagnostic algorithms aimed at improving molecular diagnosis in patients with suspected monogenic neurological disorders [3,10].

We previously developed and validated a stepwise diagnostic algorithm—"Deep Phenotyping–Based Diagnostic Algorithm for Complex Neurological Phenotypes"—specifically designed to evaluate pediatric patients presenting with CNPs. This algorithm integrates clinical data, manually curated HPO-based phenotyping, automated gene prioritization, next-generation sequencing NGS, and reverse phenotyping (Figure 1). It has been registered as an official intellectual property asset in the Republic of Kazakhstan (Certificate of Copyright Registration No. 56915, dated 17.04.2025) .

The algorithm begins with a thorough clinical evaluation, including family and perinatal history, developmental milestones, disease progression, physical and neurological examination, and pedigree analysis. These steps are paralleled by the analysis of instrumental and laboratory data, such as routine laboratory tests, metabolic screening, neuroimaging (MRI, CT), and neurophysiological studies (EEG, ENMG). All available clinical features are manually translated into standardized HPO terms, providing a structured phenotypic profile that serves as the foundation for gene prioritization [11].

The resulting HPO terms are entered into Phenomizer (https://hpo.jax.org/tools/phenomizer)—an HPO-based tool—which compares them against known gene–phenotype correlations and generates a ranked list of potential causative genes [12]. If the phenotype points to a specific disease pattern, targeted gene panels or chromosomal microarray analysis may be used. In cases of nonspecific or overlapping phenotypes, WES is performed to explore a broader range of potential etiologies. Detected variants are interpreted according to American College of Medical Genetics and Genomics (ACMG) guidelines, and reverse phenotyping is then conducted to re-examine the clinical features in light of the identified genetic changes. This comprehensive, iterative workflow facilitates accurate molecular diagnosis, enables personalized counseling of the patient and family, and lays the groundwork for potential precision medicine interventions.

The objective of the present study is to assess the clinical utility of this diagnostic algorithm in a cohort of children with CNPs, based on retrospective analysis of a regional sample from Southern Kazakhstan.

Methods

Study Design and Ethical Approval

This work represents a retrospective analysis carried out at the Diagnostic Center Clinic of Khoja Akhmet Yassawi International Kazakh-Turkish University in 2024. Clinical data

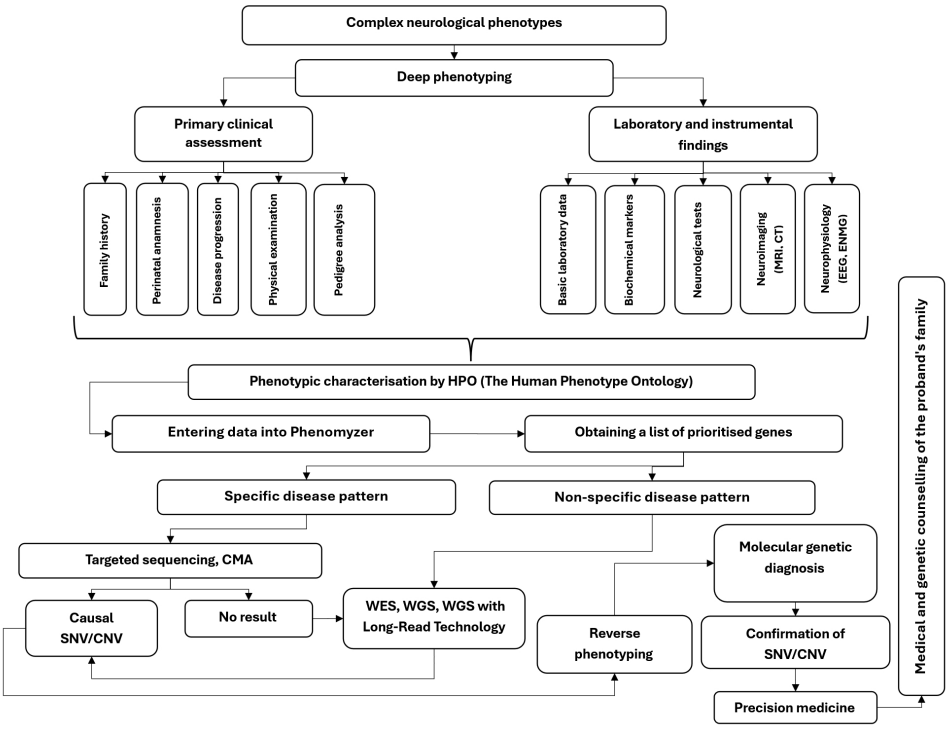

Figure 1 – Deep Phenotyping–Based Diagnostic Algorithm for Complex Neurological Phenotypes

CMA – Chromosomal Microarray Analysis, SNV – Single Nucleotide Variants, CNV – Copy Number Variations, WES – Whole Exome Sequencing, WGS – Whole Genome Sequencing

were collected from patients followed since January 2023. The study was approved by the local ethics committee (Protocol No. 16, June 2023), and informed consent was obtained from the legal guardians of all participants.

Patient Cohort

A total of 28 patients aged 0 months to 18 years with CNPs were included. Both male and female patients were represented. We included all pediatric patients (0–18 years) evaluated at our Diagnostic Center Clinic between January 2023 and December 2023 who met the definition of a CNP—namely, those with persistent involvement of two or more distinct neurological domains (motor, cognitive, behavioral, epileptic or movement), documented for at least six months in the medical record, with sufficient clinical data (neurological examination, imaging and/or electrophysiology) to enable standardized deep phenotyping, and whose legal guardians provided written informed consent. We excluded any patient presenting with only a single-domain neurological disorder, cases attributable solely to an acute or transient insult (e.g., infection, head trauma), those with an existing molecular diagnosis before January 2023, and patients lacking essential clinical documentation (such as missing neuroimaging or electrophysiological studies) required for comprehensive HPO-based phenotyping.

Diagnostic Algorithm and Phenotypic Assessment

A stepwise diagnostic algorithm was systematically applied to all cases in the cohort (Figure 1). This algorithm was designed to integrate structured clinical data, phenotype-driven genomic interrogation, and reverse phenotyping to improve diagnostic accuracy in children with CNPs. The pipeline consisted of the following major components:

Deep Phenotyping

Deep phenotyping was conducted manually by a pediatric neurologist using standardized terms from the HPO [11, 12]. Clinical data were extracted from detailed medical records, neurological assessments, neuroimaging reports, and functional evaluations. To assess motor and cognitive function, we employed the Gross Motor Function Classification System (GMFCS) and a standardized scale of intellectual functioning. Only terms that were unambiguously supported by clinical documentation were selected.

Each patient's phenotype was encoded as a set of HPO terms, covering motor, cognitive, epileptic, behavioral, neuroimaging, and dysmorphic domains. Phenotypic curation was performed using the HPO browser (https://hpo.jax.org), ensuring term validity, hierarchy consistency, and granularity.

Gene Prioritization using Phenomizer

The curated HPO term list for each patient was entered into the Phenomizer platform for phenotype-driven gene and disease prioritization. The analysis mode selected was "Rank Genes (based on OMIM disease similarity)". The tool computes semantic similarity scores between the patient's HPO profile and the phenotypic annotations of known Mendelian diseases using ontology-based inference.

For each case, Phenomizer generated a ranked list of candidate genes and associated disorders. Disease-specific patterns were identified when one or more top-ranked genes matched with the clinical hypothesis, enabling focused variant analysis. In patients with non-specific or heterogeneous features, Phenomizer results were used as a flexible guide to support exome-wide variant filtering.

All results were exported and stored in the patient file, including the list of top 5 candidate genes with semantic similarity scores and OMIM links.

Genetic Testing

WES was performed at 3billion Inc. (Seoul, South Korea) using the xGen Exome Research Panel v2 with sequencing on the Illumina NovaSeq X platform. The mean coverage was 170.99×, with 99.7% of targeted bases covered at ≥20×. Bioinformatic analysis and variant interpretation were conducted using the EVIDENCE v4.3 platform, incorporating ACMG/AMP guidelines [13, 14]. The pipeline included detection of SNVs, INDELs, CNVs, mitochondrial variants, and repeat expansions. Clinically relevant variants were reported; raw data are available upon request.

Reverse phenotyping was conducted using the OMIM (https://www.omim.org/) database to systematically compare the patient's clinical features with the known phenotypic spectrum associated with the identified gene(s). This approach enabled targeted re-examination of symptoms and refinement of the diagnostic hypothesis, particularly in cases with broad or atypical presentations [15].

Statistical Analysis

Descriptive statistical methods were used. Categorical variables were reported as absolute and relative frequencies; continuous variables were summarized using median and range. Data analysis and visualization were carried out using the Data Analyst tool.

Results

Cohort Characteristics

The cohort included 28 patients with CNPs, consisting of 14 males (50%) and 14 females (50%). The age of the patients ranged from 7 months to 17 years, with a median age of 6.5 years.

Deep Phenotyping

Manual deep phenotyping was performed for all 28 patients using standardized terms from the HPO. Clinical data were extracted from medical records, neurological examinations, neuroimaging, and functional assessments. Only features confirmed in the clinical documentation were encoded. Annotation was conducted using the official HPO browser, without automated or AI-assisted tools.

The distribution of motor impairment, assessed using the GMFCS, was as follows: Level I – 1 patient (4%), Level II – 12 patients (43%), Level III – 1 patient (4%), Level IV – 2 patients (7%), and Level V – 12 patients (43%). The majority of patients exhibited significant cognitive impairment: 22 patients (79%) were classified with severe intellectual disability. Moderate intellectual disability was observed in 2 patients (7%), profound in 2 (7%), and mild in 1 patient (4%); only one patient (4%) demonstrated no intellectual impairment.

A total of 396 HPO terms were recorded across the cohort. The average number of terms per patient was 14.1 (range: 7–24). All major neurological domains were represented in the phenotypic profiles (Table 1).

Frequency of Selected Phenotypic Features (n = 28):

1) Intellectual disability – 27 patients (96.4%);
2) Global developmental delay – 25 patients (89.3%);
3) Delayed or absent speech – 22 patients (78.6%);
4) Cerebral palsy – 18 patients (64.3%);
5) Hypotonia – 16 patients (57.1%);

Table 1

Clinical characteristics, Phenomizer-prioritized candidate genes, and confirmed genetic diagnoses in 28 pediatric patients with complex neurological phenotypes.

Case	Age (years)	Sex	GMFCS	ID Level	HPO terms	Phenomizer-predicted candidate genes	Genetic Diagnosis
1	10	Male	II	Severe	Cerebral palsy, Microcephaly, Global developmental delay, Intellectual disability, Sensorimotor aphasia, High myopia, Congenital myopia, Chorioretinal atrophy, Astigmatism, Short stature, Small hands, Small feet, Muscle dystonia, Hyperreflexia, Muscle weakness, Poor coordination, Spasticity, Strabismus, Reduced visual acuity, Sensory impairment.	VPS13B, PNPLA6, RAB3GAP1, RAB3GAP2, SPG11	VPS13B
2	15	Female	II	Moderate	Seizures, Intellectual disability, Spastic tetraparesis, Extrapyramidal movement disorder, Macrocephaly.	L2HGDH, MECP2, SLC2A1, GNAO1, PTEN	L2HGDH
3	4	Male	V	Severe	Short stature, Failure to thrive, Congenital cataract, Glaucoma, Microphthalmia, Reduced visual acuity, Dental cysts, Scoliosis, Kyphosis, Neonatal hypotonia, Areflexia, Intellectual disability, Ventriculomegaly.	OCRL, PAX6, NHS, FOXE3, LAMB1	OCRL
4	4	Male	II	Severe	Epileptic encephalopathy, Seizures, Clonic seizures, Global developmental delay, Speech delay, Nocturnal enuresis, Dystonia, Preterm birth, Low birth weight.	GNAO1, SCN2A, KCNT1, KCNQ2, STXBP1	
5	14	Male	V	Severe	Cerebral palsy, Spasticity, Global developmental delay, Delayed gross motor development, Absent speech, Tonic-clonic seizures, Epilepsy, Joint contractures, Microcephaly, Optic nerve atrophy.	SPAST, AP4B1, AP4M1, AP4E1, GNAO1	
6	16	Male	V	Severe	Cerebral palsy, Epilepsy, Tonic-clonic seizures, Global developmental delay, Delayed speech and language development, Optic nerve atrophy, Visual impairment, Dyskinesia, Abnormality of the thoracic cage, Restricted joint mobility.	GNAO1, AP4B1, TUBB4A, WDR45, KIF1A	
7	1	Female	V	Severe	Small stature, Low birth weight, Delayed skeletal maturation, Short neck, Macrocephaly, Hypertrophic cardiomyopathy, Coarse facial features, Broad face, Low forehead, Depressed nasal bridge, Epicanthus, Low-set ears, Posteriorly rotated ears, Redundant skin, Decreased skin elasticity, Hyperkeratosis, Abnormal nail morphology, Ulnar deviation of the fingers, Feeding difficulties, Poor suck reflex, Joint hypermobility, Abnormal toe morphology, Curly hair, Intellectual disability.	HRAS, PTPN11, BRAF, SOS1, RAF1	HRAS
8	5	Male	II	Severe	Obesity, Mild short stature, Macrocephaly, Scaphocephaly, Coarse facial features, Neurodegeneration, Profound intellectual disability, Learning disability, Intellectual disability, Full lips, Short neck, Widely spaced teeth, Multiple skeletal anomalies, Polydactyly, Kyphosis, Flexion contracture, Clawed hand, Hypertrichosis.	IDS, MKKS, TTC21B, BBS1, GUSB	IDS, MKKS
9	5	Male	V	Severe	Reduced visual acuity, Ophthalmoplegia, Myopathy, Muscle atrophy, Hemiparesis, Stroke-like episodes, Intellectual disability, Encephalopathy.	MTTL1, POLG, SURF1, SLC25A4, RARS2	MTTL1
10	8	Male	II	Moderate	Craniosynostosis, Brachycephaly, Frontal bossing, Maxillary hypoplasia, Mandibular prognathia, Optic atrophy, Microorbitism, Proptosis, Hypertelorism, Strabismus, Exposure keratoconjunctivitis, Reduced visual acuity, Hooked nose, Palatal swelling, Dental crowding, Coronal craniosynostosis, Sagittal craniosynostosis, Lambdoid craniosynostosis, Cervical spine abnormalities, Intellectual disability, Episodic seizures, Seizures, Frequent headaches.	FGFR2, FGFR3, TWIST1, EFNB1, TCOF1	FGFR2
11	12	Male	I	No	Ataxia, Intention tremor, Dysmetria, Impaired motor coordination, Global developmental delay, Abnormal gait, Irritability.	ATM, SPTBN2, CACNA1A, GRID2, ITPR1	
12	6	Female	II	Severe	Short stature, Coarse facial features, Facial hypertrichosis, Micrognathia, Low-set ears, Posteriorly rotated ears, Visual impairment, Strabismus, Upturned palpebral fissures, Thick eyebrows, Long eyelashes, Broad nasal tip, Macrostomia, Thin vermilion border of upper lip, Thick vermilion border of lower lip, Everted lower lip, Psychomotor developmental delay, Severe expressive language delay, Reduced speech production, Intellectual disability, Moderate hypotonia,	ARID1B, KMT2D, KDM6A, TRPS1, EHMT1	ARID1B
13	10	Female	V	Severe	Cerebral palsy, Diplegic gait, Hearing impairment, Global developmental delay, Delayed speech and language development, Dyskinesia, Delayed gross motor development, Hypotonia, Cognitive impairment.	GNAO1, AP4B1, AP4M1, FA2H, SYNE1	
14	4	Female	V	Profound	Cerebral palsy, Spasticity, Epileptic encephalopathy, Tonic seizures, Optic nerve atrophy, Delayed speech and language development, Global developmental delay, Hypotonia, Constipation, Intellectual disability.	SLC1A4, STXBP1, CDKL5, AP4E1, WDR45	

15	7	Female	II	Severe	Spina bifida, Sparse lateral eyebrow, Highly arched eyebrow, Brachymesophalangy, Short columella, Short fifth finger, Prominent ear, Macrotia, Long eyelashes, Hemivertebrae, Ectropion of the lower eyelid, Short stature, Microcephaly, Joint hypermobility, Intellectual disability, Ureteropelvic junction obstruction, Seizures	KMT2D, KDM6A, CHD7, NIPBL, PTPN11	KMT2D
16	7 months	Female	V	Severe	Epileptic encephalopathy, Tonic-clonic seizures, Eyelid myoclonia, Global developmental delay, Delayed speech and language development, Microcephaly, Hypotonia, Dystonia, Delayed gross motor development, Optic nerve atrophy.	SYNGAP1, SCN1A, SLC6A1, STXBP1, GNAO1	
17	9	Female	II	Profound	Epileptic encephalopathy, Tonic seizures, Neonatal seizures, Global developmental delay, Absent speech, Intellectual disability, Delayed gross motor development, Urinary incontinence, Fecal incontinence.	KCNQ2, SCN2A, STXBP1, CDKL5, FOXG1	
18	11	Female	IV	Severe	Cerebral palsy, Epilepsy, Tonic-clonic seizures, Global developmental delay, Delayed speech and language development, Intellectual disability, Muscular weakness, Urinary incontinence, Fecal incontinence, Optic nerve atrophy, Esotropia, Tall stature, Obesity.	WDFY3, PTEN, CNTNAP2, TSC2, AHDC1	WDFY3
19	5	Female	II	Severe	Microcephaly, Epileptic encephalopathy, Tonic seizure, Absence seizure, Global developmental delay, Absent speech, Impaired receptive language, Urinary incontinence, Fecal incontinence, Motor delay.	FOXG1, CDKL5, STXBP1, SLC6A1, MECP2	
20	4	Male	V	Severe	Tetraparesis, Global developmental delay, Motor delay, Hyperkinetic movement disorder.	GNAO1, ADCY5, ATP1A3, KMT2B, FOXP1	
21	17	Female	III	Mild	Cerebral palsy, Spasticity, Amblyopia, Hypermetropia, Exotropia, Global developmental delay, Delayed speech and language development, Intellectual disability, Delayed gross motor development, Visual impairment.	AP4M1, AP4S1, CTNNB1, SYNGAP1, TUBA1A	
22	17	Male	V	Severe	Cerebral palsy, Spasticity, Joint contractures, Epilepsy, Tonic-clonic seizures, Absent speech, Microcephaly, Global developmental delay, Delayed gross motor development, Delayed speech and language development.	AP4B1, AP4M1, KIF1A, GNAO1, STXBP1	
23	3	Female	IV	Severe	Cerebral palsy, Global developmental delay, Intellectual disability, Speech delay, Seizures, Respiratory-affective attacks, Hypotonia, Hyporeflexia, Muscle weakness, Motor development delay, Inability to walk, Small stature, Skull deformity, Low-set ears, Claw fingers, Urinary incontinence, Fecal incontinence.	ARID1B, FOXG1, STXBP1, CDKL5, SCN1A	ARID1B
24	13	Male	II	Severe	Iris hypoplasia, Pupillary ruff abnormality, Nystagmus, Mild to moderate visual impairment, Generalized hypotonia, Delayed motor development, Ataxia, Postural tremor, Severe intellectual disability, Cerebellar hypoplasia.	ITPR1, GRID2, CACNA1A, TSEN54, SLC9A6	ITPR1
25	2	Female	V	Severe	Cerebral palsy, Microcephaly, Focal epilepsy, Myoclonic seizures, Global developmental delay, Speech delay, Motor delay, Dystonia, Hypertonia, Muscular weakness, Visual impairment.	GNAO1, STXBP1, SLC2A1, KIF1A, SCN2A	
26	3	Male	V	Severe	Cerebral palsy, Epilepsy, Global developmental delay, Aphasia, Hypertonia, Muscular weakness, Urinary incontinence, Fecal incontinence, Esotropia, Hyperreflexia, Tremor.	AP4B1, AP4E1, GNAO1, STXBP1, SLC2A1	
27	7	Female	II	Severe	Intellectual disability, Craniofacial dysmorphism, Microcephaly, Hypertelorism, Strabismus, Low-set ears, Prominent ear, Bulbous nose, Macrostomia, Downturned corners of the mouth, Brachydactyly, Short stature.	DOCK8, SMARCA2, DMRT1/2/3, VLDLR	duplication 9p24.3p21.2
28	5	Male	II	Severe	Broad nasal bridge, Severe global developmental delay, Round face, Microretrognathia, Microcephaly, Low-set ears, Posteriorly rotated ears, Intellectual disability, Hypotonia, High-pitched voice, Epicanthus, Cat-like cry, Small hands, Short stature, Short neck, Intrauterine growth retardation, High-arched palate, Downslanted palpebral fissures.	SEMA5A, TERT, NSUN2, DAB2, TRIO	deletion 5p15.33p14.3

GMFCS - Gross Motor Function Classification System, ID – Intellectual disability, HPO - Human Phenotype Ontology

6) Epileptic seizures and/or epilepsy – 15 patients (53.6%);

7) Microcephaly – 12 patients (42.9%) (e.g., Case 5, Case 16);

8) Visual impairment (e.g., optic atrophy, nystagmus) – 12 patients (42.9%) (e.g., Case 8, Case 21);

9) Spasticity – 10 patients (35.7%) (e.g., Case 2, Case 10);

10) Short stature – 8 patients (28.6%) (e.g., Case 13, Case 19);

11) Joint hypermobility – 5 patients (17.9%) (e.g., Case 24);

12) Craniofacial dysmorphism – 11 patients (39.3%) (e.g., Case 3, Case 14);

13) Skeletal anomalies – 10 patients (35.7%) (e.g., Case 6, Case 27);

14) Ataxia, tremor, or coordination deficits – 6 patients (21.4%) (e.g., Case 9, Case 18);

15) Behavioral or affective symptoms – 4 patients (14.3%) (e.g., Case 25);

16) Structural brain anomalies (MRI) – 5 patients (17.9%) (e.g., Case 11, Case 23).

Each patient's phenotype included terms from at least three domains. The most commonly represented domains were cognitive (28/28), motor (28/28), epileptic (15/28), craniofacial (14/28), and visual (13/28). Dysmorphic features were

documented in 18 patients (64.3%). Neuroimaging findings were available for 20 patients (71.4%), and 5 showed structural brain anomalies.

Severe or profound neurodevelopmental impairment was prevalent. Eleven patients (39.3%) had more than 15 HPO terms annotated. Seven patients had co-occurring motor and cognitive regression (e.g., Case 7, Case 22). Five patients presented with congenital multisystemic involvement, consistent with syndromic phenotypes (e.g., Case 1 – Hunter syndrome with Bardet–Biedl-like features).

Metabolic red flags such as coarse facies, hepatosplenomegaly, and neurodegeneration were recorded in 3 patients (e.g., Case 17). Mixed tone findings (e.g., spasticity and hypotonia in same patient) were noted in 5 cases (e.g., Case 20, Case 26).

Table 2 Identified Single Nucleotide Variants and Copy Number Variations in Children with Complex Neurological Phenotypes

Case	Gene	Phenotype	ACMG criteria
1	VPS13B	Cohen syndrome	Pathogenic
2	L2HGDH	L2-hydroxyglutaric aciduria	Pathogenic
3	OCRL	Lowe syndrome	VUS
7	HRAS	Costello syndrome	Pathogenic
8	IDS / MKKS	Mucopolysaccharidosis II / Bardet-Biedl Syndrome 6	Pathogenic / Pathogenic
9	MTTL1	Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes	Pathogenic
10	FGFR2	Crouzon syndrome	Pathogenic
12	ARID1B	Coffin-Siris syndrome 1	Likely pathogenic
15	KMT2D	Kabuki syndrome 1	Pathogenic
18	WDFY3	Microcephaly 18, primary, autosomal dominant	VUS
23	ARID1B	Coffin-Siris syndrome 1	Pathogenic
24	ITPR1	Gillespie syndrome	VUS
27	duplication 9p24.3p21.2	Trisomy 9p syndrome	Pathogenic
28	deletion 5p15.33p14.3	Cri-du-chat syndrome	Pathogenic

ACMG - American College of Medical Genetics and Genomics, VUS - Variant of uncertain significance

Annotation followed strict clinical validation: terms like "Absent speech," "Gait disturbance," "Facial hypertrichosis," "Ulnar deviation of fingers," and "Cat-like cry" were retained only when documented clearly. Syndromic patterns (e.g., AP4B1, HRAS, KMT2D, STXBP1) were often reflected in dense, multisystemic HPO profiles.

The curated dataset created a structured foundation for phenotype-driven gene prioritization and downstream genomic interpretation.

Phenomizer-based Gene Prioritization

Phenotype-driven gene prioritization using the Phenomizer tool was performed systematically for all 28 patients, and the top five candidate genes identified by Phenomizer were documented in each individual case. The candidate genes listed for each patient were arranged strictly according to decreasing order of predicted relevance, with the first gene representing the highest priority, followed sequentially by the second through fifth genes. Across the cohort, a cumulative total of 120 unique candidate genes were suggested by the tool (Table 1). Frequency analysis demonstrated substantial variability, but several genes emerged consistently as frequent candidates, indicating common phenotypic patterns shared among multiple patients.

The gene GNAO1 was the most commonly prioritized candidate, appearing within the top five results in 10 separate cases (approximately 36% of the cohort). This gene is associated primarily with epileptic encephalopathies and movement disorders, reflecting the high prevalence of severe motor and epileptic features among the evaluated patients. The second most frequently prioritized gene was STXBP1, identified in 9 cases (32%), known to be linked with a spectrum of early-onset epileptic encephalopathies and profound developmental delays.

Other genes repeatedly identified by Phenomizer included members of the adaptor protein complex 4 (AP4) family, specifically AP4B1 (prioritized in 5 cases, 18%) and AP4M1 (in 4 cases, 14%). These genes are recognized for their strong phenotypic correlation with complex motor disorders, spasticity, intellectual disability, and microcephaly. Additionally, CDKL5 was prioritized in 4 patients (14%), a gene classically associated with early-onset epilepsy, profound neurodevelopmental impairment, and distinctive motor and cognitive deficits.

Several genes were prioritized less frequently but still appeared in multiple cases, suggesting important phenotypic overlaps or potential diagnostic relevance within subgroups of the cohort. These included genes such as KMT2D, ARID1B, FOXG1, SCN2A, and MECP2, which have established associations with neurodevelopmental syndromes characterized by intellectual disability, dysmorphisms, and epileptic encephalopathies.

Genetic Testing

WES identified pathogenic or likely pathogenic single nucleotide variants (SNV) in 12 of the 28 patients analyzed (42.9%) (Table 2). Among these cases, pathogenic variants accounted for 10 (83.3%), likely pathogenic variants were found in one case (8.3%), and one patient (8.3%) had dual pathogenic variants (IDS and MKKS). Additionally, variants of uncertain significance (VUS) were detected in 3 patients (10.7%).

Genetic diagnoses included various clinically established syndromes: neurodevelopmental conditions (Coffin-Siris syndrome type 1, Kabuki syndrome type 1, Cohen syndrome), metabolic disorders (L2-hydroxyglutaric aciduria, Mucopolysaccharidosis II), mitochondrial disorders (mitochondrial encephalomyopathy associated with MTTL1), craniosynostosis syndrome (Crouzon syndrome), and syndromes characterized by distinctive dysmorphic features (Costello syndrome, Bardet-Biedl syndrome type 6).

Copy number variations (CNVs), including a duplication at chromosomal region 9p24.3p21.2 (Trisomy 9p syndrome) and a deletion at region 5p15.33p14.3 (Cri-du-chat syndrome), were also identified through WES analysis in two patients (7.1%) (Table 2).

In total, approximately 50% of confirmed genetic diagnoses represented neurodevelopmental syndromes, emphasizing their prevalence in this patient cohort.

In 14 patients (50% of the cohort), WES failed to yield a molecular diagnosis. This may be due to structural variants (CNVs, small inversions) not detected by standard pipelines and the lack of parental samples for trio WES, which impedes de novo variant filtering. Additionally, pathogenic changes in non-coding regions, rare chromosomal rearrangements, or epigenetic modifications—undetectable by WES—are likely

contributors. Comprehensive approaches such as whole-genome sequencing (WGS), enhanced CNV analysis, STR screening, and methylation profiling are required to improve diagnostic yield.

Reverse Phenotyping

Reverse phenotyping was conducted for 14 patients who harbored pathogenic or likely pathogenic variants identified by WES (Cases 1, 2, 3, 7, 8, 9, 10, 12, 15, 18, 23, 24, 27, 28). For each patient, clinical features were re-evaluated against the phenotype profiles documented in OMIM for the corresponding genetic condition.

In all 14 cases (100%), the observed clinical phenotype fully matched the expected OMIM phenotype, confirming the specificity and reproducibility of the genotype–phenotype correlations. Moreover, in four patients, reverse phenotyping revealed additional clinical components not captured in the initial evaluation:

1. Case 7 (HRAS; Costello syndrome): mild neurocutaneous hyperpigmentation
2. Case 2 (L2HGDH; L2-hydroxyglutaric aciduria): subclinical ataxic gait
3. Case 23 (ARID1B; Coffin–Siris syndrome): hypoplasia of the fifth digits
4. Case 28 (5p15.33p14.3 deletion; Cri-du-chat syndrome): minor cardiac anomaly previously undocumented

Thus, reverse phenotyping not only confirmed concordance with OMIM-described features but also enriched the phenotypic characterization of select patients with novel clinical findings.

Concordance between Phenomizer Prioritization and WES Findings

Phenomizer-based gene prioritization and WES-confirmed diagnoses were compared in 14 patients. Twelve patients had SNVs, and two patients had CNVs.

• Among the 12 SNV cases, the confirmed causal gene was present within Phenomizer's top five candidates in 12/12 cases (100%).

• In both CNV-only cases (duplication 9p24.3p21.2 and deletion 5p15.33p14.3), no corresponding gene candidates were generated by Phenomizer.

Overall concordance between Phenomizer prioritization and WES findings was 12 out of 14 cases (85.7%).

Discussion

In this retrospective cohort of 28 pediatric patients with CNPs, our structured diagnostic algorithm—combining manual HPO-based deep phenotyping, phenotype-driven gene prioritization, WES, and reverse phenotyping—yielded a confirmed molecular diagnosis in 14 cases (50%). All single-nucleotide variants identified by WES were correctly prioritized within the top five Phenomizer candidates, and reverse phenotyping demonstrated perfect concordance with known OMIM phenotypes. This 50% diagnostic yield notably exceeds the 30–42% range commonly reported for WES in similar cohorts and approaches the upper limits of published trio-exome sequencing studies.

Published studies of phenotype-driven sequencing approaches in pediatric populations report diagnostic yields that generally range from 30% to 42%. A meta-analysis of 37 studies encompassing over 20 000 children found an average diagnostic yield of 36% for WES and 41% for WGS [16], while a broader meta-analysis across 161 studies reported similar rates of 38% for WES and 34% for genome sequencing (GS) in diverse cohorts [17]. Trio-based exome studies in children with developmental delay or intellectual disability have achieved yields up to 49.7%, particularly in syndromic subgroups [18].

In contrast, our multi-step algorithm produced a 50% diagnostic rate (14/28), exceeding most published WES-only frameworks (29–38%) and matching the highest yields reported for trio-WES. This elevated performance underscores the added value of our structured workflow—integrating manual HPO curation, Phenomizer prioritization, proband WES, and reverse phenotyping—in uncovering molecular diagnoses in CNPs.

Searches for identical algorithmic workflows in PubMed and other major databases yielded no results. No previously published study describes a fully integrated, end-to-end pipeline combining manual HPO-based deep phenotyping, automated gene ranking via Phenomizer, proband WES, and systematic reverse phenotyping. Although individual elements of our approach—such as ontology-driven phenotyping or WES—are well documented, and some centers report high yields with curated panels or trio-WES, none present the same comprehensive, multi-step framework.

Furthermore, this diagnostic algorithm is registered as intellectual property, underscoring its novel integration of clinical phenotyping, transparent candidate-gene prioritization, comprehensive sequencing, and iterative clinical re-evaluation. This unique combination of methods underlies the algorithm's demonstrated 50% diagnostic yield in a challenging pediatric cohort.

The diagnostic yield achieved by our multi-step algorithm demonstrates clear practical value, especially in regional settings like Kazakhstan, where access to specialized genetic services is limited [19]. Early application of WES within a structured workflow maximizes cost-effectiveness by shortening the diagnostic odyssey and reducing unnecessary investigations; singleton WES has been shown to be economically justified when used upfront in similar pediatric populations [20]. By integrating thorough clinical phenotyping with targeted sequencing, our approach facilitates timely molecular diagnoses, informs patient management, and optimizes allocation of limited healthcare resources.

It is also important to acknowledge the limitations of our study. First, the relatively small sample size (28 cases) limits statistical power and generalizability. Selection bias cannot be excluded: the cohort may have been enriched for particularly complex cases with a higher pre-test probability of genetic pathology, potentially inflating the apparent success rate. Second, the algorithm was evaluated retrospectively on existing data—prospective validation in an independent cohort is necessary to confirm its efficacy. Additionally, our focus on known genes within the algorithm means that unknown genetic factors or challenges in variant interpretation may have left some cases unresolved.

Nonetheless, even considering these limitations, our approach demonstrates a higher diagnostic yield than most published reports. This highlights its potential as a valuable clinical tool. A high rate of molecular diagnosis means that patients and families receive answers, targeted therapies, and genetic counseling more quickly. The clinical applicability of the algorithm is confirmed by its ability to deliver effective genetic diagnoses in real-world practice, even where resources are constrained. Thus, despite the limited sample size and other factors, our diagnostic algorithm has proven its merit and may serve as a foundation for improving the diagnostic process compared to existing methods. Its superiority over literature benchmarks underscores the need for broader implementation and further evaluation of this approach in larger, prospective studies.

As a form of internal validation, reverse phenotyping confirmed concordance between molecular results and clinical features in all diagnosed cases, while follow-up reassessment allowed identification of several additional traits not captured initially. In comparison with standard approaches—such as neuroimaging, EEG, metabolic testing, chromosomal microarray analysis, and targeted gene panels—the diagnostic yield appeared somewhat higher than typically reported for singleton WES, suggesting practical value in clinical settings. Importantly, in case 8 (IDS; Mucopolysaccharidosis II), the established molecular diagnosis enabled initiation of enzyme replacement therapy, directly demonstrating the algorithm's relevance for patient management.

Our study adds to the growing body of pediatric research published in the Journal of Clinical Medicine of Kazakhstan, where recent reports have described both rare neurological complications of COVID-19 in children [21] and broader pediatric health challenges such as iron deficiency anemia [22]. While the clinical contexts differ, these studies collectively highlight the importance of systematic diagnostic evaluation and early recognition of complex conditions in pediatric populations.

Conclusion

The structured diagnostic algorithm combining deep phenotyping, phenotype-driven prioritization, and WES proved highly effective in uncovering genetic etiologies in children with CNPs. Its implementation streamlines the diagnostic process, enhances clinical decision-making, and is well suited to settings with limited resources. Prospective evaluation in larger cohorts will be valuable to confirm and extend these findings.

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Efficacy and Safety of Intracavernous Administration of Platelet-Rich Plasma in the Treatment of Erectile Dysfunction in Kazakh Population: Results of a Six-Month Follow-Up

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Abstract

Erectile dysfunction (ED) is a common problem that can affect men of all ages and has a significant impact on mental and physical health and overall quality of life. In Kazakhstan, among 1,550 examined men (aged 21 to 79), 784 were diagnosed with ED (52.3%). In recent years, with the development of gene and regenerative technologies, a new promising strategy for the treatment of organic forms of ED has emerged: transplantation of mesenchymal stem cells obtained from bone marrow and mesenchymal stromal cells obtained from adipose tissue.

Autologous platelet-rich plasma (PRP) contains platelet concentrations three to seven times higher than physiological norms and is rich in growth factors, chemokines, and angiogenic factors. Therefore, a prospective study was conducted in a Kazakh population to investigate the safety and efficacy of intracavernous PRP injections in patients with moderate to severe ED.

The use of PRP therapy for the treatment of moderate and severe ED is safe but weakly effective. The activation of PRP with calcium chloride did not yield any particular results. Therefore, higher dosages may be associated with better short-term results. The PRP therapy technique requires further careful study and development of standards for the use of PRP in clinical practice, especially for the treatment of severe and moderate forms of ED. Additional prospective and randomized placebo-controlled trials are needed to finally assess the effectiveness of PRP therapy in improving erectile function.

Keywords: platelet-rich plasma, erectile dysfunction, intracavernous injections.

Introduction

The erectile dysfunction (ED) is defined as the inability to achieve or maintain sufficient penile stiffness for successful sexual intercourse. It is a common problem that can occur in men of all ages, but is particularly common in older men and in patients with diabetes mellitus. This condition has a significant impact not only on mental and physical health, but also on a man's overall quality of life [1-4].

The rate of incidence of the erectile dysfunction varies in different reports. The frequency of erectile dysfunction among men aged 40-70 years is 52% (Massachusetts Male Aging Study, Feldman HA et al., 1994). In another study, the National Health and Social Life Survey (Laumann et al., 1999), erectile dysfunction was reported in 31% of men aged 18 to 60 years [5-6]. Overall, the incidence of erectile dysfunction is 19.2% among men aged 30 to 80 years. In the Republic of Kazakhstan in 2007, 2203 men aged 18-74 years were examined. The prevalence of erectile dysfunction was 50.8% [7]. A similar study of sexual function in 769 men aged 18 to 69 years in Kazakhstan was conducted in 2021-2022 [8].

The risk of developing erectile dysfunction increases in men with diabetes mellitus, arterial hypertension, dyslipidaemia, obesity, sedentary lifestyle and smoking [9-13]. Numerous studies on the pathogenesis of ED have shown that most cases of erectile dysfunction are of vascular origin (50-70% of cases), regardless of the causes of its occurrence and are associated with impaired blood flow to the cavernous bodies. This form of erectile dysfunction is called vascular erectile dysfunction [14-17].

Currently, there are several methods of local treatment of erectile dysfunction: vacuum therapy, intracavernous and transurethral pharmacotherapy [18-19]. If these methods are ineffective or the patient prefers a radical solution to the problem, surgical implantation of penile prostheses is indicated [20].

In recent years, with the development of gene and regenerative technologies, a new promising strategy for the treatment of organic forms of erectile dysfunction has emerged. Such approaches include transplantation of bone marrow-derived mesenchymal stem cells [21-24] and adipose tissue-derived mesenchymal stromal cells [25-26]. These cells have regenerative, anti-apoptotic and anti-fibrotic properties [27]. The studies have shown that mesenchymal stem cells promote tissue regeneration by secreting exosomes and fibroblast growth factors such as hepatocyte growth factor, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and others. In addition, they express high levels of factors that regulate haematopoietic cell function, such as stromal cell growth factor (CXCL12), vascular cell adhesion molecules, interleukin-7, angiopoietin-1 (Ang-1) and osteopontin [28]. However, it should be noted that autologous stem cell transplantation may increase the risk of neoplasms [29].

Autologous platelet-rich plasma (PRP) has been used in various fields of medicine for decades. PRP contains platelet concentrations three to seven times higher than physiological norms [30], it is rich in growth factors, chemokines and angiogenic factors. The use of PRP to accelerate repair of damaged tissues focuses on regulating inflammation and stimulating regeneration. Autologous PRP has proven to be safe and effective in accelerating wound healing, soft tissue reconstruction and bone augmentation due to the release of multiple growth factors such as platelet-derived growth factor, transforming growth factor-beta, fibroblast growth factor, insulin-like growth factors 1 and 2, vascular endothelial growth factor (VEGF) and epidermal interleukin-8, keratinocyte growth factor and connective tissue growth factor [31].

Autologous PRP, due to destroyed platelets, contains alpha-granules which, when activated, release transforming growth factor-beta (TGF-β), VEGF, and EGF. These components stimulate collagen formation, accelerate tissue regeneration and promote vascular and endothelial growth, which makes PRP a promising method for the treatment of erectile dysfunction [32]. In addition, some publications mention the use of PRP with the addition of calcium chloride, which binds platelets at the injection site, creating a longer lasting effect. Calcium chloride converts fibrinogen into fibrin, forming a platelet-rich fibrin matrix [33].

Prospective studies of intracavernosal PRP injections in patients with erectile dysfunction not amenable to first-line treatment (combination of daily Tadalafil 5mg with Vardenafil 20mg on demand) show improvement in erection quality after PRP treatment [34-37].

Given that the use of intracavernous PRP in clinical practice is relatively recent, available literature reviews on its clinical effects remain limited and inconsistent. Currently, there are no regulated and standardised protocols for the preparation of material for intracavernous PRP, including centrifugation, activation, dosage and frequency of injection. In Kazakhstan, there have been no clinical studies of intracavernous administration of PRP for organic erectile dysfunction so far as well. Therefore, a prospective study was conducted in a Kazakh patient population to investigate the safety and efficacy of intracavernous PRP injections in patients with erectile dysfunction. It should also be added that for this study were selected patients with organic form of erectile dysfunction, resistant to drugs of first-line therapy. The prevalence of organic erectile dysfunction resistant to first-line therapy reaches up to 30% among ED patients [38]. In this regard, there is a need to survey the results of clinical studies of intracavernous application of PRP in the Kazakh population to assess the potential of this method.

Methods

This case-control study was conducted at National Scientific Medical Centre JSC (Urology Department) and Medi-Art Medical Centre. We used specific inclusion and exclusion criteria for patients, as listed in the Table 1. Eighty men aged

Table 1 The inclusion and exclusion criteria for participants of the study.

Inclusion criteria	Exclusion criteria
Erectile dysfunction lasting at least half a year	Age under 18 years
Diagnosed organic erectile dysfunction based on ultrasound dopplerography and/or dynamic infusion cavernosography	Blood coagulation disorders
Baseline International Index of Erectile Function-5 (IIEF-5) score of less than 15	Severe concomitant pathology (decompensated diseases of the endocrine system, decompensated forms of diabetes mellitus, severe diseases of the cardiovascular system)
Ineffectiveness or extremely poor clinical response to oral first-line therapy (phosphodiesterase type 5 inhibitors)	Non-organic causes of erectile dysfunction (psychogenic, endocrine)
The patient's willingness and readiness to refuse other treatments for erectile dysfunction	Drug-induced erectile dysfunction
Erection Hardness Score (EHS) 2-3	Presence of penile endoprosthesis
	Anatomical penile abnormalities (Peyronie's disease, congenital penile curvature) or suffered priapism with the outcome in sclerosis of cavernous bodies
	Previous penile surgery for erectile dysfunction or premature ejaculation

36 to 65 years and suffered from organic form of erectile dysfunction (ED) were examined between 2023 and 2024 and included to the study. All patients were resistant to therapy with first-line drugs (phosphodiesterase type 5 inhibitors).

The patients were randomised using block randomization into the following four groups (n = 20 men each):

- Group 1 (control group): The patients did not undergo PRP procedure (they refused injectable treatments and did not consider the option of penile endoprosthesis). They continued to receive standard treatment with phosphodiesterase type 5 inhibitors taking tadalafil 5 mg daily.
- Group 2: The patients underwent auto sampling of 22 ml of venous blood from the cubital vein with 1.2 ml of citrate-phosphate-glucose (CPG) added. This was followed by routine one-stage centrifugation of venous blood on a baguette bowl centrifuge for 20 minutes at 2000 rpm. The obtained autologous platelet-rich plasma (concentration of at least 1 × 106 platelets per 1 µl, in total 4 × 109 platelets were administered intracavernosally in a volume of 4 ml.
- Group 3: The patients underwent auto sampling of 33 ml of venous blood from the cubital vein with 1.8 ml of CPG added. After centrifugation for 20 minutes at 2000 rpm, autologous platelet-rich plasma (concentration of at least 1 × 106 platelets per 1 µl, in total 6 × 109 platelets) was administered intracavernosally in a volume of 6 ml.
- Group 4: The patients underwent auto sampling of 33 ml of venous blood from the cubital vein with 1.8 ml of CPG added. After centrifugation for 20 minutes at 2000 rpm, the obtained autologous platelet-rich plasma (concentration of at least 1 × 106 platelets per 1 µl, in total 6 × 109 platelets) was administered intracavernosally in a volume of 6 ml. Before injection, 10% calcium chloride solution was added to PRP in the volume of 0.6 ml to form fibrin matrix.

Injection procedure. Under aseptic conditions, an injection solution containing at least 1 × 106 platelets per 1 µl was injected into each cavernous body at two points using a 30G needle. Fifteen minutes before injection, a spray of 10% lidocaine solution (up to 5 times total exposure 15 minutes containing 4.6 mg lidocaine in one dose (EGIS, Hungary) was applied to the injection site. A Stockmann penile clamp (Storz, Tuttlingen, Germany) was used for 20 minutes to slow the release of PRP from the injected area. Intracavernosal PRP therapy was performed twice 3 weeks apart, the injection sites varied by 1 cm in the mid-penile region. None of the patients showed any induration or infiltrates after the injections. Mild pain at the time of injection was noted in 11% of patients. Due to the limited number of studies on PRP for erectile dysfunction, there are no clearly recommended protocols, so this study used two doses of PRP and an interval of 3 weeks.

Efficacy assessment and ethical issues. Treatment efficacy was evaluated using the International Index of Erectile Function-5 (IIEF-5) and Erection Hardness Scale (EHS) scales. All participants were informed in detail about the efficacy and possible complications of the treatment and signed informed voluntary consent to participate in the study.

Statistical analysis. Statistical Package for the Social Sciences (SPSS) software version 22.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analyses. Data were presented as median values with ±95% confidence interval for continuous variables and as percentages for categorical variables. Data analysis was conducted using repeated measures analysis of variance to assess the International Index of Erectile Function-5 (IIEF-5) and Erection Hardness Scale (EHS) scores.

Table 2 Comparison of groups by age, body mass index, and duration of erectile dysfunction

Parameters	Group 1 (Control)	Group 2	Group 3	Group 4
Age (years)	49,0 [46,0; 53,9]	51,0 [45,8; 54,7]	55,5 [49,1; 56,8]	50,5 [47,4; 54,8]
BMI (kg/m2)	28,1 [25,1; 30,6]	29,2 [28,3; 33,3]	27,0 [26,0; 31,3]	28,7 [27,1; 33,8]
Duration of ED (years)	3,0 [2,9; 4,0]	3,0 [2,8; 3,9]	3,0 [3,07; 4,1]	3,0 [3,0; 4,0]

Note: BMI – body mass index, ED – erectile dysfunction. The values are shown as median and confidence interval [-95%, +95%].

Cochran's Q-test was used to compare data on dichotomous dependent variables. Statistical significance of the results was assessed at the $p < 0.05$ level. Results of single-factor analysis of intragroup comparisons to identify statistically significant intragroup dynamics in IIEF-EF and EHS indicators by analysis-of-variance method (Fisher's F-criterion) and non-parametric Kruskal-Wallis method (non-parametric analogue of analysis-of-variance method, factor analysis). Data on visits/observations were taken as the grouping factor in the calculations. Results of double-factor analysis of intergroup comparison to identify statistically significant differences in the dynamics between groups in terms of visit/observation intervals by IIEF-EF and EHS indicators. The analysis of variance and the non-parametric Kruskal-Wallis method were also used. The factors used to evaluate comparisons were "Group Factor" and "Visit/observation interval factor".

Results

During intracavernous injections in patients in Group 3 and Group 4, short-term pain sensations were noted in two and three patients, respectively. The duration of pain sensations was less than a minute. These pain sensations were noted after one injection. Hematomas and inflammatory reactions were not observed. In Group 2, no pain sensations were reported.

There were no dropouts or protocol violations in the present study and all 80 patients were able to follow-up after 1.5, 3, and 6 months post-treatment. Table 2 summarizes the median values and confidence intervals for age, body mass index, and duration of erectile dysfunction as obtained for the patients in all groups. No significant difference in any parameter was observed indicating uniform distribution of patients between the groups.

Within-group comparison. When all groups were compared in terms of treatment efficacy after 1.5, 3, and 6 months, no statistically significant differences were found between the average or median values of the groups. A similar picture is observed when assessing the indicator of the Erection Hardness Scale or EHS. The results of comparative analysis depending on the time point of observation show that the effectiveness of PRP-therapy after 1.5 months leads to a tendency of improvement of IIEF-EF and EHS scores. After 3 months, the indices reach a peak, but no significant differences in average or median values compared to the initial data were found. On the sixth month, a decrease in the values of these scales is observed.

However, it was found that the patients in each group can be separated into two distinct subgroups: those which were non-reactive within the whole period of observation (during

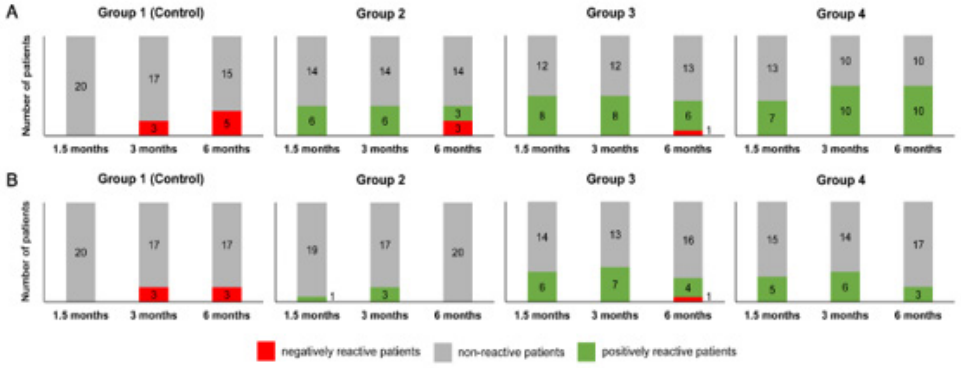

Figure 1 – The bar diagrams showing the proportion of non-reactive, positively reactive, and negatively reactive patients in all groups (as indicated in diagram labels) in regard to IIEF-EF score (panel A) and EHS score (panel B).

6 months) and those which were reactive either positively or negatively. Figure 3 summarizes this proportionality for all four groups in regard to either IIEF-EF score or EHS score. Note that only in Group 1 (control) there were no patients with positive reaction to the treatment (tadalafil), with the majority being non-reactive and only up to five negatively reactive patients to the end of observation (both for IIEF-EF and EHS). In contrast, in experimental Group 2 and Group 3, there was an absolute majority of non-reactive patients while the part of positively reactive patients was substantial, up to 40% and 35% of total number of patients in regard to IIEF-EF and EHS, respectively. Moreover, in experimental Group 4 we found equal proportion between non-reactive and positively reactive patients at 3-month and 6-month post-treatment moment for IIEF-EF, with no negatively reactive patients. In regard to EHS, Group 4 also did not have negatively reactive patients but the ratio of positively reactive patients to total number of patients was lower and peaked at 30% in 3-month after the treatment (Figure 1: A and B for Group 4). In general, the efficacy of the treatment in all experimental groups was a little bit higher in regard to IIEF-EF compared to EHS.

The statistical analysis for significance in the prevalence differences between different time points and within the same group was performed using Chi-square test. For IIEF-EF, in control Group 1 it was found that the prevalence of negatively reactive patients was significant only on 6th month post-treatment compared to the situation before treatment ($p = 0,017$). Similarly, in Group 2 the prevalence of positively reactive patients was significant 1.5 and 3 months after the treatment, compared to the situation before ($p = 0,008$). In the same group on 6th month, the proportion between non-reactive and reactive was also significantly different vs. before treatment ($p = 0,029$). In Group 3 and Group 4, the significant difference for distribution of patients between non-reactive and reactive was found for all pairs of time moments (e.g. before vs. 1.5 month, before vs. 3 months, and before vs. 6 months), too. However, for EHS the significant difference in such distribution was observed less often, especially for comparisons between before and 6th month post-treatment. In fact, the time point on the 3rd month was the point for most often observation of peaked segregation between non-reactive and reactive patients.

Next, we analysed the individual dynamics in IIEF-EF and EHS scores for all patients in all groups. For both score systems, we found distinct segregation of patients to non-reactive and reactive subgroups, and typically the segregation remained sustained along the observation period. In other words, if a patient displayed an initial dynamics, it was more likely that the same patient had dynamical changes later. The non-reactive patients (e.g. during 1.5 months) generally remained non-reactive during the rest of observation.

In regard to both IIEF-EF and EHS scores, the distinct subgrouping to non-reactive and reactive patients was accompanied by a very remarkable feature, as shown in Figure 2 for IIEF-EF. First, in control Group 1 we never observed positively reactive patients along the whole period of observation, but in all experimental groups at least at initial stage of observation we observed such positively reactive men. Also, as shown in Figure 2, most of the positively reactive patients had higher initial level of IIEF-EF score, compared to non-reactive patients. The similar feature was found for dynamical changes in EHS score while we do not show the data due to little visual representation of EHS scores which changed between 2 to 4 over the time of observation. In general, Group 4 displayed most prominent dynamic changes in IIEF-EF and EHS scores as well as most remarkable segregation between non-reactive and reactive patients.

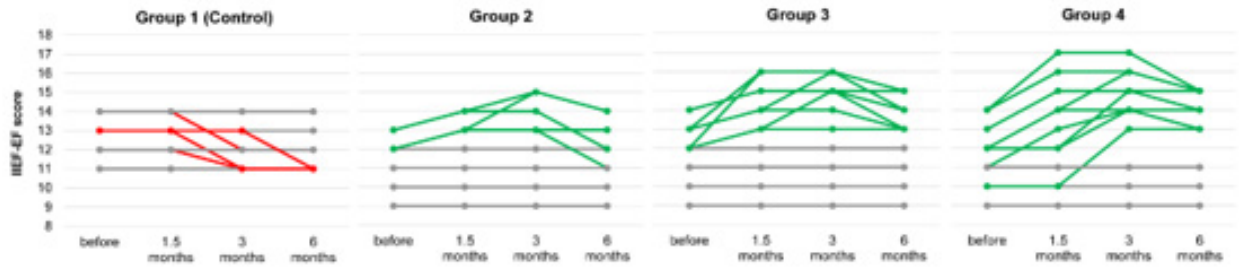

Figure 2 – The diagrams for dynamic changes in IIEF-EF score within the observation period per individual patient

Grey lines represent patients with no dynamical changes during the whole observation period, red lines represent patients that have never raised but only decreased IIEF-EF score, and green lines represent patients which at least once declared the increase in the IIEF-EF score. Note that those patients with positive reactivity all have generally higher initial IIEF-EF score. Some lines for different patients may superimpose. Y-axis scale is the same for all panels.

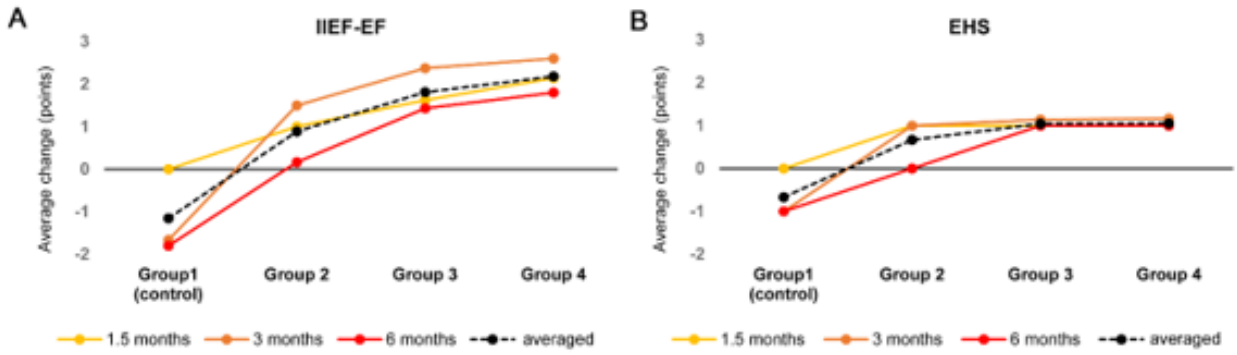

Figure 3 – The comparative changes in IIEF-EF (panel A) and EHS (panel B) scores in the groups of patients in different time points of observation

The average change is the summed up change in score (for all patients), the negative values indicates the drop in the score and the positive values indicate the increase in the score. In control group, no positive changes in the scores were observed irrespective to the time point. Average values were obtained by averaging data for all time points.

It should be noted that the observed changes in the dynamics of IIEF-EF and EHS scores were insensitive to the age or body mass index of the patients. We therefore conclude that these physiological factors unlikely be an important contributors to the reactivity or non-reactivity to the PRP treatment. Rather, we would speculate about the existence of two pools of patients, where some are non-responsive and others have initial "responsiveness" to the treatment by PRP. The propensity for this reactivity may be linked to the hormonal background or other factors that need to be elucidated.

Intergroup comparison. According to the data shown above, the patients in the groups displayed dynamics to segregate to non-reactive and reactive: in control Group 1 the reactive patients were solely negative, in experimental groups there were mostly positively reactive patients and their proportion was substantial. To compare the distributions statistically, we performed Chi-square test for inter-group analysis. Due to the lack of reactive patients in control Group 1 at 1.5 months post-treatment, we performed the tests only for 3-month and 6-month time points. In regard to IIEF-EF, the patients in experimental Group 2 were distributed significantly different from the distribution in Group 1 for 3-month time point ($p = 0,01$) but not for 6-month time point. In Group 3 vs. Group 1, the distribution of patients was significantly different for both time points ($p < 0,012$). Very similar in Group 4 vs. Group 1, the distribution of patients was significantly different for both time points ($p < 0,0006$). When we compared experimental groups, we found that Group 2 and Group 3 were not different for 6-month time point in regard to patients' distribution, as well as Group 3 vs. Group 4. However, Group 2 and Group 4 had significantly different distribution between patients for 6-month time point ($p = 0,024$). Note that for EHS score, the significantly different segregations between any two groups were observed at lower rate, most likely due to the much less changes in EHS score over time of observation as compared to the changes in IIEF-EF score.

The inter-group differences in average effect of treatment (i.e. for all patients) on IIEF-EF and EHS scores are shown in Figure 3. Here, the values represent summed up changes in the score within a group for a certain time point. Zero value corresponds to no average changes observed in the group whole some patients may have increased score and other patients may have decreased score. For example, if two patients had the increase by 1, and one patient had the decrease by 2, the overall change in the score is zero (no net changes). Negative average value corresponds to average decrease in the score, i.e. when three patients had the decrease by 1, one patient had the increase by 1, and all other patients had no changes. Positive average values, in turn, correspond to overall increase in the score.

As shown in Figure 3, in control Group 1 there were no positive changes in both IIEF-ED and EHS because all average values were equal or below zero. In contrast, all experimental groups showed positive effect of the treatment as evaluated by the average change in the IIEF-EF and EHS scores. Note that the Figure 3 contains also data obtained by averaging score changes along all observation time points (the integral effect of therapy, see dashed black lines). The treatment in Group 4 was generally most effective in the averaged increase in IIEF-EF score (Figure 3A). Also, quantitative change was most expressed on 3rd month of observation in all experimental groups. However, EHS score did not show any difference between Group 3 and Group 4 while smaller increase was attained in Group 2 (Figure 3B). This may simply reflect that EHS score has changed in smaller range (2 to 4 points) compared to IIEF-EF score (9 to 17 points). In other words, the accuracy for tracking the treatment-induced changes is higher for IIEF-EF vs. EHS.

Discussion

Erectile dysfunction (ED) is a common problem in patients of all age groups. Currently, none of the existing conservative methods of treatment of organic ED provides complete cure. Our study included patients with organic ED in whom resistance to first-line therapy – phosphodiesterase type 5 inhibitors – was established. Patients with an IIEF-5 index of less than 15 and with ineffectiveness or extremely poor clinical response to first-line oral drugs who were willing and ready to refuse other methods of treatment were included in the study.

All participants had EHS scores between 2-3, indicating a severe to moderate form of ED. No significant adverse events such as haematomas, infections or pain symptom were reported during PRP intervention. After completion of PRP therapy, none of the patients who completed two sessions of intracavernous PRP injection within 6 months achieved an EHS score of 4.

Note however that EHS score has much smaller range of changes: in our cohort, the score varied from 2 to 4 points. In contrast, IIEF-EF score had much greater variability, from 9 to 17 points. This may affect the accuracy for evaluation of treatment efficiency. For the purpose of accurate evaluation of

minor or moderate improvements in erectile function, it is better to use IIEF-EF scale instead of EHS scale.

Thus, the results of comparative analysis of the groups by duration of observation show that the use of PRP therapy after 1.5 months tended to improvement of IIEF-EF and EHS scores. After 3 months, the values reach a peak, but no significant differences were found compared to the initial data. On the sixth month, a decrease in the values of these scales was observed, but this negative dynamics is also not statistically significant. The results of this study suggest that PRP therapy is a safe treatment option for men with ED, but its effectiveness is controversial.

Many experts have expressed scepticism about the technique. For example, the Sexual Medicine Society of North America (SMSNA) has not accepted PRP therapy as a standard treatment [39, 40]. Due to the lack of definitive standards, evidence of the efficacy of PRP in ED and recommendations reported by various clinics give unclear conclusion about the treatment by PRP as a universal approach for ED. The debate about the use of PRP therapy for the treatment of ED continues. The Sexual Medicine Society of North America (SMSNA) has published a consensus statement indicating that new technologies such as PRP and Low intensity extracorporeal low energy shock wave therapy (LiESWT) should be used only in clinical studies [41].

One of the most recent publications on the results of applying PRP in ED was published in the Journal "Urology" of the American Urological Association on July 1, 2023. This prospective, double-blind, randomised, placebo-controlled study showed that two injections of platelet-rich intracavernous plasma 1 month apart in men with mild to moderate erectile dysfunction are safe, but no significant difference in efficacy was found between PRP and placebo [42].

For further widespread use of PRP in treatment of ED, it is necessary to standardise the technique, develop protocols for PRP activation and determine the optimal dosages and frequency of intracavernous injections [43]. Our study may indicate a safety of PRP therapy (as we did not observe any complication during the period of 6 months) but the results of its efficacy were non-significant and we therefore unable to conclude about the clinical efficacy of PRP treatment for ED. However, PRP has been successfully applied in other fields of medicine [44-46].

The results of our study show that PRP therapy can lead to improvement of IIEF-EF and EHS indicators in the short term up to 3 months but with no significant long-term effect. Therefore, it is reasonable to perform repeated intracavernous PRP injections 1.5-2 months after the previous sessions, with a total course of 4 injections. More pronounced tendency to improvement was observed in groups with PRP volume of 6 ml. Consequently, higher dosages may be associated with better short-term results. Activation of PRP with calcium chloride showed no particular results.

According to the study by Shaher H et al. (2023), PRP therapy demonstrated satisfactory results in the treatment of mild and moderate ED with marked improvement on 1st and 3rd months of observations which decreased slightly on 6 months period ($P < 0.001$) [47,48]. However, in our study, PRP therapy for severe and moderate ED showed only a slight short-term improvement.

Conclusion

The application of PRP therapy for the treatment of moderate and severe ED is safe but not sufficiently effective for all patients. However, in selected patients this therapy shows prominent improvement in erectile function. Moreover, one should take into account the existence of distinct pools of patients which can respond differently to the therapy. We call this in our paper as non-reactive and reactive patients. The PRP therapy requires further careful study and development of standards for its use in clinical practice especially for the treatment of moderate to severe ED, assuming that some patients may not be reactive. Additional prospective and randomised placebo-controlled studies are needed to definitively assess the efficacy of PRP therapy in improving the erectile function.

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Comparative Evaluation of Minimally Invasive Surgical Methods for the Treatment of Varicose Vein Disease in the Lower Extremities

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Abstract

Background: Varicose vein disease (VVD) of the lower extremities is a common vascular disorder that significantly impairs quality of life and may lead to serious complications such as thrombophlebitis, deep vein thrombosis, and chronic venous insufficiency. The development of minimally invasive surgical techniques, including endovenous laser ablation (EVLA), radiofrequency ablation (RFA), and sclerotherapy, has offered alternatives to traditional open surgery, with potential advantages in reducing postoperative complications, shortening recovery, and improving functional and cosmetic outcomes.

Objective: to perform a comparative evaluation of the efficacy, safety, and outcomes of minimally invasive surgical methods (EVLA, RFA, sclerotherapy) versus traditional surgery (ligation and vein stripping with or without phlebectomy) in the treatment of varicose vein disease of the lower extremities.

Methods: A retrospective study was conducted at the National Scientific Center of Surgery named after A.N. Syzganov in Almaty, Kazakhstan, between January 2019 and December 2024. The study included 481 patients diagnosed with VVD, divided into two groups: Group 1 (TM, n=305) underwent traditional surgical treatment (ligation and vein stripping), while Group 2 (MIM, n=176) received minimally invasive interventions (EVLA, RFA, or sclerotherapy). No other endovenous techniques (such as mechanochemical ablation or cyanoacrylate closure) were available in our center during the study period, and therefore were not performed. Conservative treatment (compression therapy and venoactive medications) was routinely used in non-operative patients but was not included in the present interventional cohort. Data on demographics, CEAP classification, Doppler ultrasound parameters, intraoperative characteristics, postoperative complications, and recovery time were analyzed. Statistical analysis was performed using t-test, chi-square test, and logistic regression ($p<0.05$).

Results: Minimally invasive methods showed significantly shorter operative times (43.2 vs. 78.6 min), less intraoperative blood loss (48.9 vs. 160.3 ml), and fewer complications (6.8% vs. 16.9%). Postoperative pain (VAS) and hospital stay were significantly lower in the minimally invasive group. However, traditional surgery was more often used in advanced cases (C3–C4 stages).

Conclusion: Minimally invasive surgical methods for VVD are associated with fewer complications, faster recovery, and greater patient satisfaction. However, traditional approaches remain essential in severe cases. Individualized treatment planning based on clinical stage and patient characteristics is key to achieving optimal outcomes.

Keywords: varicose veins, minimally invasive methods, sclerotherapy, surgical treatment, thrombosis, lower extremities.

Introduction

Varicose vein disease of the lower extremities (VVLE) is among the most common vascular disorders, characterized by venous dilation, elongation, and tortuosity. This condition significantly impairs patients' quality of life and is a major cause of chronic venous insufficiency. According to epidemiological data, symptoms of varicose vein disease are observed in 25–30% of women and 10–20% of men [1, 2]. Non-modifiable risk factors for VVLE include age and sex. Studies show that prevalence increases with age: among individuals aged 40, 50, and 60 years, the disease affects 22%, 35%, and 41% of the population, respectively [2]. One of the key contributors to increased risk in women is the hemodynamic and hormonal changes occurring during pregnancy, during which the incidence of newly developed varicose veins may reach up to 28%. Genetic predisposition is another important factor: the risk of developing VVLE in individuals whose both parents have the disease reaches up to 90% [3].

Modifiable risk factors include excess body weight, which can be assessed both qualitatively (body habitus) and quantitatively using the Body Mass Index (BMI). Lifestyle factors such as physical inactivity and prolonged standing or sitting also contribute to the risk of VVD. Women with a body mass index (BMI) over 30 kg/m² demonstrate a significantly higher likelihood of developing VVLE, while this correlation is less pronounced in men. Studies also confirm an independent association between occupational posture and the risk of varicose veins: the condition is diagnosed in 27% of individuals with sedentary jobs and in 36% of those whose work involves prolonged standing [4].

VVLE is associated with complications that substantially impair quality of life, leading to chronic pain, leg swelling, skin changes, and ulceration, and in severe cases may result in life-threatening events and mortality. One of the most common complications is superficial thrombophlebitis, occurring in 5–30% of cases and characterized by venous wall inflammation with thrombus formation. The risk of thrombus propagation into deep veins is estimated at 10–20%. Deep vein thrombosis (DVT), occurring in 3–5% of patients with advanced disease, may lead to pulmonary embolism (PE), with a mortality rate ranging from 10% to 30% if left untreated. PE is diagnosed in 1–2% of patients with VVLE and DVT, with mortality rates between 5% and 10% depending on severity. Chronic venous insufficiency (CVI) develops in 30–50% of cases, presenting with edema, skin changes, and venous ulcers affecting 15–20% of patients, requiring prolonged care. Hemorrhage from varicose veins occurs in 2–5% of cases and may result in severe blood loss and up to 3% mortality. Although VVLE is rarely the direct cause of death, complications such as PE and infected venous ulcers significantly increase mortality risk, reaching 5–10% [3, 4–7].

Traditional open surgical techniques, including high ligation and vein stripping, are still widely employed for managing large varicose trunks and complex cases. These methods achieve vein closure rates of 85–90% at one year, although recurrence may reach 10–20% within five years [6].

Minimally invasive techniques have demonstrated higher efficacy and improved patient comfort. Endovenous laser ablation (EVLA) achieves vein closure in 93–98% of cases within 12 months, with a recurrence rate of 2–7% at two years [7]. Radiofrequency ablation (RFA) offers similar results, with closure rates of 90–96% and recurrence rates of 3–5% [8]. Sclerotherapy is effective in 80–85% of cases, though recurrence can reach 20% within three years [9].

Traditional surgery in this study refers primarily to high ligation with great saphenous vein stripping, often combined with phlebectomy when indicated. These procedures are associated with higher complication rates: hematomas and wound infections occur in 15–25% of cases, while postoperative pain requiring prolonged analgesia is reported in 30–40% of patients. The recovery period following open surgery typically lasts from 2 to 4 weeks [6].

Minimally invasive methods are less traumatic and yield superior outcomes. Superficial thrombophlebitis occurs in less than 1% of EVLA and RFA procedures, versus 8–10% with open surgery [7]. DVT after minimally invasive interventions is reported in less than 1% of cases, compared to 3–5% following traditional procedures. Postoperative recovery time for minimally invasive approaches is typically 3–7 days [8].

Objective: to perform a comparative evaluation of the efficacy, safety, and outcomes of minimally invasive surgical methods (EVLA, RFA, sclerotherapy) versus traditional surgery (ligation and vein stripping with or without phlebectomy) in the treatment of varicose vein disease of the lower extremities.

Methods

A retrospective study was carried out among 481 patients who underwent inpatient treatment for varicose vein disease of the lower extremities (VVLE) in the Department of Vascular Surgery at the National Scientific Center of Surgery named after A.N. Syzganov, Almaty, Kazakhstan, between January 1, 2019, and December 31, 2024.

Inclusion criteria were: adults aged 18 years or older, hospitalized with a diagnosis of VVLE, who underwent surgical treatment according to an individualized intervention strategy. Exclusion criteria were: patients with infectious diseases, active or advanced malignancies (e.g., gastrointestinal, hepatobiliary, or hematological cancers), or contraindications to surgery, including severe cardiovascular, renal, or hepatic failure.

The process of patient selection is summarized in Figure 1. Out of 562 patients initially assessed for eligibility, 81 were excluded based on the above criteria, leaving 481 patients

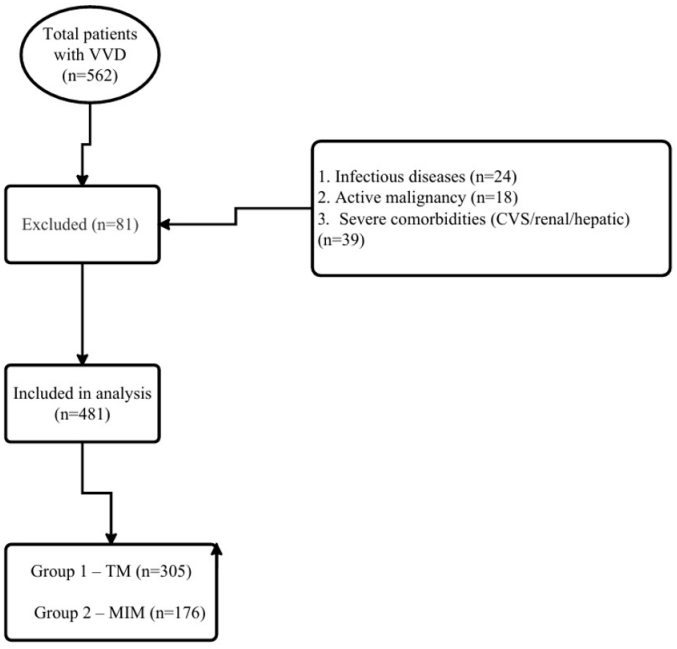

Figure 1 – Flowchart of patient selection.

for final analysis. Of these, Group 1 (TM, n=305) underwent traditional surgical treatment (ligation and vein stripping), while Group 2 (MIM, n=176) received minimally invasive interventions (EVLA, RFA, or sclerotherapy).

Clinical decision-making process: Patients were allocated to either traditional surgery or minimally invasive treatment based on clinical presentation, venous anatomy, and disease stage as assessed by duplex ultrasound. Traditional open procedures (high ligation and vein stripping) were chosen for patients with large varicose trunks, recurrent or complicated varicose veins, advanced venous dilation, or when endovenous access was technically limited. Minimally invasive techniques (EVLA, RFA, or sclerotherapy) were preferred in cases of isolated truncal reflux, less extensive disease, favorable anatomy for catheter-based intervention, or when reduced perioperative trauma was desirable. Patient preference and the availability of endovenous technologies were also taken into account.

Thus, treatment assignment was individualized, balancing disease severity, anatomical considerations, comorbidities, and patient-specific risk factors, in line with contemporary clinical guidelines [1].

Patients were divided into two groups: Group I – traditional methods (n = 305, 63.45%), which included high ligation and vein stripping; Group II – minimally invasive methods (n = 176, 36.55%), comprising EVLA, RFA, or sclerotherapy. No combined procedures (simultaneous use of traditional and minimally invasive techniques in the same patient) were performed. Each patient underwent a single method of treatment according to the individualized strategy.

The study also used ICD-10 codes related to venous disease:

- I80.0 – Phlebitis and thrombophlebitis of superficial vessels of lower extremities
- I80.1 – Phlebitis and thrombophlebitis of femoral vein
- I80.2 – Phlebitis and thrombophlebitis of other deep vessels of lower extremities
- I83.0 – VVLE with ulcer
- I83.2 – VVLE with ulcer and inflammation
- I83.9 – VVLE without ulcer or inflammation

All patients underwent comprehensive preoperative evaluation, including medical history and physical examination, duplex ultrasound to assess disease stage and severity, Doppler ultrasound was performed to assess venous patency, visualize blood flow patterns, identify reflux and venous insufficiency, and detect thrombophlebitis, providing essential information for determining the severity of disease and guiding treatment strategy. Laboratory tests, including complete blood count, biochemistry, and D-dimer, were also conducted to assess thrombotic risk.

Outcome Measures:

1. Vein closure rates confirmed by ultrasound post-intervention.
2. Recurrence rates tracked during hospitalization, at discharge, and during 1–3 year follow-up.
3. Postoperative outcomes included duration of hospitalization, pain levels (VAS scale), analgesic use, and rehabilitation time.
4. Complication rates, including thrombophlebitis, DVT, bleeding, and infections.
5. Surgical metrics such as operative time, intraoperative trauma, and blood loss.

Continuous variables were expressed as mean ± standard deviation (M ± SD). Normality of distribution was tested using the Shapiro–Wilk test. In cases where data were normally distributed, comparisons between groups were performed using the Student's t-test. For categorical variables, the chi-square test (χ^2) was applied. Logistic regression analysis was conducted to identify risk factors for adverse outcomes, with odds ratios (OR) and 95% confidence intervals (CI) reported. A p-value < 0.05 was considered statistically significant. All analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

Risk factor analysis for adverse outcomes (mortality, recurrent bleeding, reoperation) was conducted using logistic regression, with odds ratios (OR) and 95% confidence intervals (CI). Statistical significance was set at $p < 0.05$.

The study protocol was reviewed and approved by the Local Ethics Committee of the National Scientific Center of Surgery named after A.N. Syzganov, Almaty, Kazakhstan (Approval №7, dated April 25, 2025). Written informed consent was obtained from all patients prior to participation.

Results

A total of 481 patients were included in the study. The mean age was 55.4 ± 13.2 years (range: 18–87 years), with no statistically significant differences between treatment groups (p = 0.82). Gender distribution revealed 132 men (27.4%) and 349 women (72.6%), with female predominance in both groups (p = 0.02). Detailed demographic distribution by year of treatment is provided in Annex Table 1 (Table 1).

The mean age across the entire cohort was 55.4 ± 13.2 years (range: 18–87 years). The lowest mean age was recorded in 2022 (46.13 ± 17.6 years), and the highest in 2020 (53.80 ± 12.8 years). However, the differences in age distribution between years were not statistically significant (p = 0.82).

Gender distribution revealed 132 men (27.44%) and 349 women (72.56%). Females predominated in all years, particularly in 2024, where they constituted 72% (n=108) of cases. Statistical analysis showed significant gender differences by year (p = 0.02).

A comparative analysis of sex distribution, age, and body mass index (BMI) was performed between the two treatment groups. In TM (traditional methods), the proportion of female patients was significantly higher—237 women (77.7%) vs. 68 men (22.3%). In MIM (minimally invasive methods), female representation decreased to 112 (63.6%) compared to 64 men (36.4%).

Table 1 Demographic data of patients by year

Year	Total Patients (%)	Mean Age (years)	Men n (%)	Women n (%)
2019	66 (13,73%)	54,97 ± 13,2 [22; 87]	23 (34,85%)	43 (65,15%)
2020	80 (16,64%)	53,80 ± 12,8 [29; 83]	25 (31,25%)	55 (68,75%)
2021	66 (13,73%)	55,0 ± 11,9 [30; 85]	14 (21,21%)	52 (78,79%)
2022	69 (14,36%)	46,13 ± 17,6 [18; 75]	15 (21,74%)	54 (78,26%)
2023	50 (10,41%)	54,52 ± 13,8 [19; 85]	13 (26%)	37 (74%)
2024	150 (31,21%)	54,37 ± 11,8 [18; 78]	42 (28%)	108 (72%)
Total	481 (100%)	55,4 ± 13,2 [18; 87]	132 (27,44%)	349 (72,56%)

Table 2 Sex distribution, mean age, and BMI by treatment group

Parameter	TM (n=305)	MIM (n=176)	Total (n=481)
Men n (%)	68 (22.3%)	64 (36.4%)	132 (27.44%)
Women n (%)	237 (77.7%)	112 (63.6%)	349 (72.56%)
Mean Age (years)	55.2 ± 13.4 [18–87]	55.7 ± 12.2 [18–77]	55.4 ± 13.2 [18–87]
Mean BMI (kg/m²)	28.1 ± 4.3	27.8 ± 3.9	27.9 ± 4.1
Obesity (BMI ≥ 30) n (%)	69 (22.6%)	34 (19.3%)	103 (21.4%)

Note: BMI – Body Mass Index.

The mean age was 55.2 ± 13.4 years [18–87] in TM and 55.7 ± 12.2 years [18–77] in MIM, with no statistically significant difference between the groups ($p > 0.05$).

Obesity was considered a key risk factor in this study. The mean BMI in TM was 28.1 ± 4.3 kg/m², while in MIM it was 27.8 ± 3.9 kg/m². Overall, the mean BMI across all patients was 27.9 ± 4.1 kg/m². Obesity (BMI ≥ 30 kg/m²) was more frequent in TM (29.2%) than in MIM (24.4%), though this difference was not statistically significant ($p > 0.05$) (Table 2).

Table 3 presents the distribution of risk factors, including female sex, obesity, ≥4 pregnancies, arterial hypertension, and diabetes mellitus, across the two treatment groups. Female sex was significantly more prevalent in the traditional surgery group (77.7%) compared with the minimally invasive group (63.6%), confirming its role as a non-modifiable risk factor for varicose vein disease (OR = 1.92; 95% CI: 1.25–2.93; $p = 0.003$).

Obesity was observed in 22.6% of TM and 19.3% of MIM patients, with an overall prevalence of 21.4%. Although more frequent in the traditional group, the difference was not statistically significant (OR = 1.22; 95% CI: 0.78–1.89; $p = 0.36$).

The frequency of ≥4 pregnancies was higher in the minimally invasive group (18.8%) than in the traditional group (8.9%), and this difference reached statistical significance (OR = 0.42; 95% CI: 0.25–0.69; $p = 0.001$).

Arterial hypertension was detected in 53.4% of TM and 58.5% of MIM patients, while diabetes mellitus was present in 24.6% and 28.4%, respectively. Neither of these factors showed a statistically significant impact on treatment allocation ($p = 0.35$ and $p = 0.32$, respectively).

Overall, female sex and multiparity (≥4 pregnancies) were the strongest predictors differentiating treatment groups, whereas hypertension and diabetes mellitus did not demonstrate significant associations.

Table 3 Sex distribution, mean age, and BMI by treatment group

Risk Factor	TM (n=305)	MIM (n=176)	Total (n=481)	OR (95% CI)	p-value
Female sex	237 (77.7%)	112 (63.6%)	349 (72.5%)	1.92 (1.25–2.93)	0.003
Obesity (BMI ≥ 30)	69 (22.6%)	34 (19.3%)	103 (21.4%)	1.22 (0.78–1.89)	0.36
≥4 Pregnancies	27 (8.9%)	33 (18.8%)	74 (15.4%)	0.42 (0.25–0.69)	0.001
Arterial Hypertension	163 (53.4%)	103 (58.5%)	266 (55.3%)	0.83 (0.60–1.16)	0.35
Diabetes Mellitus	75 (24.6%)	50 (28.4%)	125 (26.0%)	0.84 (0.60–1.18)	0.32

The average disease duration in TM was 10.5 ± 5.3 years, significantly longer than in MIM (8.3 ± 4.7 years, $p = 0.01$). According to CEAP (Clinical, Etiological, Anatomical, Pathophysiological) [1], most patients in TM were classified as C3 (25%) or C4 (40%), while MIM patients were predominantly in stages C2 (45%) and C3 (35%) ($p = 0.03$). No TM patients were in early stages (C0–C1). The majority were at C3 (122 patients, 40%), followed by C2 (76; 25%) and C4 (107; 35%). In contrast, MIM had a higher proportion of patients in earlier stages, primarily C2 and C3. Overall, the most common CEAP stages were C3 (38%), C2 (32%), and C4 (30%) ($p = 0.03$).

According to the Saveliev classification of chronic venous insufficiency (CVI) stages [18], in the TM group the compensated stage was identified in 90 patients (29.5%), the subcompensated stage in 132 (43.3%), and the decompensated stage in 83 (27.2%). In the MIM group, these stages were recorded in 52 (29.5%), 75 (42.6%), and 49 (27.9%) patients, respectively. Statistical analysis using the χ^2 test revealed no significant differences between the groups ($\chi^2 = 0.028$, $p = 0.986$).

To evaluate the condition of the venous system, all patients underwent preoperative duplex ultrasound (DUS). The key parameters assessed included valvular competence, patency of deep and superficial veins, presence of reflux, diameter of major superficial veins, and signs of thrombosis.

Preoperative DUS findings revealed that patients who underwent traditional surgical treatment exhibited more pronounced disturbances in venous hemodynamics compared to those who received minimally invasive interventions (Table 4). In TM, the average diameter of the great saphenous vein (GSV) was 8.1 ± 1.8 mm, which was 0.9 mm greater than in MIM (7.2 ± 1.05 mm, $p = 0.032$). A similar trend was observed for the small saphenous vein (SSV), with an average diameter of 5.5 ± 1.1 mm in TM versus 4.9 ± 0.73 mm in MIM ($p = 0.048$)

The duration of pathological reflux was also significantly longer in the traditional surgery group. In the GSV, it averaged 5.4 ± 0.83 seconds in TM versus 4.6 ± 0.62 seconds in MIM ($p = 0.021$). In the SSV, reflux lasted 3.9 ± 0.59 seconds in TM versus 3.4 ± 0.68 seconds in MIM ($p = 0.039$).

Hemodynamic impairments were more severe in the traditional surgery group: the mean venous flow velocity was 7.8 ± 1.7 cm/s, which was 0.7 cm/s lower than in MIM (8.5 ± 1.6 cm/s, $p = 0.044$).

A significant difference between the groups was noted in the rate of valvular incompetence: 97.4% in TM versus 85.2% in MIM, a 12.2% difference ($p < 0.001$).

Table 4 Preoperative duplex ultrasound parameters by surgical technique

Parameter	TM (n=305)	MIM (n=176)	Total	p-value
GSV diameter (mm)	8.1 ± 1.8	7.2 ± 1.05	7.7 ± 1.5	0.032
SSV diameter (mm)	5.5 ± 1.1	4.9 ± 0.73	5.2 ± 0.92	0.048
Reflux in GSV (s)	5.4 ± 0.83	4.6 ± 0.62	5.0 ± 0.72	0.021
Reflux in SSV (s)	3.9 ± 0.59	3.4 ± 0.68	3.7 ± 0.64	0.039
Venous flow velocity (cm/s)	7.8 ± 1.7	8.5 ± 1.6	8.2 ± 1.65	0.044
Valvular incompetence (%)	97.4% (n=297)	85.2% (n=150)	92.5% (n=447)	<0.001
Signs of thrombosis (%)	18.4% (n=56)	0% (n=0)	11.6% (n=56)	<0.001

GSV – great saphenous vein; SSV – small saphenous vein.

Additionally, signs of thrombosis were present in 18.4% of patients in the traditional surgery group and were absent in the minimally invasive group ($p < 0.001$).

These preoperative ultrasound findings suggest that patients undergoing traditional surgery exhibited more severe venous hemodynamic changes, including increased vein diameters, longer reflux durations, reduced flow velocity, and a higher frequency of valvular incompetence. These findings imply that minimally invasive techniques are more often selected for patients with less advanced disease, while traditional surgical interventions are typically reserved for more severe forms of lower extremity varicose vein disease.

Analysis of intraoperative data demonstrated statistically significant differences between the groups (Table 5). The mean operative time in the traditional surgery group was 78.6 ± 10.9 minutes, which was nearly 1.8 times longer than in the minimally invasive group (43.2 ± 8.4 minutes, $p < 0.001$; Table 5).

Intraoperative blood loss was also significantly greater in TM (160.3 ± 35.7 mL) compared to MIM (48.9 ± 12.8 mL, $p < 0.001$; Table 5). The intraoperative complication rate was 9.5% (n = 29) in the traditional group and 2.3% (n = 4) in the minimally invasive group ($p = 0.011$; Table 5). Common complications included bleeding (6 cases [2.0%] in TM vs. 1 case [0.6%] in MIM), vein perforation (4 cases [1.3%] in TM vs. none in MIM), nerve and lymphatic injury (8 cases [2.6%] in TM vs. 2 cases [1.1%] in MIM), intraoperatively detected DVT (3 cases [1.0%] in TM vs. none in MIM), and other events such as hematomas, seromas, and anesthesia reactions (8 cases [2.6%] in TM vs. 1 case [0.6%] in MIM; Table 5).

Anesthesia modality also differed significantly: regional anesthesia was used in 89.2% of minimally invasive procedures compared with only 15.7% of traditional surgeries ($p < 0.001$; Table 5).

Thus, traditional methods were associated with longer operative time, greater blood loss, and higher intraoperative complication rates, whereas minimally invasive interventions were less traumatic and demonstrated superior intraoperative safety.

Analysis of postoperative complications revealed statistically significant differences between groups (Table 6). The overall complication rate was significantly higher in the traditional surgery group (16.9%) compared to the minimally invasive group (6.8%, $p < 0.001$).

The most common complication in both groups was hematoma formation, which occurred more frequently after traditional procedures (7.5% vs. 2.9%, $p = 0.012$). The incidence of infiltrates was also higher in TM (4.2% vs. 1.5%, $p = 0.041$), likely due to greater tissue trauma.

Lymphorrhea was observed in 2.8% of traditional surgeries vs. 0.9% of minimally invasive cases ($p = 0.034$), and postoperative wound infections occurred in 2.3% vs. 0.6%, respectively ($p = 0.027$). Deep vein thrombosis occurred in 1.9% of TM and 0.5% of MIM patients ($p = 0.039$).

Early recurrence of varicose veins (within 30 days post-op) was more common in the traditional group (4.5% vs. 2.5%, $p = 0.076$). Pulmonary embolism (PE) was documented in one patient (0.6%) from the minimally invasive group; the patient was successfully resuscitated and discharged on postoperative day 11. No PE cases were reported in TM ($p = 0.321$). There were no fatal outcomes in either group.

These findings confirm that traditional surgical methods are associated with higher rates of postoperative complications, supporting the advantages of minimally invasive techniques characterized by lower trauma and more favorable postoperative recovery.

Table 5 Intraoperative parameters by surgical method

Parameter	TM (n=305)	MIM (n=176)	p-value
Operative duration (min)	78.6 ± 10.9	43.2 ± 8.4	<0.001
Intraoperative blood loss (mL)	160.3 ± 35.7	48.9 ± 12.8	<0.001
Intraoperative complications (%)	9.5% (n=29)	2.3% (n=4)	0.011
Use of regional anesthesia (%)	15.7% (n=48)	89.2% (n=157)	<0.001

Table 6 Postoperative complication rates in the compared groups

Complication	TM, % (n)	MIM, % (n)	p-value
Overall complication rate	16.9% (52)	6.8% (12)	<0.001
Hematomas	7.5% (23)	2.9% (5)	0.012
Infiltrates	4.2% (13)	1.5% (3)	0.041
Lymphorrhea	2.8% (9)	0.9% (2)	0.034
Surgical site infections	2.3% (7)	0.6% (1)	0.027
Deep vein thrombosis (DVT)	1.9% (6)	0.5% (1)	0.039
Recurrent varicose veins (within 30 days)	4.5% (14)	2.5% (4)	0.076
Pulmonary embolism (PE)	0.32% (1)	0% (0)	0.321
Mortality	0% (0)	0% (0)	—

The average length of hospital stay in the traditional surgery group was 5.2 ± 1.4 days, which was significantly longer compared to 3.1 ± 0.9 days in the minimally invasive group ($p < 0.001$). Pain intensity, assessed using the Visual Analog Scale (VAS), also differed significantly between the groups. On the first postoperative day, the mean pain score in TM was 6.8 ± 1.2, whereas in MIM it was considerably lower—4.2 ± 1.1 ($p < 0.001$). By postoperative day three, pain levels had decreased in both groups, however remained more pronounced in patients treated with traditional surgery (4.5 ± 1.0 vs. 2.1 ± 0.8, $p < 0.001$). These results further support the advantage of minimally invasive techniques in terms of lower postoperative morbidity, shorter hospitalization, and reduced pain levels in the early recovery period.

Discussion

Traditional surgical approaches to treating varicose veins, such as combined phlebectomy, remain widely used in clinical practice despite the development of minimally invasive techniques. According to current European guidelines, the primary classical surgical interventions include Babcock-Narat saphenectomy, the Varady-Müller modification, miniphlebectomy, short and total stripping, and bilateral crossectomy [9,10]. These methods effectively eliminate venous reflux and remove pathologically altered veins but are associated

with high trauma, extended hospitalization, and greater postoperative pain compared to endovenous procedures [11].

The evolution of minimally invasive technologies, including endovenous laser ablation (EVLA) and radiofrequency ablation (RFA), has made it possible to reduce the invasiveness of treatment while maintaining high efficacy. According to Woźniak W. (2016), EVLA and RFA are associated with lower rates of postoperative complications and shorter rehabilitation times compared to traditional phlebectomy [12]. Studies also confirm that patients undergoing minimally invasive procedures report less pain on the Visual Analog Scale (VAS), quicker return to work, and improved cosmetic outcomes [13,14].

EVLA and RFA demonstrate high efficacy in eliminating reflux in superficial veins, achieving durable vein closure in 87–100% of cases [12]. However, recurrence remains a concern. Literature reports recurrence rates of 8.9% after EVLA and 11.1% after RFA, potentially attributable to thermal exposure variability, planning errors, and patient-specific anatomical factors [15,16].

Postoperative complication rates vary by treatment method. Sensory disturbances due to subcutaneous nerve injury occur in 23–40% of patients after total saphenectomy and in 7–19% after subtotal procedures, compared to only 3.7% with RFA and 1.8% with EVLA [12]. Skin hyperpigmentation is most frequent after sclerotherapy (up to 30%) but occurs in just 3.6% and 6.8% of cases following EVLA and RFA, respectively [15,16].

Open surgeries such as crossectomy and stripping are associated with a higher rate of early postoperative complications, including hematomas (up to 1.2%), thrombosis of preserved veins (2.4%), neurological disorders (2.4%), and hyperpigmentation (3.6%) [17]. Long-term recurrence rates following open surgery range from 11.9% to 13.5% [17].

Compared to endovenous techniques, traditional surgical procedures provoke more pronounced postoperative inflammation due to mechanical trauma and vascular injury. Unlike EVLA and RFA, which achieve vein closure via thermal coagulation with minimal impact on surrounding tissues, traditional surgeries are linked to higher rates of early complications such as seromas and lymphorrhea [15,16].

Nevertheless, in cases of severe venous insufficiency—especially those involving large varicose clusters or deep vein incompetence—combined phlebectomy remains the treatment of choice. The optimal surgical strategy must consider patient-specific characteristics, the extent of venous pathology, and comorbidities. In recent years, a growing trend toward combining traditional and minimally invasive methods has emerged, enabling a reduction in operative trauma while enhancing treatment efficacy [17].

Thus, traditional surgery continues to hold clinical relevance, offering effective reflux elimination but with greater invasiveness. A hybrid approach incorporating both traditional and minimally invasive techniques provides an optimal balance between procedural radicality and reduced complication risk, ultimately improving outcomes and quality of life.

Based on the results of this study, it is recommended that surgical treatment selection for varicose veins be guided by individual patient characteristics, disease severity, comorbid conditions, and expected postoperative outcomes. Traditional surgery is generally indicated for severe cases with large varices or deep venous insufficiency. EVLA and RFA are effective and less traumatic alternatives in patients with less advanced disease; however, their applicability may be limited in complex cases due to anatomical or technical constraints, rather than solely because of recovery time or cosmetic considerations. Improved preoperative diagnostics and tailored surgical planning based on anatomical variations are essential to optimize treatment outcomes. Implementing early rehabilitation protocols and enhancing postoperative management will help reduce complications and shorten recovery time.

Limitations

This study has several limitations. First, its retrospective design may limit data completeness and accuracy. Second, the absence of randomization introduces the potential for selection bias. The research was conducted over a five-year period (2019–2024), restricting assessment of long-term outcomes. Furthermore, data were collected at a single institution (National Scientific Center of Surgery named after A.N. Syzganov, Almaty), limiting generalizability. Patient heterogeneity, anatomical variation, physical activity levels, and comorbidities may have influenced outcomes. The limited follow-up duration prevents evaluation of 5–10-year recurrence rates. Additionally, procedures were performed by different surgeons, potentially affecting complication rates and outcomes.

Although gender was included as a demographic and risk factor variable in our analysis, occupational data were not consistently documented in the medical records, and therefore could not be analyzed. We recognize that occupational posture (sedentary work or prolonged standing) is an important risk factor for varicose vein disease, as supported by previous studies, and this represents a limitation of our study.

Despite these limitations, the findings provide clinically meaningful insight into the comparative effectiveness of traditional versus minimally invasive surgical treatments for varicose veins. Future studies should adopt randomized designs, longer follow-up periods, and multi-center collaboration to improve external validity.

Conclusion

The choice of surgical method for treating varicose veins should be based on individual patient characteristics, severity of venous pathology, and comorbidities. Traditional surgery remains relevant for complex cases and contraindications to minimally invasive procedures. In contrast, EVLA and RFA are preferred for patients prioritizing rapid recovery and minimal cosmetic impact.

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Prognostic Role of Breast Architecture in Imaging, Histopathology, and Breast Cancer Outcome

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Abstract

Aims. This study investigates the prognostic significance of mammographic density in breast cancer detection, histopathological patterns, treatment outcomes, and the diagnostic efficacy of different imaging modalities.

Methods. A retrospective analysis was conducted using data from approximately 7,000 women (20% of the annual screening cohort of 35,000) over the 10-year period. Breast density was classified using BI-RADS categories (A–D). The frequency of breast cancer detection in each density category was analyzed. Imaging methods included mammography, ultrasound, and MRI. Recommendations for women with high-density breasts were developed based on findings.

Results. High breast density (BI-RADS C and D) was identified in approximately 60% of screened women. Breast cancer detection rates were significantly higher in BI-RADS C (55%) and BI-RADS D (60%) groups compared to BI-RADS A (12%) and BI-RADS B (15%). Tumor sizes were larger in high-density breasts (2.5 cm for BI-RADS C, 2.8 cm for BI-RADS D). Mammography sensitivity decreased in dense breasts, whereas ultrasound and MRI showed higher sensitivity (90–97%). Based on these findings, guidelines for women with high-density breasts were proposed, emphasizing supplemental imaging and personalized screening.

Conclusion. High mammographic density is associated with aggressive tumour types, larger tumour sizes, and poorer outcomes.

Keywords: Mammographic density, Invasive ductal carcinoma, Tumor recurrence, Risk assessment, Histopathology.

Introduction

Breast cancer remains the most commonly diagnosed cancer and a leading cause of mortality among women worldwide, with increasing incidence rates attributed to lifestyle changes, environmental factors, and reproductive behaviours [1, 2]. In Kazakhstan, breast cancer accounts for 13.3% of all female cancer diagnoses, with approximately 4,000 new cases reported annually [3, 4]. Early detection and accurate diagnosis are critical for improving patient outcomes, yet challenges persist, particularly in women with dense breast tissue [5, 6].

Breast density is categorized into four types: Type A, in which the breast structure consists primarily of fatty tissue; Type B, where the breast contains fatty tissue but up to 25% fibroglandular tissue; Type C, characterized by 50% fibroglandular tissue, making it moderately dense; and Type D, where 75% or more of the breast structure consists of fibroglandular tissue, indicating extreme density. High-density breasts are associated with an increased risk of breast cancer and reduced mammographic sensitivity, necessitating alternative screening methods such as ultrasound and MRI to improve early detection rates.

Research Problem

Mammographic density refers to the proportion of fibroglandular tissue relative to fatty tissue in the breast, as visualized on mammograms. High mammographic density is a well-established independent risk factor for breast cancer, with women in the highest density categories (BI-RADS C and D) facing a four to six times greater risk compared to those with predominantly fatty breasts (BI-RADS A) [7]. Dense breast tissue not only increases cancer risk but also reduces the sensitivity of mammography, as tumours can be obscured by the dense parenchyma, leading to delayed diagnosis and larger tumour sizes at detection [8, 9].

Research focus

This study evaluates the diagnostic efficacy of three key imaging modalities—mammography, ultrasound, and magnetic resonance imaging (MRI)—in women with varying breast densities. Mammography, the standard screening tool, is less effective in dense breasts due to reduced sensitivity. Ultrasound complements mammography by improving lesion detection in dense tissue, while MRI offers superior sensitivity and specificity, particularly for high-risk patients and those with dense breasts [9-11]. The integration of these modalities enhances diagnostic accuracy and facilitates early detection, especially in challenging cases. Beyond mammographic density, several factors influence breast cancer risk, including age, hormonal status, genetic predisposition, and lifestyle choices such as obesity and alcohol consumption [12, 13]. Recent advancements in imaging technology, such as digital breast tomosynthesis (DBT) and contrast-enhanced mammography, have improved diagnostic capabilities, particularly for women with dense breasts [14]. Additionally, artificial intelligence (AI)-driven tools are being explored to enhance image analysis and risk prediction, offering promising avenues for personalized screening and treatment strategies [15-17].

Research Aim and Research Questions

The primary aim of this study is to evaluate breast density distribution among women screened for breast cancer in the Aktobe region over the period 2014–2024. The research seeks to analyze the frequency of breast cancer detection across different breast density categories, identifying the density types most commonly associated with malignancy. Additionally, the study aims to provide recommendations for women with high breast density, focusing on further diagnostic steps, optimal screening approaches, and preventive strategies.

Methods

Study Design

This observational retrospective cohort study analyzed data from 7,000 women screened between 2014-2024 in the Aktobe region. Recurrence rate data were obtained from the Aktobe Regional Cancer Registry through probabilistic record linkage using unique patient identifiers. Registry data included histologically confirmed recurrence events with standardized follow-up protocols. The sample size (n=7,000) was calculated to achieve 80% power (α=0.05) to detect a 15% difference in cancer detection rates between BI-RADS categories, based on the annual screening cohort of 35,000 women in the Aktobe region. A stratified random sampling approach ensured proportional representation across all density categories. Breast density was classified using the BI-RADS system (A–D) based on mammographic findings, and cases were supplemented with ultrasound and MRI for improved detection in high-density cases. Imaging results from mammography, ultrasound, and MRI were utilized to provide a comprehensive evaluation of breast cancer risk profiles and tumour characteristics. This multi-modal approach allowed for a detailed assessment of diagnostic efficacy, particularly in women with dense breast tissue, where mammography alone may be less effective.

Inclusion and Exclusion Criteria

To evaluate the prognostic role of breast density, a retrospective cohort was assembled from the Aktobe screening population (2014–2024) using random sampling stratified by BI-RADS density categories (A–D). The sample size (n=7,000) was calculated to achieve 80% power to detect a 15% difference in cancer detection rates between density groups, with proportional representation of each category (A: 15%, B: 25%, C: 40%, D: 20%) mirroring regional screening data. This design avoids overrepresentation of high-density groups and ensures unbiased assessment of density's prognostic value. Included participants were randomly selected from the screening cohort, with no density-based exclusion, to maintain a distribution reflective of the general population: approximately 15% BI-RADS A (fatty breasts), 25% BI-RADS B (scattered fibroglandular tissue), 40% BI-RADS C (heterogeneously dense), and 20% BI-RADS D (extremely dense). All cases required histopathological confirmation of breast cancer (invasive ductal carcinoma [IDC], invasive lobular carcinoma [ILC], or ductal carcinoma in situ [DCIS]) and complete imaging records, including mammography supplemented by ultrasound or MRI. Complete imaging records, including mammography, ultrasound, and MRI scans, were mandatory for inclusion to facilitate a comprehensive evaluation of breast density and tumour features. Women were excluded from the study if they had a diagnosis of cancers other than breast cancer (e.g., ovarian, lung, or metastatic cancers to the breast) or if their imaging or histopathological records were incomplete. Participants who had undergone prior treatment for breast cancer, such as chemotherapy, radiation, or surgery, before the study period were also excluded to avoid confounding effects on tumour characteristics and outcomes. Additionally, women who were pregnant or lactating at the time of screening were excluded, as hormonal changes during these periods can alter breast density and imaging results. Non-residents of the Aktobe region or those who underwent screening outside the specified study period were not included to maintain geographic and temporal consistency. Finally, cases with imaging findings confirmed as benign (e.g., fibroadenomas or cysts) without malignant transformation were excluded to focus solely on malignant breast cancer cases.

Research Methods

Several diagnostic and imaging tools help researchers inspect both breast tissue densities and tumour attributes. Through BI-RADS classification, mammography assists physicians in identifying women with dense breasts that could hide tumours. The examination with ultrasound allows doctors to study the structure of fibroglandular tissue, which helps identify both tumour position and tissue density. Tumor characteristics are confirmed through MRI testing, which provides enhanced insights into lesion size and morphology assessment. The analysis of breast tumour histotypes through histopathological methods helps understand the relationship between breast density observed on mammograms and tumour pathology.

Statistical Methods

Multiple statistical approaches were employed to analyze the relationships between mammographic density and histopathological outcomes. Continuous variables (e.g., tumor size) were assessed for normality using Shapiro-Wilk tests and Q-Q plots, with non-parametric alternatives (Kruskal-Wallis, Dunn's tests) used when appropriate. Categorical associations between BI-RADS categories (A-D) and tumor histotypes (IDC, ILC, DCIS) were evaluated using chi-square tests with Yates' correction and effect size measures (Phi, Cramer's V). Multivariable logistic regression with Firth's bias reduction calculated adjusted odds ratios (aORs) for cancer risk factors while controlling for age, BMI, menopausal status, HRT use, and reproductive history, with model diagnostics including VIF (<5) and Hosmer-Lemeshow tests. Survival outcomes were analyzed via Kaplan-Meier curves and Cox proportional hazards models, verified by Schoenfeld residuals. All analyses were two-sided (α=0.05) with Benjamini-Hochberg correction for multiple comparisons, implemented in R v4.3.1 (packages: rms, survival) following TRIPOD guidelines, and complete reproducible code will be made publicly available.

Results

Among the 7,000 women screened, 15% were classified as BI-RADS A, 25% as BI-RADS B, 40% as BI-RADS C, and 20% as BI-RADS D. Breast cancer detection rates were notably higher in BI-RADS C and D groups, with 55% and 60% of cases, respectively, compared to 12% in BI-RADS A and 15% in BI-RADS B. Tumor size at diagnosis was also correlated with breast density, with an average size of 1.4 cm in BI-RADS A, 1.6 cm in BI-RADS B, 2.5 cm in BI-RADS C, and 2.8 cm in BI-RADS D (Figure 1). These findings suggest that dense breast tissue may contribute to delayed detection and larger tumor sizes at the time of diagnosis. The ages of women across different mammographic density categories matched up since statistical analysis showed no meaningful differences within the density groups. The research indicates age acts independently as a non-predicting factor since mammographic density patterns in these individuals seem unaffected by age, which requires investigators to examine alternative factors such as hormones, genetic backgrounds and lifestyle choices while evaluating breast density characteristics.

Risk Assessment in Women with High Breast Density

The tool generated breast cancer probability predictions through a combination of patient risk factors and density classifications. The research showed that women with BI-RADS C density faced a probability of 18% developing breast cancer throughout the following 10 years. Research demonstrated that the risk of breast cancer development at 22% over a ten-year period was the highest among female individuals who had extremely dense breasts with BI-RADS D classification. Breast tissue classified as less dense presented a decreased probability of developing cancer. Women with BI-RADS A density demonstrated a 6% 10-year breast cancer risk (Figure 2). The risk rose to 9% when women received a BI-RADS B classification.

The research findings show that women with denser breast tissue face increased danger of developing breast cancer. When combined with BI-RADS C and D categories, the increased density enhances the challenges for early tumor detection by mammography, so ultrasound and MRI provide better alternative screening tools for accurate diagnosis and prompt intervention. Knowledge about risk variables helps scientists create specific programs for early breast cancer detection and prevention strategies.

Correlation Between Mammographic Density and Histopathological Patterns

Higher breast tissue density correlated strongly with the development of more aggressive histological breast cancer subtypes, consistent with established literature (Kerlikowske et al., 2011; Shawky et al., 2019). Women with BI-RADS C

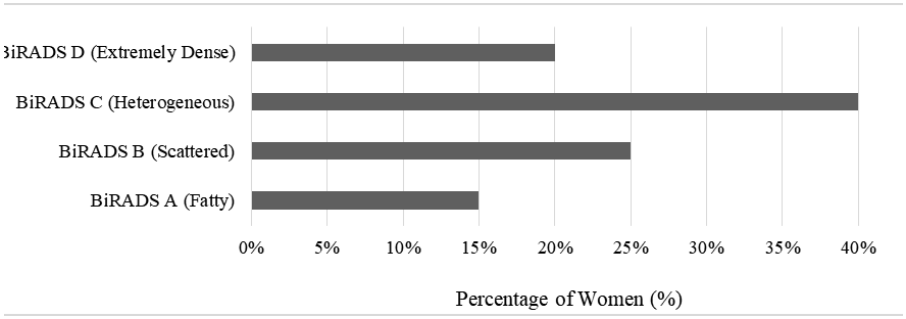

Figure 1 – Distribution of Mammographic Density in Women Aged 40-70 Years

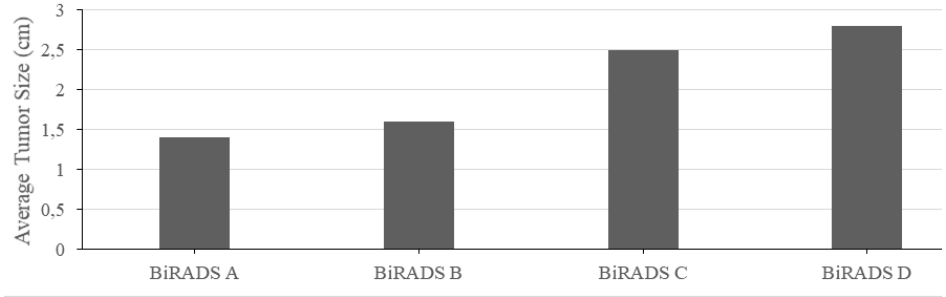

Figure 2– 10-Year Risk of Breast Cancer Based on Mammographic Density

and D densities showed significantly higher rates of invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) compared to lower density categories, supporting the association between dense tissue and tumor aggressiveness. Mammographic density levels BI-RADS C and BI-RADS D lead women to develop invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC), which represent two serious forms of breast malignancy. Breast density classifications showed different pathological patterns in the analyzed data. Fifty-five per cent of BI-RADS C-density women had IDC (aOR = 3.2, 95% CI: 2.1–4.8, $p < 0.001$), while their diagnoses included ILC at a rate of 35% (aOR = 2.8, 95% CI: 1.8–4.3, $p = 0.002$). Extremely dense breasts classified as BI-RADS D had 60% IDC cases together with 25% ILC cases (IDC: aOR = 4.1, 95% CI: 2.7–6.2, $p < 0.001$; ILC: aOR = 2.5, 95% CI: 1.6–3.9, $p = 0.008$), which highlights the association of dense tissue with cancer aggressiveness. The presence of BI-RADS A density correlated with less dangerous cancer types because 12% of cases revealed ductal carcinoma in situ (DCIS). The outbreak pattern of BI-RADS B density varied because 15% of analyzed cases had tumour categories that were not as aggressive (Table 1).

Table 1 Correlation between Mammographic Density and Histopathological Patterns

Mammographic Density	Histopathological Type	Percentage of Cases (%)	aOR (95% CI)	p-value
BiRADS C	Invasive Ductal Carcinoma	55%	3.2 (2.1–4.8)	<0.001
BiRADS C	Invasive Lobular Carcinoma	35%	2.8 (1.8–4.3)	0.002
BiRADS D	Invasive Ductal Carcinoma	60%	4.1 (2.7–6.2)	<0.001
BiRADS D	Invasive Lobular Carcinoma	25%	2.5 (1.6–3.9)	0.008
BiRADS A	Ductal Carcinoma in Situ	12%	1.0	-
BiRADS B	Other Types	15%	1.0	-

Treatment Outcomes and Recurrence Rates

The data suggest a poorer prognosis and lower survival rates for women with BI-RADS C and BI-RADS D densities, who had higher recurrence rates [10]. This was followed by an analysis including the first 20 years of follow-up, which showed that women with BI-RADS C density had a recurrence rate of 15%, whereas those with BI-RADS D density showed a recurrence rate of up to 18%. They also had a 3% recurrence rate, with the BI-RADS A group exhibiting the lowest, while the BI-RADS B group showed a recurrence rate of 8 % (Table 2).

Women who had higher mammographic density showed lower survival rate proportions. Overall, there was a substantial difference between the groups regarding survival rates. Greater breast density could impede early detection due to tumor masking, which, in turn, leads to widespread aggressive diseases as a side effect. These data support the development of more targeted treatment schemes based on prognosis, considering mammographic density a significant concern. Implementing better screening and other methods could turn the increased risks posed by higher breast density into improved patient outcomes.

Table 2 Recurrence Rates and Overall Survival Rates by Mammographic Density

Mammographic Density	Recurrence Rate (%)	Overall Survival Rate (%)
BiRADS A	3%	98%
BiRADS B	8%	92%
BiRADS C	15%	85%
BiRADS D	18%	80%

Mammographic Density and Tumor Size at Diagnosis

While considering women who have tumour sizes at diagnosis, it was noted that women who had BiRADS C and BiRADS D density had larger tumour sizes as well. The average size of tumours for BiRADS C women was 2.5 cm, and BiRADS D women had an average size of 2.8 cm. This is significantly greater than the average size of tumours for BiRADS A at 1.4 cm and BiRADS B at 1.6 cm, as shown in Figure 3. This result indicates that breast tissue composition may predispose some women to have a greater degree of undiagnosed breast cancer at presentation, which is why women in the C and D categories have tumours detected at a later stage.

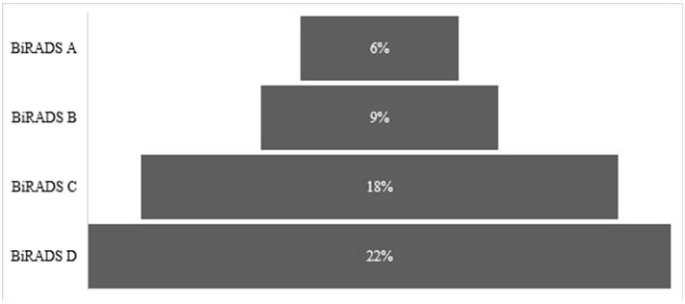

Figure 3 – Tumor Size Distribution by Mammographic Density

Comparison of Screening Methods and Diagnostic Efficiency

The assessment sought to determine the degree to which mammography and subtypes of breast ultrasonography, including MRI breast, could diagnose breast cancer at different grades of mammographic density. From the results obtained, there were identified and disturbing gaps in sensitivity between these modalities due to the density of the breast tissue. Mammography recorded varying sensitivity rates on breast density with a 70 per cent efficiency for women with breast BI-RADS A and 80, 85, and 90 per cent for BI-RADS B, C, and D, respectively. Even though mammography is and will remain the best first-line screening test for breast cancer, with higher breast density comes greater difficulty in detecting lesions and correctly diagnosing cancers due to the over-masking effect mammographically dense tissue tends to exhibit.

Compared to mammograms, ultrasound examinations are more sensitive, especially in women with denser breast tissues. The sensitivity for BI-RADS A was noted at 80%, 85% for BI-RADS B, 90% for BI-RADS C, and 92% for BI-RADS D. Thus; ultrasound would be considered an important additional method of diagnosis in situations where the result from the mammogram is ambiguous or inconclusive. For all grades of breast cancer density, MRI remained the most sensitive imaging modality.

In this study, it was found that MRI sensitivity for BI-RADS A was 85%, 90% for BI-RADS B, 95% for BI-RADS C, and 97% for BI-RADS D. These outcomes demonstrate further the advanced MRI diagnostic capabilities, particularly for patients with high mammographic density, where traditional readings of mammograms are less beneficial (Table 3).

Table 3 Diagnostic Efficiency of Screening Methods

Mammographic Density	Recurrence Rate (%)	Overall Survival Rate (%)
BiRADS A	3%	98%
BiRADS B	8%	92%
BiRADS C	15%	85%
BiRADS D	18%	80%

Discussion

The findings indicate that a notable percentage of women possess dense breast tissue, with 40% categorized as BI-RADS C and 20% as BI-RADS D. This distribution aligns with earlier research that has shown the commonness of high mammographic density in women and its effects on breast cancer detection and outcomes [18, 19]. The link between higher breast density and increased cancer risk highlights the importance of tailored screening methods, especially for women with denser breast tissue [20].

Women classified with BI-RADS C and D densities showed notably increased 10-year breast cancer risks of 18% and 22%, respectively, in contrast to the lower risks observed in women with BI-RADS A (6%) and B (9%). These findings are consistent with recent studies that have repeatedly shown that women with denser breasts face a greater risk of developing breast cancer, possibly because of the heightened epithelial and stromal elements that create a microenvironment favourable for tumour growth [21]. Research conducted in the last five years has corroborated this discovery, indicating that mammographic density continues to be one of the most significant independent predictors of breast cancer, even when considering genetic and lifestyle influences [18, 22].

Histopathological analysis also reinforces the idea that increased breast density correlates with more aggressive subtypes of breast cancer. In this research, women with BI-RADS C density had 55% of diagnoses as invasive ductal carcinoma (IDC) and 35% as invasive lobular carcinoma (ILC), whereas BI-RADS D density exhibited 60% IDC and 25% ILC. These results align with earlier studies that have shown a greater incidence of IDC in women with dense breast tissue, possibly arising from enhanced cellular growth and hormonal effects in dense breasts [23]. The existence of aggressive tumour types in denser breasts further underscores the difficulties in early detection since denser tissue can obscure tumours on standard mammograms, resulting in late diagnoses and worse outcomes [24].

Another critical impact on treatment outcomes, considering that the study focused on treatment results and the rates of recurrence related to different densities, should be mentioned: a higher rate for the denser group was evident-15% in BI-RADS C versus 18% in BI-RADS D-and a very poor prognosis was present in BI-RADS A at 3% and B at 8%. These results are in agreement with previous literature that has suggested high mammographic density is a risk not only for developing breast cancer but also for recurrence and overall survival [25]. Notably, emerging evidence from multiple studies indicates that breast density may influence tumour biology and response to treatment, with denser breasts often associated with higher tumour grades and lower response rates to neoadjuvant chemotherapy [26, 27]. These findings again underscore the importance of close monitoring and aggressive management strategies in patients with dense breasts for improved long-term outcomes [28].

Tumor size at diagnosis also showed a strong association with breast density. In this case, tumour sizes were 2.5 cm and 2.8 cm in BI-RADS C and D densities, respectively, compared with 1.4 and 1.6 cm in A and B, respectively. This implies that dense breast tissue contributes to the delayed detection of a tumour, as has widely been reported in the recent literature. This, in turn, limits the possibility of detecting tumours at smaller sizes, as dense tissue obscures the mammogram and thus leads to diagnosis when the stage is more advanced [29, 30]. This again brings into sharp focus the importance of supplemental imaging techniques such as ultrasound and MRI in women with dense breasts-which have been shown to improve early detection rates [31].

This study further evaluated the accuracy of the diagnoses reached using different imaging techniques. In the case of mammography, the sensitivity rates were 70% for BI-RADS A and 90% for BI-RADS D. On the other hand, ultrasound and MRI had even greater sensitivity, where ultrasound showed a range between 80% in BI-RADS A and 92% in BI-RADS D. As for MRI, sensitivity was noted to be 85% in BI-RADS A and 97 % in BI-RADS D. This puts into perspective the effectiveness of imaging techniques in comparison to straight-forward mammography for dense breast tissues [32]. In the past five years, prior studies corroborate on other imaging techniques, which has resulted in MRI being favoured in women with denser breast tissues. The development of contrast-enhanced mammography alongside AI evaluation of the images is likely to further advance the accuracy of the diagnosis made, but more tests need to be done to confirm this [33-35].

Recent advances in breast cancer screening and detection have supported personalized screening strategies related to the density of the breast. The increasing use of AI-based screening tools in mammography has shown promise for better detection rates, especially in dense breast tissue [36, 37]. The NHS's recent initiative to launch the world's largest AI trial for breast cancer diagnosis reflects the growing recognition of AI's role in overcoming the limitations of conventional imaging [38]. If successfully implemented, AI-assisted screening could reduce radiologists' workload and improve detection rates, particularly for women with dense breasts [39]. Besides, the recent FDA mandate on including breast density information in mammogram reports is a serious step toward patient awareness and individualized discussions on screening [40-42]. FDA-approved AI tools (e.g., QuantX, Transpara) improve density-based risk stratification in prospective studies, though regional validation is needed. This regulatory change will increase the likelihood that more women with dense breasts will seek additional screening options, thus improving early detection and reducing mortality.

Limitations and Future Directions

The present study contributes importantly to knowledge about the relationship of mammographic density with the risk of breast cancer. This study, however, has numerous limitations

that need consideration for future studies. The population was geographically limited, affecting the generalisation of findings to broader populations with diverse genetic, environmental, and lifestyle influences. Also, though mammographic density has been clearly defined as one of the risk factors, this analysis did not consider all confounding variables like hormonal therapy, menopausal status, and reproductive history that could have affected observed associations. Further studies need to be directed at these newer imaging modalities, assessing their cost-effectiveness and researching the underlying molecular mechanisms that link high breast density with greater tumour aggressiveness by transcriptomic and proteomic studies. AI and machine learning also promise to better detect breast cancer, reduce diagnostic errors, and develop optimal risk-based screening strategies. However, further research is needed regarding their clinical integration and ethical considerations. Recent regulatory changes that mandate the reporting of breast density also create a need for research into their impact on patient awareness, screening behaviours, and cancer outcomes.

Conclusion

The study results demonstrate a significant correlation between high mammographic density and larger tumor size at diagnosis. Supplemental ultrasound/MRI improved detection in dense breasts. Future work should validate AI tools in diverse cohorts and explore cost-effective screening protocols. Implementing personalized screening strategies, integrating advanced imaging techniques, and promoting patient awareness can significantly improve early detection and treatment outcomes. Future advancements in AI-based imaging analysis and risk prediction models may further enhance breast cancer screening efficacy, ultimately reducing mortality rates associated with dense breast tissue.

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Comparative Genomics of Zoonotic Pathogens: Genetic Determinants of Host Switching and Cross-Species Transmission

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Abstract

Emerging infectious diseases of animals that spread to humans (zoonoses) continue to represent a major threat to public health throughout the world. Knowledge of genetic elements that enable pathogens to navigate host shifts and species jumps is crucial for predicting and preventing zoonotic cross-species transmission. Comparative genomics, comparing whole-genome sequences across a range of hosts and pathogens, provides a powerful means to dissect the molecular determinants of zoonotic emergence. Here, we summarize and discuss recent advances in the understanding of genetic modifications that enable host switching and cross-species transmission by selected zoonotic pathogens. We review how comparative genomic analyses revealed the critical importance of factors such as receptor-binding domain evolution, immune evasion genes, and virulence determinants in improving pathogen fitness in new hosts.The review also highlights the integration of genomic data into One Health surveillance frameworks, enabling real-time monitoring, early detection, and improved outbreak response. Despite these advances, challenges including sampling bias, incomplete genomic databases, misannotation, and the complexity of predicting phenotype from genotype limit the field's potential. Furthermore, ethical and biosafety concerns in studying high-risk zoonotic pathogens necessitate careful governance. We outline future directions, emphasizing the need for expanded wildlife sampling, longitudinal studies of host-pathogen co-evolution, and the application of artificial intelligence in zoonotic risk assessment. Building comprehensive, globally accessible genomic databases is essential for coordinated pathogen tracking and risk mitigation. Ultimately, comparative genomics is indispensable for understanding and managing zoonotic threats, and its continued advancement, integrated within interdisciplinary, data-driven One Health strategies, will be critical to preventing future pandemics.

Keywords: Comparative Genomics; Zoonotic Pathogens; Host Switching; Cross-Species Transmission; One Health Surveillance.

1. Introduction

Zoonotic diseases—those transmitted from animals to humans—pose a persistent and growing threat to global public health. Emerging infectious diseases, such as COVID-19, Ebola, Nipah virus infection, and avian influenza, have underscored the devastating consequences of pathogens breaching species barriers [1]. The frequency and impact of these events are also rising due to an interplay among ecological, environmental, and anthropogenic drivers such as deforestation, wildlife trade, climate change, and intensification of agriculture. Despite these dangers, the molecular and evolutionary basis for how pathogens can cross-species boundaries is still not well resolved [2].

Comparative genomics can provide a valuable tool to unravel the genetic basis of host switching and cross-species transmission. By conducting the systematic comparison and analysis of zoonotic pathogens' genomes among divergent hosts, several genetic factors that drive adaptation to new hosts have been recognized and described, such as mutations in receptor-binding proteins, genomic recombination, and horizontal gene transfers [3]. These comparative methods also allow for the detection of signatures of selective pressure, gene gain and loss, and genome plasticity that could drive host range dynamics.

This review integrates existing knowledge on the genetic determinants underpinning zoonotic emergence, highlighting key molecular mechanisms identified through comparative genomics. We also discuss how advances in sequencing and bioinformatics have revolutionized pathogen genome analysis, illustrated with case studies from high-impact zoonotic viruses and bacteria. Importantly, in the post-COVID-19 era, renewed global attention to zoonoses makes this review timely, as it emphasizes the value of comparative genomics for surveillance, prediction, and prevention of future pandemics within a One Health framework.

2. The Conceptual Framework of Zoonosis and Host Switching

Zoonoses are infectious diseases caused by pathogens that have been transmitted from vertebrate animals to humans. Host switching, or host jump, is a pivotal evolutionary event in zoonotic emergence, wherein a pathogen establishes infection in a new host species, often following genetic or ecological changes [4]. Although many cross-species transmission events are known, successful host switching that results in pathogen infection and transmission in a new host population is rare and involves a complex interaction of biological, ecological, and evolutionary forces [5].

The possibility for host switching requires, as a key pre-requisite, ecological opportunity: direct transmission of the reservoir host to a new host. These interfaces have been greatly expanded by human activities, including habitat destruction, intensive agriculture, wildlife trade, and urbanisation, which have created the conditions under which spillover of zoonotic pathogens becomes more likely [6]. But exposure to the environment isn't enough. The pathogen must also have or develop genetic compatibility with the new host, be it the ability to bind to host cell receptors, avoid immune responses, and efficiently replicate.

Host shifting is typically depicted as a multistep process of (1) pathogen and novel host encounter, (2) infection and cellular colonization, (3) within-host adaptation, and (4) onward transmission within the new host population [7]. Every single step can be affected by the ecology of the host, the biology of the parasite, and environmental factors. Furthermore, the pathogen's evolvability, that is, the ability to mutate, recombine, or acquire novel genes can strongly affect the likelihood of a host jump being successful [8]. To make this process clearer, we have included a schematic diagram (Figure 1) that visually summarizes the sequential steps involved in host switching, from ecological opportunity to sustained transmission in a new species.

Evolutionarily, host switching is achieved via standing genetic variation, adaptive mutations, or horizontal gene transfer, depending on the organism. For example, RNA viruses, including coronaviruses and influenza viruses, have high mutation rates and recombination frequencies that allow for rapid adjustment to newly encountered hosts. By comparison, bacterial zoonoses often involve the gain of genes by plasmid or bacteriophage [9].

Various theoretical models have been developed to interpret and assess the dynamics of zoonotic emergence. Network strategies like pathogen-host interaction networks enable the identification of species with high potential to share pathogens. Evolutionary frameworks integrating host phylogenies, transmission ecology, and genomic information provide more detailed knowledge on how and why some pathogens jump across species [10].

Knowledge of the ecological and evolutionary context of zoonosis and host switching is essential for predicting the risk of spillover and for directing surveillance. Combining ecological context with genetic mechanisms, researchers will be better able to predict the potential emergence of events and to design specific strategies for intervention [11].

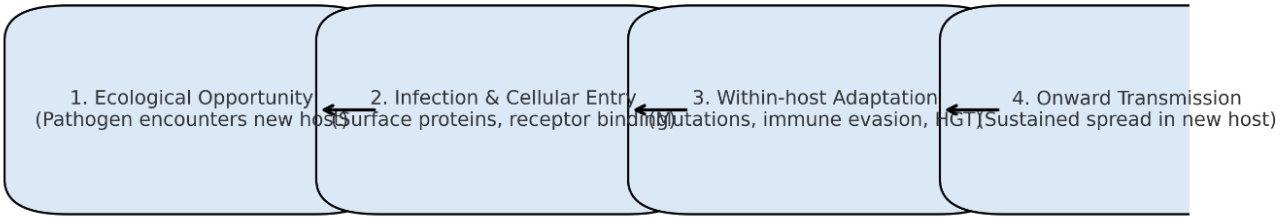

Figure 1 – Multistep Process of Host Switching in Zoonotic Pathogens

This schematic illustrates the sequential stages by which zoonotic pathogens cross species barriers. Step 1: Ecological opportunity — the pathogen encounters a novel host due to environmental or anthropogenic drivers. Step 2: Infection and cellular entry — mediated by surface proteins and receptor-binding compatibility. Step 3: Within-host adaptation — supported by mutations, immune evasion strategies, and horizontal gene transfer that enhance survival in the new host. Step 4: Onward transmission — sustained spread occurs within the new host population, leading to establishment of infection.

Table 1 Comparison of Genome Sequencing Technologies Used in Comparative Genomics of Zoonotic Pathogens

Technology	Read Length	Accuracy	Turnaround Time	Cost per Gb	Advantages	Limitations
Illumina (Short-read) [16]	150–300 bp	>99.9%	Fast (hours–1 day)	Low	High accuracy, high throughput	Difficulty resolving repetitive regions
Oxford Nanopore (Long-read) [17]	Up to 2 Mb (variable)	~90–98%	Real-time (minutes–hours)	Low–moderate	Portability, ultra-long reads, real-time output	Lower base-level accuracy than Illumina
PacBio (HiFi Reads) [18]	10–25 kb (HiFi)	>99.9% (HiFi)	Moderate (1–2 days)	Higher	Long reads with high accuracy	Higher cost and infrastructure needs

This table summarizes key characteristics of major sequencing platforms (Illumina, Oxford Nanopore, and PacBio HiFi). It compares read length, accuracy, cost, and turnaround time, highlighting strengths and limitations that influence data quality, genome completeness, and downstream analyses such as phylogenomics, pan-genome construction, and mutation tracking.
Note: Choice of platform depends on the genome size, complexity, and required resolution for comparative analysis.

3. Comparative Genomics: Tools and Approaches

The use of comparative genomics is the basis of many studies discerning the genetic determinism of host switching and zoonotic transfer. Researchers can identify the genetic differences contributing to pathogenicity, host range, and adaptation by analyzing and comparing genomes from different strains, species, or host-associated lineages. The current development of high-throughput sequencing methodologies and bioinformatics tools has dramatically improved our ability to dissect pathogen genomes in large numbers and detail [12].

The advent of modern genome sequencing technologies (both short-read technologies, such as Illumina, and long-read technologies such as Oxford Nanopore and PacBio) allows high-quality pathogen genomes to be generated from diverse sample types [13] (Table 1). Long-read sequencing, in particular, has improved the assembly of complex genomic regions, including those rich in repetitive sequences or mobile elements, which are often crucial to virulence and host adaptation [14].

Following sequencing, comparative analysis pipelines are used to align, annotate, and compare genomes. Tools such as MAUVE, ProgressiveMauve, and MUMmer allow for whole-genome alignments and detection of structural variations. Orthology detection tools (e.g., OrthoFinder, OrthoMCL) are used to identify shared and unique genes across species or strains, helping define core, accessory, and unique genome components [15]. These insights are particularly valuable for understanding how gene gain or loss contributes to host specialization.

Functional annotation databases such as Pfam, KEGG, COG, and Gene Ontology (GO) enable researchers to infer the biological roles of specific genes and pathways. When combined with transcriptomic and proteomic data, comparative genomics can uncover regulatory networks and gene expression patterns that change in response to new hosts or environments [19].

The pan-genome concept is particularly useful in zoonotic pathogen studies. A pan-genome includes the total gene repertoire across all strains of a species, divided into core genes (shared by all strains) and accessory genes (variable between strains) [20]. Within pathogens such as Salmonella, Escherichia coli, and Streptococcus, the accessory genome frequently consists of virulence genes, resistance genes, and mobile elements, which have an impact on host range and zoonotic capability [21].

Further levels of insight are afforded by single-nucleotide polymorphism (SNP) analysis and phylogenomic reconstruction [22]. Comparisons based on SNPs aid in the identification of mutations associated with host adaptation, while phylogenetic trees describe evolutionary relationships and patterns of transmission across host species. For that, tools like IQ-TREE, RAxML, or BEAST are often used [23].

Lastly, GWAS and machine learning algorithms are more and more incorporated into comparative genomics pipelines. This strategy can link genomic characteristics to phenotypes such as host specificity and virulence, with predictive potential for zoonosis emergence [24]. Combined, these tools and approaches create a powerful framework to unravel the genetic underpinnings of host switching. They are not only crucial for retrospective study, but also for real-time monitoring and prediction of zoonotic risk.

4. Genetic Determinants of Host Specificity and Switching

The ability of zoonotic pathogens to cross interspecies barriers is shaped by several genetic determinants. These include changes in surface proteins, immune evasion strategies, genomic plasticity, and regulatory elements that enable adaptation to new cellular environments [25] (Figure 2). Genome comparisons across diverse pathogens have been central to identifying these mechanisms and clarifying their roles in host adaptation.

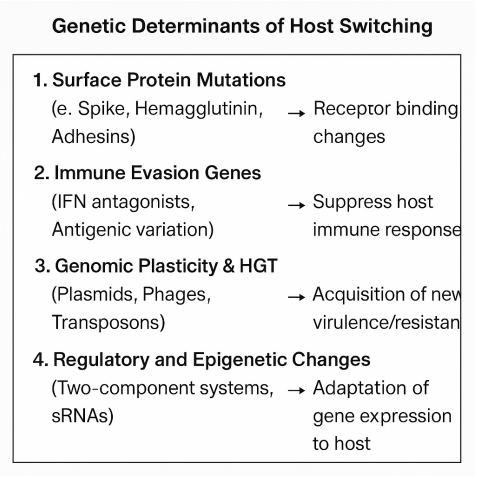

Figure 2 –Genetics of Host Switching in Zoonotic Pathogens

This figure summarises four principle genetic mechanisms that can facilitate host switching in zoonotic pathogens: (1) mutations in surface proteins that allow binding to the receptors of new hosts; (2) immune evasion genes that limit the host's ability to resist infection; (3) acquisition of new genes by HGT (horizontal gene transfer) resulting in genomic plasticity, and (4) regulatory and epigenetic changes that facilitate gene expression in response to the host environment. Discovered through comparative genomics, these genetic loci are important for understanding the molecular mechanism of inter-species transmission.

4.1 Surface Proteins and Receptor Binding Domains

One of the factors most significantly influencing host specificity is the binding of a pathogen to a host receptor (via binding of its surface proteins to host cell receptors). For viruses, host tropism is governed by receptor binding domains (RBDs) of envelope or spike proteins [26]. In coronaviruses, for example, the binding affinity of the spike (S) protein RBD to the angiotensin-converting enzyme 2 (ACE2) receptor is affected by mutations and is a critical determinant of zoonotic SARS-CoV and SARS-CoV-2 transmission [27]. Hemagglutinin mutations are also found in the influenza viruses, where they can influence association with sialic acid residues; avian-adapted adapted specifically identify α2,3-linked receptors, while human strains prefer α2,6 [28]. In bacterial pathogens, the adhesins and the pili mediate the attachment to tissues of hosts, and, for example, Campylobacter jejuni counts on surface proteins that adhere to gangliosides on intestinal epithelial cells, defining the range of hosts [29].

4.2 Immune Evasion and Modulation Genes

To establish infection in a new host, pathogens must evade innate and adaptive immune defenses. Genes encoding for immune modulators, such as interferon antagonists, complement inhibitors, or surface antigen variants, are often under positive selection during host transitions [30]. Viruses like Nipah and Ebola encode proteins (e.g., V and VP35) that suppress interferon signaling, facilitating replication in diverse hosts. Antigenic variation systems, such as those in Trypanosoma or Borrelia, allow evasion of host antibodies and enable chronic infection, increasing the chance of interspecies transmission [31].

4.3 Genomic Plasticity and Horizontal Gene Transfer

Genomic plasticity—enabled by high mutation rates, recombination, and horizontal gene transfer (HGT)—is a key driver of host switching. RNA viruses, with their error-prone polymerases, accumulate mutations rapidly, promoting adaptability. HGT in bacteria involves the acquisition of new virulence factors and new metabolic pathways through plasmids, phages, or transposons [32]. For example, zoonotic E. coli frequently gain pathogenicity islands and Shiga toxin genes through phage infection, promoting their capacity to colonize human hosts [33]. Comparative genomics often reveals such large genomic islands that encode secretion systems, toxins, or resistance genes, and often are flanked by mobile elements, indicative of these elements being recently acquired and adapted [34].

4.4 Transcriptional Regulation and Epigenetics

Reprogramming of gene expression is also part of the adaptation to a new host environment. Through comparative transcriptomics, expression of virulence genes has been demonstrated to be host-dependent [35]. These alterations are regulated by regulatory proteins, two-component systems, and small RNAs. As a case in point, the expression of host-specific virulence genes in S. enterica is dependent upon the PhoP/PhoQ regulatory system and other regulatory systems [36]. Epigenetic modifications such as DNA methylation also contribute to the regulation of gene expression patterns associated with host adaptation, although less so in the context of zoonosis [37]. Together, these genetic determinants make up the molecular basis that allows zoonotic pathogens to cross host barriers. It is important to learn what the role of this diversity is and how it has evolved, to be able to predict spillover events, and to design control strategies.

5. Case Studies in Zoonotic Pathogens

Comparative genomics provides a powerful toolkit to identify the genetic underpinnings of host switching and adaptation in zoonotic pathogens. Detailed analysis of both viral and bacterial pathogens has revealed a plethora of mechanisms used by pathogens to cross interspecies hurdles [38]. Within viral pathogens, SARS-CoV-2, Influenza A, and Nipah virus demonstrate unique yet intersecting forms of genomic adaptation that contribute to successful interspecies transfer—receptor-binding domain mutations, genome reassortment, and immune evasion [39]. It further indicates the fact that these viruses have crossed multiple reservoir/intermediate hosts and the zoonotic emergence is in continuous dynamic. In Figure 3, the phylogenetic heatmap is shown, which depicts the phylogenetic relationships shared by these viruses and their hosts, revealing proximity patterns that explain the ability to jump from host to host.

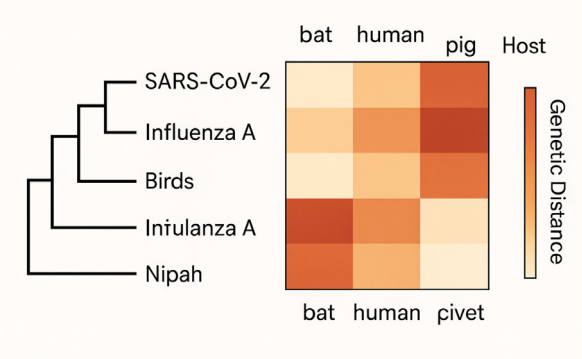

Figure 3 –Phylogenetic Heatmap Showing Genetic Relationships Between Zoonotic Viruses and Their Hosts

This diagram illustrates the genetic relatedness of three major zoonotic viruses—SARS-CoV-2, Influenza A virus, and Nipah virus—across their key host species: bats, humans, pigs, and civets. The left panel shows a simplified phylogenetic tree highlighting the evolutionary distance between these viruses. The accompanying heatmap quantifies genetic similarity between virus isolates and host species, where darker shades indicate greater similarity (shorter genetic distances). The visualization underscores how closely related viral strains can emerge in genetically distant hosts, aiding cross-species transmission.

5.1 Viruses

SARS-CoV and SARS-CoV-2

Coronaviruses have become emblematic of zoonotic potential, especially after the emergence of SARS-CoV in 2002 and SARS-CoV-2 in 2019. Comparative genomic analysis of SARS-related coronaviruses in bats, civets, and humans revealed that changes in the spike (S) protein, specifically within the receptor-binding domain (RBD), enabled efficient binding to the human ACE2 receptor [40]. A key finding was the presence of a polybasic furin cleavage site in SARS-CoV-2—a feature absent in most closely related bat viruses—that enhanced viral entry and expanded tissue tropism [41]. Ongoing surveillance of bat coronaviruses has identified several strains with RBDs similar to SARS-CoV-2, suggesting that minimal mutational changes may suffice to enable future spillover events [42]. Comparative genomics has also revealed recombination events between different coronavirus lineages, underscoring the role of genome shuffling in host adaptation.

Influenza A Virus

Influenza A virus (IAV) is a paradigmatic example of a virus capable of repeated zoonotic transmission. Its segmented genome allows for reassortment, whereby gene segments from avian, swine, and human strains mix during co-infection in intermediate hosts like pigs [43]. Comparative analyses of human, avian, and swine IAV strains have shown that host switching often involves mutations in the hemagglutinin (HA) and neuraminidase (NA) proteins, which mediate receptor binding and release. For example, the 2009 H1N1 pandemic strain was a reassortant containing gene segments from North American swine, Eurasian swine, avian, and human IAVs [44]. Additionally, polymerase gene mutations such as PB2 E627K have been linked to enhanced replication in mammalian hosts [45].

Nipah Virus

Nipah virus, a paramyxovirus harbored by fruit bats (Pteropus spp.), causes severe respiratory and neurological disease in humans [46]. Comparative genomic studies of Nipah strains from bats, pigs, and humans revealed high sequence conservation across hosts but notable variation in genes involved in immune evasion, such as the V, W, and C proteins [46]. These proteins antagonize host interferon responses and may be under strong positive selection during host shifts. Genomic analysis also suggested that the adaptation of the viral fusion (F) protein contributes to differences in cell tropism and pathogenesis between strains isolated from different host species [47].

5.2 Bacteria

Salmonella enterica

Salmonella enterica comprises multiple serovars with distinct host ranges, making it a model organism for studying host adaptation. Comparative genomic studies have shown that host-restricted serovars, such as S. Typhi (human-specific) and S. Dublin (cattle-adapted), have undergone genome degradation through pseudogenization and loss of function in metabolic and virulence genes [48]. These losses are thought to reflect specialization to the host environment. Conversely, broad-host-range serovars like S. Typhimurium retain a more flexible genetic toolkit, including a larger accessory genome. Horizontal gene transfer, particularly of virulence plasmids and pathogenicity islands (e.g., SPI-1 and SPI-2), has played a central role in shaping host interactions [49]. Regulatory differences also influence virulence expression; for instance, the PhoP-PhoQ and SsrA-SsrB systems show altered activity across serovars, affecting intracellular survival in different hosts [50].

Brucella spp.

Brucella species are intracellular pathogens responsible for brucellosis in humans and animals. Each species exhibits host preferences: B. melitensis infects goats and sheep, B. abortus infects cattle, and B. suis infects pigs [51]. Comparative genomics has revealed over 98% genomic identity across species, yet small-scale genomic variations—especially in outer membrane proteins, transcriptional regulators, and stress response genes—are implicated in host specificity [52]. Reductive evolution and genome streamlining appear to have accompanied the transition to intracellular lifestyles. Genomic islands like the virB operon, encoding a type IV secretion system, are conserved across Brucella species and crucial for intracellular survival and replication [53].

5.3 Parasites and Fungi

Toxoplasma gondii

Toxoplasma gondii is a protozoan parasite capable of infecting all warm-blooded animals, though felids are its definitive host. Comparative genomics of different clonal lineages (Types I, II, III) and atypical strains reveals genetic variation in secretory effector proteins (e.g., ROP and GRA proteins) that modulate host immune responses [54]. These effectors show signatures of positive selection and are linked to strain-specific virulence in humans and mice. Recent studies also suggest that minor genetic differences among strains result in significant variation in tissue tropism and transmission efficiency.

Histoplasma capsulatum

This dimorphic fungus causes histoplasmosis and is transmitted through inhalation of spores from soil contaminated with bat or bird droppings [55]. Comparative genomic studies across geographic isolates have shown variation in genes encoding cell wall components, heat-shock proteins, and secreted enzymes. These differences correlate with strain virulence and adaptation to environmental versus host-associated niches [56]. These case reports have revealed the wide range of genomic strategies zoonotic pathogens use for host adaptation. Whereas viruses tend to take advantage of high mutation rates, recombination to switch hosts, bacteria favor gene acquisition, loss, and regulatory adaptation. Host specificity can also be altered as a result of the evolution of effector proteins and stress responses, even among eukaryotic parasites. Comparative genomics continues to reveal the molecular signatures of these transitions, yielding critical information for disease prediction, prevention control.To complement these examples, we provide a summary table (Table 2) that highlights the key similarities and differences across the viral, bacterial, and eukaryotic pathogens discussed.

Table 2 Comparative Genomic Strategies of Zoonotic Pathogens Across Major Groups

Pathogen Group	Representative Examples	Key Genomic Strategies for Host Switching	Distinctive Features
Viruses	*SARS-CoV-2, Influenza A, Nipah virus*	High mutation rates; recombination; receptor-binding domain (RBD) adaptation; genome reassortment	Rapid adaptation through small genetic changes; frequent spillovers from wildlife reservoirs
Bacteria	*Salmonella enterica, Brucella spp.*	Horizontal gene transfer (plasmids, phages, pathogenicity islands); genome degradation; regulatory adaptations	Host-restricted vs. broad-host-range lineages shaped by accessory genomes
Parasites	*Toxoplasma gondii*	Variation in secretory effector proteins (e.g., ROP, GRA) under positive selection	Strain-specific virulence linked to immune modulation
Fungi	*Histoplasma capsulatum*	Genetic variation in cell wall proteins, heat-shock proteins, and enzymes	Adaptation to both environmental niches and host-associated infections

6. Evolutionary and Phylogenomic Insights into Cross-Species Transmission

The evolutionary dynamics of zoonotic pathogens are crucial for unraveling the mechanisms and patterns of cross-species transmission. Phylogenomics, the combination of whole-genome data with phylogenetics, allows for the derivation of pathogen ancestry, their host-switching history, and molecular evidence for adaptation [57]. This section discusses the evolutionary processes that drive host transitions and illustrates how phylogenomic approaches are helping to identify transmission routes and estimate zoonotic potential.

6.1 Phylogenetic Reconstruction of Host Jumps

Researchers can use phylogenies derived from whole-genome or selected sequence data to infer the evolutionary relatedness of pathogen strains and monitor the movement of pathogens across host species barriers. For zoonotic viruses, including Ebola, coronaviruses, and influenza, phylogenetic trees are indicative of several spillovers from wildlife reservoirs into humans, frequently associated with environmental/ ecological drivers.

For SARS-CoV-2, early phylogenetic analyses indicated a close relationship to the bat coronavirus RaTG13, suggesting a bat origin. However, an intermediate host (often speculated as a pangolin) was speculated on the basis of the phylogenetic clustering of some of the regions of the spike protein. Recombination analyses together with molecular clock dating have been adopted to predict the schedule of divergence events, thus leading to a focus on the most realistic period for zoonotic transmission [58].

In influenza A viruses, host jumps can be visualized through phylogenetic incongruence between different genomic segments due to reassortment. Phylogenetic analysis of hemagglutinin and neuraminidase genes, for instance, helped identify the avian and swine sources of the 2009 H1N1 pandemic strain [59]. To illustrate these concepts, we provide a schematic overview (Figure 4) highlighting how phylogenetic analysis is applied in tracing host jumps and cross-species transmission.

This diagram demonstrates the role of phylogenetic analysis in understanding cross-species transmission. The left panel shows a phylogenetic tree of viral strains from different hosts (bats, civets, pigs, humans). The middle panel depicts incongruences between host and pathogen phylogenies, which are indicative of host-switching events. The right panel summarizes key applications of phylogenetics, including reconstructing evolutionary origins, identifying intermediate hosts, dating cross-species transmission using molecular clocks, and predicting future spillover risk through comparative evolutionary models.

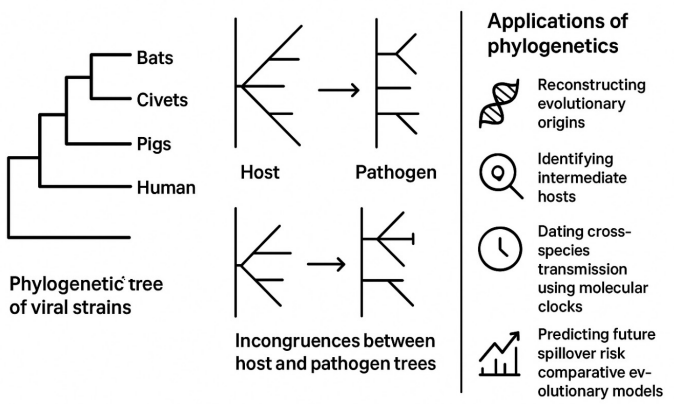

Figure 4 – Role of Phylogenetic Analysis in Tracing Host Jumps

6.2 Molecular Evolution and Selective Pressures

Cross-species transmission events often exert strong selective pressures on pathogens, particularly in genes involved in host receptor binding, immune evasion, and replication. Comparative genomic studies frequently identify positive selection ($dN/dS > 1$) acting on such genes during or after host jumps [60].

In RNA viruses, mutations in receptor-binding domains (RBDs) of surface glycoproteins are often under strong directional selection. For example, amino acid changes in the spike protein of SARS-CoV-2, such as N501Y and D614G, were found to enhance human ACE2 binding and transmissibility. These mutations arose independently in multiple lineages, suggesting convergent evolution toward improved adaptation to human hosts [61].

In bacterial pathogens, adaptive evolution is observed in genes involved in nutrient acquisition, stress responses, and host immune modulation. For instance, Salmonella enterica serovars adapted to specific hosts show pseudogenization of genes that are no longer required in their restricted niches—a form of genome degradation known as pathoadaptation [62].

6.3 Host-Pathogen Co-evolution and Phylogenetic Compatibility

Host-pathogen co-evolution shapes zoonotic potential through a balance of molecular compatibility and ecological opportunity. Some pathogens demonstrate phylogenetic host fidelity, meaning they tend to jump between closely related host species. This pattern has been observed in lentiviruses (e.g., SIV to HIV) and in rabies virus, where spillover is more likely between taxonomically related mammals [63].

Comparative phylogenetic methods, such as cophylogeny mapping (e.g., using tools like Jane or TreeMap), can reveal congruent patterns of evolution between hosts and their pathogens, suggesting co-speciation. Conversely, host-switching events appear as incongruences in these trees [64]. These methods are increasingly applied to infer the likelihood of future host jumps based on evolutionary constraints.

6.4 Molecular Clocks and Zoonotic Timing

Molecular clock models allow researchers to estimate the timing of divergence and cross-species transmission events based on the rate of nucleotide substitutions. In zoonotic viruses, these models have been used to trace the emergence timelines of various outbreaks.

For example, molecular clock dating helped establish that HIV-1 group M emerged in humans in the early 20th century, following a cross-species transmission from chimpanzees (SIVcpz) in Central Africa. In the case of SARS-CoV-2, dating analyses placed the most recent common ancestor of circulating strains in late 2019, corroborating epidemiological evidence [65].

Accurate molecular dating depends on high-quality genomic data and appropriate calibration points, such as known outbreak dates or fossil records (in long-evolving host species). Bayesian tools like BEAST, MrBayes, and TreeTime are commonly used to integrate molecular clocks with phylogenetic uncertainty [66].

6.5 Predictive Evolutionary Modeling

A key frontier in zoonotic research is using phylogenomic data for predictive modeling. By analyzing patterns of host switches in known pathogens, researchers have begun developing machine learning models trained on genomic, ecological, and evolutionary features [67]. These models aim to identify pathogens with high zoonotic potential before spillover occurs.

Tools such as SpillOver, developed by EcoHealth Alliance, use multi-parametric data—including host range, phylogenetic breadth, and viral traits—to rank the risk posed by known viruses. Similarly, evolutionary algorithms are being used to simulate possible mutational pathways that might enable future host adaptation [68].

Although promising, these predictive endeavours are constrained by patchy surveillance, particularly in wildlife reservoirs, and uncertain inferences about the nature of ecological cross-reactivity and the available evolutionary potential. However, (re)adding phylogenomics and ecological data analysis is the best approach to pandemic preparedness. Phylogenomics provides a powerful evolutionary lens for zoonotic emergence [69]. Phylogenetic and molecular evolutionary methods increase our understanding of the source, spread, and adaptation of pathogens as they have emerged, diverged, and adapted over time [70]. These approaches do not just shed light on historical outbreaks; however, they also offer the potential for predicting future zoonoses. As genomics becomes more prevalent and computational tools advance, this capability to identify early signals of host adaptation and emergence will be more central to global public health.

7. Challenges and Limitations in Comparative Genomics of Zoonoses

Although comparative genomics has been adopted as an imperative approach to elucidating the processes of zoonotic origin and host adaptation, some inherent limitations and challenges limit its fullest utility. Such adverse effects might cause inaccuracy of inferences drawn, vulnerability of evolutionary models, and even the general relevance of genomic findings for public health practice.

7.1 Sampling Bias and Incomplete Databases

In the field of comparative genomics, one of the most important obstacles is bias in the sampling, and in particular, underrepresentation in the genomic databases of wildlife pathogens. Pathogen discovery has been biased towards human and domestic animal infections; thus, we have not collected a representative dataset of the true diversity of microbes in wild reservoirs. This bias is particularly troubling for viruses and bacteria carried by wildlife, such as bats, rodents, and primates, which are frequently the origin of spillover infections [71].

Due in part to these incomplete DBs, accurate prediction of phylogeny/restriction MODs cannot be made. For instance, the actual zoonotic origin of agents such as SARS-CoV-2 is still challenging to determine in the absence of an extensive genetic survey of their probable intermediate hosts. Additionally, using reference genomes derived from a few strains may obscure within-species variation, which is important in the interpretation of host specificity and virulence [72].

7.2 Misannotation and Sequence Errors

Genomic data is of no better quality than the annotation of that data. Because of inaccurate annotations, especially for hypothetical and poorly defined genes, functional and evolutionary interpretations of genes can be misleading. Although automated annotation pipelines are efficient, they frequently convey errors from pre-existing databases, and they do not allow gene function to be elucidated in the case of uncharacterized organisms [73].

In addition, sequencing errors, particularly in low-quality or highly degraded samples, may lead to false variants or obscure biologically significant mutations. These mistakes can lead to comparative analysis errors, phylogenetic tree errors and even errors in site detection by selection. Strict QC, manual curation, and cross-validation with transcriptomic or proteomic data are important, yet work (resource)-intensive procedures [74].

7.3 Difficulty in Predicting Phenotype from Genotype

An enduring problem of zoonotic genomics is the gap between genotype–phenotype. Although comparative genomics can reveal genetic differences related to host-adaptation or virulence, it is less easy to use these data for functional predictions. The existence of a mutation does not always reflect in a phenotypic effect, and epistatic interactions or environmental conditions may modulate gene expression and function [75].

For example, if there are receptor-binding mutations in the viral spike proteins that are identified, it may appear to be indicative of an improved host compatibility, but in the absence of functional assays or animal models, such assumptions remain speculative. Likewise, even after acquisition, such virulence factors, when released by bacterial cells, need to be regulated in virulence networks, and predicting where and when in a dataset one might encounter such virulence factors under these complex transcriptional regulations is not straightforward based only on sequence data [76].

7.4 Ethical and Biosafety Issues in Studying High-Risk Pathogens

Working with high-consequence zoonotic pathogens raises significant ethical and biosafety issues and concerns, especially when genomic studies involve synthetic biology or gain-of-function (GoF) research. These studies are designed to identify the mutational pathways that would enhance transmissibility or pathogenic potential, but also pose dangers of deliberate or accidental release [77].

The debate over GoF research was reignited over COVID-19, emphasising the importance of transparent risk/benefit analyses and stringent adherence to BSL protocols. Ethical considerations further apply to wildlife sampling with the potential for ecosystem imbalance as well as pathogen exposure risks when not done with care [78].

International agendas such as the WHO's R&D blueprint and the Global Health Security Agenda highlight the need for responsible genomic research. Yet, differences in biosafety capacity, regulatory implementation, and data governance between countries are significant challenges [79]. Notwithstanding its incredible implications, zoonosis comparative genomics is extremely constrained by sampling, data quality, functional lack of prediction, and ethical limitations [80]. Solutions to these challenges will require joint investment in global genomic surveillance, enhanced bioinformatics pipelines, cross-disciplinary training, and a strong ethical framework.

Addressing these barriers will increase the applicability of genomics in predicting and addressing zoonotic threats.

To emphasize the main barriers in this field, we summarize the key challenges of comparative genomics in zoonotic pathogen research:

- Sampling bias: wildlife pathogens are underrepresented, limiting accurate predictions.
- Incomplete databases: gaps in genomic reference data obscure true diversity.
- Annotation and sequencing errors: can mislead functional and evolutionary interpretations.
- Genotype–phenotype gap: mutations do not always translate into clear phenotypic effects.
- Ethical and biosafety concerns: especially with high-risk pathogens and gain-of-function studies.

These limitations highlight the need for improved sampling strategies, better data quality, cross-validation with functional studies, and stronger governance frameworks.

8. Implications for Public Health and One Health Strategies

Comparative genomics has fundamentally transformed our comprehension of zoonoses and provides a unique tool for surveillance, prediction, and containment of pathogen emergence. Its implications for public health are vast, particularly when used in the context of One Health, which combines human, animal, and environmental health. This section highlights four main areas in which comparative genomics directly contributes to public health and One Health approaches: surveillance and early warning, zoonotic risk prediction, and targeted.

8.1 Surveillance and Early Detection via Genomic Monitoring

One of the most immediate applications of comparative genomics is in real-time surveillance and early outbreak detection. By sequencing pathogen genomes from diverse hosts and environments, public health agencies can rapidly identify novel or re-emerging zoonoses. This was exemplified by the SARS-CoV-2 pandemic, where global genomic surveillance enabled the detection of new variants, assessment of transmissibility, and timely policy adjustments [81].

Genomic monitoring allows for the identification of mutations associated with host adaptation, antimicrobial resistance, or immune escape. Through comparative analyses, scientists can distinguish between endemic strains and those that signal a shift in host range or virulence—information crucial for initiating containment measures [38].

The decreasing cost of high-throughput sequencing and the proliferation of portable sequencers (e.g., Oxford Nanopore) have made genomic monitoring more accessible, including in resource-limited settings. Integration of sequencing into frontline diagnostics and veterinary surveillance allows for earlier recognition of zoonotic signals before they reach human populations [17].

8.2 Zoonotic Risk Prediction Models

Predicting zoonotic spillover risk is a major public health priority. Comparative genomics provides the molecular foundation for zoonotic risk models, which evaluate the likelihood of host jumps based on genetic, ecological, and evolutionary parameters.

Genomic features such as receptor-binding domains, codon usage bias, and sequence similarity to known zoonotic strains are now incorporated into computational models to assess zoonotic potential. For example, viral risk-ranking tools like SpillOver and Zoonotic Rank utilize genomic inputs alongside ecological and host data to score animal viruses by their likelihood of crossing into humans [82].

Furthermore, machine learning algorithms trained on genomic datasets can detect patterns predictive of host range expansion or transmission efficiency. When combined with host phylogenies and environmental exposure data, these models offer a more holistic and accurate risk stratification system [83].

However, the success of such models hinges on the availability of diverse, high-quality genomic data from multiple species—a challenge that underscores the need for coordinated global sampling and open data sharing.

8.3 Targeted Interventions and Vaccine Development

Comparative genomics also informs focused public health interventions, such as vaccines and therapies. Conserved and divergent regions in pathogen genomes can be used for the development of broadly based or variant-specific vaccines and for forecasting antigenic drift.

In much the same way that flu vaccine composition is determined based on genomic surveillance of circulating strains, updates are made annually to reflect the most likely dominant subtypes. Likewise, the snowballing of mRNA COVID-19 vaccines into existence was precipitated by instant access to the SARS-CoV-2 genome, with ongoing genomic comparisons overseeing booster design [84].

In bacterial zoonoses, comparative genomics has enabled to detection of virulence islands and resistance genes, which are candidates for the development of new antimicrobials. Genomic information also helps in developing diagnostics that can differentiate between closely related human pathogens or identify important pathogenomic markers [85]. In addition, comparative analysis can be used to find high-risk lineages, and control is then concentrated (i.e., targeted culling, wildlife vaccination, environmental sanitation) in hotspot areas.

8.4 Integrating Genomic Data into One Health Frameworks

The end goal of comparing genomes is to integrate them into One Health surveillance and response activities. Genomic approaches can integrate data from humans, animals, and the environment, leading to a coherent picture of pathogen ecology.

Initiatives like the Global Virome Project, PREDICT, and the WHO's International Pathogen Surveillance Network explicitly encourage cross-sectoral data integration. At the intersection of these fields, comparative genomics provides a lingua franca and has facilitated coordination of work in pathogen discovery, outbreak response, and capacity building [86].

Equitable integration will depend upon aligning bioinformatics platforms, establishing standard metadata protocols, and providing access to sequence technologies for all. The advancement of genomic literacy in veterinarians, public health practitioners, and ecologists is also critical.

Moreover, ethical issues (data ownership, benefit sharing, and the risk of stigmatization of regions harboring high-risk agents) will also need to be considered in the context of any integrated genomic surveillance effort. Comparative genomics provides important support to public health through improved

surveunlock new windowance, predictive models, focused interventions, and One Health collaboration. With greater dissemination of genomic technologies and the enhancement of data integration, these tools are likely to take on a more central role in the prevention, detection, and control of zoonotic diseases at the global level [87].

9. Future Directions and Research Gaps

While there has been tremendous progress in comparative genomics and its use for the study of zoonoses, several large gaps do exist that currently prevent us from having a full understanding of and thus the ability to control risks for zoonotic spillover. Filling these gaps is central to strengthening international health security and the development of predictive capabilities. This section outlines major directions ahead and some research needs in the area.

9.1 Need for Better Sampling in Wildlife and Understudied Reservoirs

In zoonotic comparative genomics, sampling of wildlife hosts and other putative reservoirs has long been a weakness. Most genomic data are dominated by a narrow subset of species, which tend to be biased in favor of domestic animals and human pathogens, with much of the rest of biodiversity poorly characterized. Several novel zoonotic diseases are caused by wildlife species- bats, rodents, and non-human primates- that carry complex viral and bacterial populations that are still poorly understood [88].

Expanding surveillance and genomic sequencing in these understudied reservoirs is essential for capturing the true diversity of zoonotic agents and understanding their evolutionary trajectories. This includes not only species known to harbor zoonoses but also ecological niches where pathogen spillover risk may be high due to environmental changes, human encroachment, or wildlife trade [89].

Innovative sampling approaches—such as environmental DNA (eDNA) surveillance, non-invasive sampling methods, and metagenomic sequencing—offer promising avenues to increase the breadth and depth of pathogen discovery while minimizing ecological disruption. Collaborative global networks and capacity building in biodiversity hotspots are also critical for sustained and equitable genomic sampling efforts [90].

9.2 Longitudinal Studies of Host-Pathogen Co-evolution

Static snapshots of pathogen genomes provide valuable insights, but to fully understand zoonotic emergence, longitudinal studies tracking host-pathogen co-evolution over time are necessary. These studies can elucidate how selective pressures, host immune responses, and ecological factors drive pathogen adaptation, diversification, and host specificity.

Repeated sampling of both pathogens and hosts in the field also permits detection of early genetic changes in both microbes and hosts preceding spillover, as well as the dynamics of persistence and transmission of the pathogen. For instance, to track viral evolution within bat populations between seasons or years, mutation rates, models of recombination, and the appearance of variants with different potential for zoonotic transmission can be inferred [91].

Such efforts to integrate ecological and genomic data collection by sampling repeatedly over defined host populations are challenging logistically but essential in predictive modeling. They also help to disentangle how pathogens are maintained in reservoirs, and when spillover is more probable [92].

9.3 AI and Machine Learning in Zoonotic Risk Assessment

Artificial Intelligence (AI) and machine learning (ML) have become transformative methodologies to analyze complex, high-dimensional genomic data in predicting zoonotic risk. These methods can reveal nuanced patterns and interactions among pathogen genomes and between pathogens and hosts that are not easily discerned using a naive analysis [93].

By teaching algorithms on various data, from viral sequences to host phylogenies to ecological variables to epidemiological accounts, AI models can rank pathogens according to their zoonotic potential, predict the likelihood of host switching, and forecast evolutionary paths in different scenarios. These predictive analytics can improve early alert systems and prioritize surveillance and intervention [93].

But building fair and trustworthy AI models relies on rich, predictive data to train on and transparent algorithms. Integrating ML with experimental validation and ecological context is necessary to reduce false positives and ensure actionable outputs. Continued investment in interdisciplinary collaborations combining genomics, computer science, ecology, and public health will accelerate the maturation of AI-driven zoonotic risk assessment tools [93,94].

9.4 Global Genomic Databases for Zoonotic Pathogen Tracking

The effectiveness of comparative genomics hinges on access to comprehensive, standardized, and openly accessible genomic databases. Current repositories such as GenBank and GISAID have revolutionized pathogen tracking but remain fragmented, unevenly populated, and often lacking critical metadata such as host species, geographic origin, and sampling date [95].

Developing global genomic databases dedicated to zoonotic pathogens—integrating data from human, animal, and environmental sources—is essential to enable timely, coordinated responses to emerging threats. These databases should facilitate real-time data sharing, support standardized annotation protocols, and enable integration with epidemiological and ecological datasets [38].

Finally, the development of fair data-sharing policies that respect sovereignty and support benefit-sharing, particularly for nations with diverse faunal populations, is necessary for the establishment of global cooperation. Simple, cloud-based applications with intuitive interfaces and robust analytics will democratize access, and local users will be able to effectively take part in surveillance initiatives. Initiatives to develop these databases need to be complemented with the building of expertise in bioinformatics and data governance to maintain genomic surveillance infrastructure globally, now and in the long term [96].

The future of zoonotic comparative genomics lies in expanding the scope and extent of surveillance, embracing longitudinal evolutionary studies, using AI-driven predictive models, and developing global genomic databases. Filling in these knowledge gaps will improve our capacity to predict and mitigate zoonotic spillovers and increase global health resilience to emerging infectious diseases.

Conclusion

This review has emphasized the breakthrough effect of comparative genomics on our knowledge of zoonotic pathogens in relation to the genetic factors that are responsible for host adaptation and cross-species transmission. Through identification of genomic differences and examination of the fundamental evolutionary forces underlying pathogen spillover, comparative genomics offers unprecedented views of the complex interactions between pathogens and hosts. Important genetic determinants, including receptor-binding specificity and immune escape strategies that contribute to the transmission and virulence emergence of zoonotic coronaviruses, have been characterized.

The most notable development has been the integration of genomic data into surveillance systems, transforming outbreak detection and response, and allowing public health to follow pathogen evolution almost in real-time. The progress on zoonotic risk prediction models and genomic signature-based models for predicting spillover events. Additionally, comparative genomics has directly assisted in interventions to combat zoonotic pathogens through the development of vaccines and treatments and, therefore, has played a critical part in the reduction of the zoonotic disease burden.

Yet, trait prediction faces several challenges, such as sampling biases, annotation errors, and the complexity of relatedness from genotype to phenotype. Ethical and biosafety issues must be the priorities, especially when it comes to research on dangerous pathogens. Addressing these limitations requires strong interdisciplinary collaborations and an improved global genomic infrastructure.

In the future, continued investigations into research gaps—through more extensive wildlife sampling, longitudinal studies of host–pathogen co-evolution, and the use of artificial intelligence—will sharpen our predictive abilities. Building universal, accessible genomic databases is also essential for coordinated One Health surveillance initiatives. With these advances, the field is now better equipped than ever to detect emerging threats early, guide targeted interventions, and ultimately prevent future pandemics.

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Contemporary Methods for Evaluating the Health Impact of Chrysotile-Asbestos on Workers: Scoping Review

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Abstract

Analysis of literature data on delayed effects (remote consequences) of exposure to mineral fibers on the body, including chrysotile asbestos, to study the mechanisms by which mineral fibers cause adverse effects in both animals and humans, is a hot topic of interdisciplinary research, many aspects of which are yet to be clarified. The conducted review of scientific literature allowed us to establish a list of pathological conditions in humans and animals that develop later after cessation of contact with various mineral fibers, including chrysotile asbestos. Such conditions (diseases) include pleural mesothelioma, lung cancer, asbestosis, pneumofibrosis, pulmonary emphysema, chronic dust bronchitis, etc. According to literary data, it has been established that workers at enterprises with high occupational dust risks, especially older and elderly people, may develop dust pathology after dismissal, which should be considered as delayed effects of exposure to unfavorable factors. Such production requires more careful attention, study and in-depth analysis of morbidity with temporary loss of ability to work, the results of periodic professional examinations for a quantitative assessment of the magnitude of health risks in various professional groups, the use of modern epidemiological, ecological-hygienic and clinical-diagnostic studies to prevent the development of diseases, premature mortality aimed at increasing the life expectancy of people in industrial regions of the country and after the end of work.

Keywords: mining industry, extraction and enrichment of mineral fibers, chrysotile asbestos production, asbestos cement materials, chrysotile asbestos-containing dust.

Introduction

According to the modern classification of pneumoconiosis (1996), asbestosis is categorized as a form of silicosis—an occupational disease caused by inhalation of dust containing silicon dioxide bound with various elements, including aluminum, magnesium, iron, and calcium [1]. Asbestosis is recognized as one of the most severe forms of pneumoconiosis [2], resulting from prolonged exposure to asbestos dust, a fibrous, structureless hydrosilicate known for its resistance to high temperatures [3].

Pneumoconiosis caused by weakly fibrogenic dust—defined as dust with a free silicon dioxide content of less than 10% or completely absent—such as asbestosis, typically presents with moderate pneumofibrosis and follows a benign, slowly progressive course. However, it is often complicated by chronic bronchitis and secondary infections, which significantly contribute to disease severity. In the International Classification of Diseases, 10th Revision (ICD-10), asbestos-induced pneumoconiosis is classified under category J61.

Radiographic manifestations of asbestosis include focal, linear, or irregular opacities that initially appear in the lower lung fields and progressively extend to the middle and upper lung zones. Importantly, radiological

changes are not exclusive to individuals with asbestosis; they are also observed in asymptomatic individuals with a history of asbestos exposure. Such findings include pleural plaques—localized thickening or calcification of the parietal pleura, typically affecting the lower costal pleura, diaphragmatic pleura, and the pleural transition to the mediastinum. In the absence of additional radiological abnormalities or clinical symptoms, pleural plaques alone are not considered indicative of asbestosis. As fibrosis advances, complete obliteration of acini may occur, leading to the development of a characteristic "honeycomb lung" appearance on imaging—marked by interspersed focal opacities of varying shapes and sizes along with small cystic formations (7–10 mm in diameter) [4].

The definitive diagnosis of occupational asbestosis relies on detecting asbestos fibers in the workplace environment, as well as identifying asbestos (iron) bodies in biological samples or tissue specimens. Numerous studies have established the role of asbestos fibers in the pathogenesis of pulmonary fibrosis, lung cancer, and pleural malignancies [5–8].

Asbestos dust exerts a moderate fibrogenic effect and a pronounced irritant effect on the respiratory tract. Asbestosis is characterized by diffuse pulmonary fibrosis involving the peribronchial and perivascular interstitial tissue, interlobular septa, and alveolar walls [9].

According to Litvyakov A.M. and Shchupakova A.N. [9], in addition to its moderate fibrogenic properties, asbestos dust exerts a pronounced irritating effect on the respiratory tract. Asbestosis is characterized by diffuse pulmonary fibrosis involving peribronchial and perivascular interstitial tissue, interlobular septa, and alveolar walls.

The term "asbestos" is a commercial or collective designation for a group of silicate minerals that differ in mineralogical structure, physicochemical properties, and biological activity. These minerals are classified into two main groups: serpentines and amphiboles, which share a fibrous structure, leading to certain common industrial applications. Based on the crystalline lattice structure, amphibole asbestos belongs to the ribbon silicate group, while serpentine asbestos belongs to the layered silicate group [10]. The amphibole group includes crocidolite, amosite, anthophyllite, tremolite, and actinolite, which exhibit significantly higher toxicity, fibrogenicity, and carcinogenicity compared to chrysotile asbestos [11].

Due to its fire-resistant, durable, and dielectric properties, asbestos is widely used in construction (e.g., asbestos-cement products such as pipes, panels, and roofing materials) and industry (e.g., asbestos-based plastics and technical products, including brake pads). It also plays a crucial role in fire protection through asbestos textiles. Major industrial sources of asbestos are located in Russia, Canada, China, and Mediterranean countries. Canada, which possesses the largest global asbestos reserves, has a particularly high prevalence of asbestosis. In the construction sector alone, approximately five million people are exposed to asbestos daily. Among a cohort of 17,000 insulation workers in Canada and the United States, 38% were diagnosed with asbestosis [12].

Recent advancements have enabled the quantitative measurement of dust accumulation in the lungs or the biological dose expressed as the number of fibers per gram of dry lung tissue. X-ray energy-dispersive spectroscopic analysis (EDXA) provides precise identification of fiber type [13]. Although standardization of results across laboratories remains a challenge, the application of these methods in epidemiological research has allowed for several key findings:

From an initial pool of records identified through the search process, a final total of 94 studies met the inclusion criteria and were included in the review.

Key data were extracted from each selected source, including study design, population characteristics, exposure duration and level, health outcomes reported, and methodological quality. Where available, quantitative risk estimates such as standardized mortality ratios (SMR), relative risks (RR), and odds ratios (OR) were recorded.

The extracted data were synthesized descriptively, with findings organized according to major disease outcomes and exposure patterns. Particular attention was paid to dose-response relationships, latency periods, and variation across geographic and industrial contexts, including specific focus on data from Kazakhstan and post-Soviet regions where chrysotile asbestos remains in use.

Although qualitative analysis were conducted, a formal meta-analysis was not feasible due to the high heterogeneity of studies, exposure assessment methods, and outcome definitions in the available epidemiological data. Many studies employed inconsistent exposure metrics or lacked the detailed statistical information required for data pooling. Nevertheless, quantitative data were extracted to support the descriptive analysis.

Quantitative Risk Estimates from Major Studies

For a long time, asbestos dust assessments primarily considered only long, thin fibers [17]. Even today, most researchers assert that the carcinogenic potential of asbestos is determined not by its mineralogical type but by fiber length. Specifically, fibers longer than 5 μm are generally not considered carcinogenic, whereas fibers shorter than 3 μm exhibit a pronounced carcinogenic effect. The prevailing hypothesis suggests that long asbestos fibers, which are not effectively cleared from the respiratory tract, accumulate in the bronchioles and cause tissue injury during respiratory movements. However, asbestosis has also been observed following exposure to short asbestos fibers, indicating that fiber length alone does not fully explain disease development [18].

Inhalation of asbestiform tremolite has been linked to an increased incidence of mesothelioma in certain mining environments. Exposure-response relationships for tremolite asbestos and mesothelioma have been extensively evaluated in high-exposure occupational settings, and exposure assessments have been developed for various product-use scenarios [19–21].

Currently, chrysotile asbestos production is concentrated in Russia, Kazakhstan, and China. Chrysotile remains the only type of asbestos still commercially available and has historically accounted for 95% of global asbestos sales over the past century. The asbestos industry, particularly through its public relations organization, the International Chrysotile Association (ICA), continues to actively promote asbestos use. The ICA is funded by asbestos mining companies in Russia, Kazakhstan, and Zimbabwe, as well as asbestos manufacturers in India and Mexico [22].

Mathematical models have been developed to assess the relationship between asbestos dose and inhalation exposure in the respiratory system [23]. These models provide insights into the sequence of events from pollutant inhalation to tissue damage. Fiber size plays a critical role in the pathogenesis of asbestos-related lung diseases. Long asbestos fibers, typically exceeding the diameter of alveolar macrophages, are less likely

1. Confirmation of the biological persistence of amphibole fibers in the lungs compared to chrysotile fibers.

2. Detection of asbestos fibers in the lungs of individuals with no known history of significant asbestos exposure.

3. Demonstration of differences in fiber burden based on geographical location (urban vs. rural) and occupational exposure.

4. Establishment of correlations between fiber load and asbestos-related diseases [14].

Exposure to high concentrations of quartz-containing dust for relatively short durations can lead to delayed-onset pneumoconiosis, a distinct form of the disease that may develop 10–20 years after cessation of dust exposure. Such cases are characterized by a short occupational exposure duration, typically 4–5 years [15].

According to Bushueva T.V. [16], statistical analyses ($p=0.05$) indicate a significantly higher prevalence of early respiratory symptoms in workers exposed to chrysotile asbestos who had a history of pneumonia.

The aim of this review is to analyze contemporary methods for assessing the delayed health effects of chrysotile asbestos exposure in workers, with a focus on identifying pathological conditions, evaluating risk factors, and exploring modern diagnostic and preventive strategies.

Methods

This scoping review was conducted using a structured and transparent approach to identify and analyze existing evidence on the delayed health effects of occupational exposure to chrysotile asbestos.

A comprehensive search was performed across multiple scientific databases, including PubMed, Scopus, Web of Science, Google Scholar, and regional platforms such as the Russian Scientific Electronic Library (eLIBRARY.RU). The search also incorporated reports, monographs, and technical documents from authoritative international bodies such as the World Health Organization (WHO) and the International Labour Organization (ILO), ensuring inclusion of both peer-reviewed and grey literature.

The search strategy included combinations of keywords and MeSH terms such as “chrysotile asbestos”, “occupational exposure”, “mesothelioma”, “lung cancer”, “asbestosis”, and “long-term health effects”. No restrictions were imposed on publication year, but the search was limited to literature published in English and Russian. The final search was conducted in June 2025.

Inclusion criteria comprised:

- Studies addressing the long-term health outcomes of occupational exposure to chrysotile asbestos;
- Epidemiological studies, cohort and case-control designs, toxicological analyses, and government or institutional reports;
- Articles discussing outcomes such as lung cancer, mesothelioma, asbestosis, and chronic respiratory disease.
- Exclusion criteria included:
- Studies focused exclusively on non-occupational exposure (e.g., environmental or para-occupational sources);
- Reports addressing short-term effects only or lacking outcome data;
- Editorials, opinion pieces, or studies without accessible full texts.

to be completely phagocytized. Fibers that are not rapidly cleared by the mucociliary escalator may penetrate the interstitium of alveolar walls, be transported via lymphatic pathways, or migrate to the pleura and other extrapulmonary sites [24]. In cases where fibers are not effectively removed from the lungs, physicochemical processes such as ion leaching, dissolution, and degradation can facilitate clearance. These processes may occur extracellularly in the lung lining fluid or intracellularly within alveolar and interstitial macrophages.

Of particular interest are recent studies focused on mathematical modeling of mineral fiber deposition and clearance in the respiratory system (Figure 1). Using this model, the estimated average elimination coefficient for crocidolite was 0.099 (compared to the published average of 0.092), for amosite 0.169 (compared to 0.19), and for chrysotile 6.45. The study demonstrated that lung fiber burden varies linearly with exposure intensity and superlinearly with exposure duration. Modeling of three distinct exposure events over three decades indicated that lung fiber burden was predominantly influenced by the most recent exposure event ($R=0.967$, $p<0.05$) and only weakly correlated with the earliest exposure event ($R=0.032$, $p<0.05$). Despite certain limitations, mechanistic modeling of asbestos exposure provides a valuable tool for risk assessment and disease prediction [25].

Table 1 Summary of Epidemiological Risk Assessments Based on Key Studies of Chrysotile Asbestos

Study / Source	Outcome	Risk Estimate (95% CI)	Comments
Boffetta et al., 2019	*Lung cancer*	SMR 1.60 (1.30–1.95)	Meta-analysis of 55 cohorts; chrysotile-specific
Magnani et al., 2022	*Mesothelioma (all types)*	RR 4.78 (3.62–6.32)	Pooled multicenter case-control study
IARC Monograph 100C, 2012	*Lung cancer (all asbestos)*	–	Concluded sufficient evidence for chrysotile carcinogenicity
IARC (2021 Summary Update)	*Mesothelioma*	–	Reaffirmed Group 1 classification for chrysotile
Bernstein et al., 2022	*Lung cancer*	OR 1.10 (0.85–1.35)	Study funded by industry; methodological critiques noted
Marinaccio et al., 2022	*Pleural mesothelioma*	OR 5.30 (3.90–7.00)	Based on national registry data (Italy)

Several large-scale studies and authoritative reviews have quantitatively assessed the health risks associated with exposure to chrysotile asbestos. In a meta-analysis by Boffetta et al. (2019), which included 55 occupational cohorts, the standardized mortality ratio (SMR) for lung cancer associated with chrysotile asbestos exposure was estimated at 1.60 (95% CI: 1.30–1.95). Similarly, a pooled analysis by Magnani et al. (2022) reported a relative risk (RR) of 4.78 (95% CI: 3.62–6.32) for mesothelioma. In contrast, Bernstein et al. (2022) reported a modest and statistically non-significant odds ratio (OR) of 1.10 for lung cancer; however, this study has been criticized for potential conflicts of interest and methodological concerns. Independent organizations, including the International Agency

for Research on Cancer (IARC), continue to classify chrysotile as a Group 1 human carcinogen based on robust epidemiological and mechanistic evidence [25, 60] (Table 1).

Following inhalation, asbestos fibers that reach the alveolar region of the respiratory tract may either be excreted or retained. Retained fibers can exert direct cytotoxic effects on alveolar epithelial cells or induce a chronic inflammatory response, ultimately leading to epithelial cell proliferation and fibrosis. It has been suggested that fibers penetrating the alveolar lining and entering the interstitium may migrate via the lymphatic system to regional lymph nodes. Accumulation of asbestos fibers in subpleural lymphatics is believed to contribute directly or indirectly to diffuse visceral pleural fibrosis and pleural effusion. Asbestos-induced pleural effusions are thought to result from cytokine release, particularly interleukin-8, by mesothelial cells, triggering an inflammatory response and fluid accumulation in the pleural cavity. Inhaled fibers may reach the pleural cavity through three potential pathways: direct transpleural penetration, lymphatic translocation, or hematogenous transport. Recent studies on refractory ceramic fibers in hamsters have demonstrated rapid translocation of short fibers into the pleural cavity, followed by inflammation and mesothelial cell proliferation [26, 27]. Additionally, focal entrapment of long asbestos fibers at pleural lymphatic drainage sites has been implicated in the pathogenesis of mesothelioma [28].

Fiber biopersistence is considered a key determinant of pathogenicity, particularly for interpreting short-term studies designed to assess the toxicity of newly proposed fibers [29]. The relative biopersistence of fibers of varying lengths has been evaluated through changes in mean lung fiber load, determined by electron microscopy at intervals of 3 days, 1 month, 6 months, and 12 months after intratracheal instillation in rats. Observed differences in fiber persistence support the hypothesis that short fibers are primarily cleared through cellular mechanisms, whereas long fibers undergo clearance through dissolution and fragmentation. Both fiber size and solubility significantly influence biopersistence [30].

Figure 1 illustrates the key biological mechanisms underlying the pathogenicity of fibrous materials, with a particular focus on fiber biopersistence, fiber dimensions, and toxicological outcomes.

The left side of the illustration shows the lung environment post-exposure, highlighting two primary types of fibers: short fibers (cleared more rapidly by macrophages) and long fibers (retained longer in lung tissue). Short fibers undergo phagocytosis and enzymatic degradation, while long fibers, due to their length and durability, resist clearance and are removed primarily through slow dissolution and fragmentation. This difference in biopersistence is critical in determining the duration of tissue exposure to fibrous particles, a key factor in disease development.

On the right, the diagram depicts experimental models used to assess fiber toxicity. In vivo studies involving rats show fiber clearance over time (3 days, 1 month, 6 months, and 12 months), with electron microscopy used to measure lung fiber load. In vitro assays using RAW 264.7 macrophages and BEAS-2B bronchial epithelial cells evaluate cytotoxicity and apoptosis for various materials (e.g., SWCNTs vs. chrysotile asbestos). The findings reveal that neither chrysotile nor carbon nanotubes caused direct cell death or apoptosis in these specific assays, underscoring the need for long-term exposure models to capture true pathogenic potential. Overall, the image emphasizes that fiber type, length, solubility, and persistence are all pivotal in determining carcinogenic risk.

Fiber type, dimensions, deposition, dissolution, and migration all play crucial roles in mineral fiber carcinogenesis. Experimental studies involving direct injection of mineral dust into the pleural or peritoneal cavities indicate that the most carcinogenic samples—producing the highest incidence of mesotheliomas—contain the longest and thinnest fibers [9]. The cytotoxicity of various fibrous materials, including two types of unpurified single-walled carbon nanotubes (SWCNT-1 and SWCNT-2) differing in length and morphology, was assessed in vitro using RAW 264.7 macrophages and BEAS-2B human bronchial epithelial cells, with chrysotile asbestos serving as a positive control. Neither SWCNTs nor chrysotile asbestos exhibited direct cytotoxicity in the MTS assay, nor did they induce apoptosis based on Western blot analysis of RAW 264.7 macrophages and BEAS-2B epithelial cells [31].

Figure 2 illustrates the biological fate of inhaled mineral fibers based on their length and solubility. Short fibers are efficiently cleared by alveolar macrophages via phagocytosis and mucociliary transport, while long fibers persist longer and

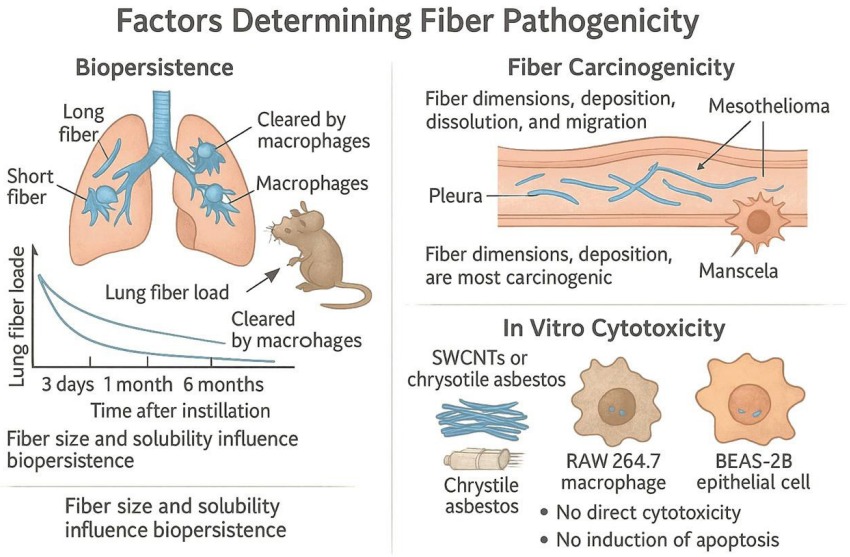

Figure 1 – Factors determining fiber pathogenicity

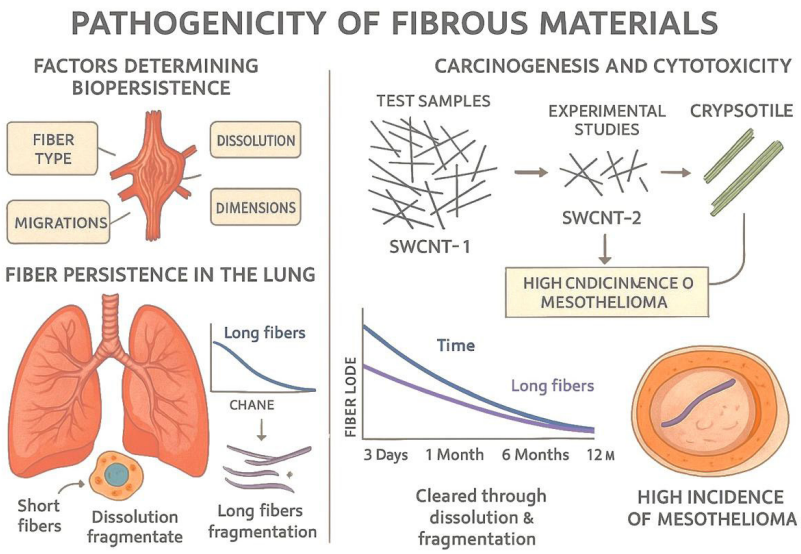

Figure 2 – Pathogenicity of fibrous materials

are eliminated through gradual dissolution and mechanical fragmentation. The time-course of fiber clearance in rat lungs (3 days, 1 month, 6 months, and 12 months after intratracheal instillation) reflects differences in biopersistence. Both fiber size and solubility significantly influence the retention time and, ultimately, the health risks associated with exposure. This schematic outlines how fiber type, length, and diameter affect carcinogenic potential, with longer and thinner fibers exhibiting higher mesothelioma incidence in animal studies. It also incorporates findings from in vitro assays involving RAW 264.7 macrophages and BEAS-2B bronchial epithelial cells exposed to chrysotile asbestos and two types of single-walled carbon nanotubes (SWCNT-1 and SWCNT-2). Neither material showed direct cytotoxicity or induced apoptosis, underscoring that fiber pathogenicity depends more on physical and physicochemical properties than on immediate cellular toxicity.

A postmortem analysis of lung tissue from 72 deceased individuals with confirmed occupational or environmental asbestos exposure (primarily to crocidolite and chrysotile) was conducted to assess the quantity, size distribution, and mineralogical composition of retained asbestos fibers. Correlations were examined between lung asbestos burden, fiber dimensions, exposure duration, latency period in cases of malignant mesothelioma, survival time, and time elapsed since cessation of exposure. Findings revealed that in 62.5% of cases, asbestos fiber concentrations in lung tissue were below the threshold indicative of occupational exposure. In 29.1% of cases, asbestos fibers were undetectable, and chrysotile fibers were rarely identified. Notably, the mean fiber length and the length-to-width ratio were significantly associated with cumulative asbestos exposure duration [32].

Long amphibole fibers exhibit prolonged biopersistence, migrate rapidly (within seven days) into the pleural cavity, and contribute to interstitial fibrosis and pleural inflammation. Quantitative reviews of epidemiological studies comparing chrysotile and amphibole asbestos fibers have confirmed distinct differences in their pathogenic potential, particularly regarding lung cancer and mesothelioma risk. While significant and long-term exposure to chrysotile has been associated with lung cancer, low-dose exposures appear to pose minimal health risks [33, 34].

Inhaled fibers with high aspect ratios are targeted by alveolar macrophages, which attempt to engulf them, releasing inflammatory mediators in the process. When macrophages fail to clear these fibers, chronic inflammation and disease development may follow. The exact biophysical and biochemical mechanisms by which fiber length influences macrophage response remain unclear. However, recent studies have assessed the role of fiber length in phagocytosis and molecular inflammatory pathways, enabling the development of quantitative, length-based risk models. These models suggest that fiber length alone can be used as a predictive factor for disease risk, independent of other physicochemical properties of the fiber [35].

Despite widespread asbestos bans, many developing countries still use asbestos or asbestos-containing materials. Due to the well-documented hazards of asbestos, researchers have been developing substitute materials for decades. However, comprehensive data on the health risks associated with asbestos substitutes remain limited. While fibrous materials have been used in asbestos-free products since before 1980, the toxicity of many alternatives remains insufficiently studied. Only a subset of substitute materials has undergone formal health hazard evaluations, and worker safety data remain sparse. Consequently, efforts should focus on minimizing occupational exposure not only to asbestos but also to its replacements [36].

Increased mortality from malignant mesothelioma and lung cancer has been reported in several epidemiological cohort studies of Italian workers involved in the manufacture of asbestos-containing products. Lung inorganic fiber loads were analyzed in post-mortem tissue samples using scanning electron microscopy (SEM) equipped with an energy-dispersive spectrometer. Notably, most lung samples from mesothelioma patients contained minimal asbestos fibers, and chrysotile was often undetectable. The most abundant fibers identified were crocidolite, amosite, tremolite/actinolite, and anthophyllite asbestos. The quantity of crocidolite and amosite fibers varied significantly depending on the type of occupational exposure [37–41].

The mechanisms by which mineral fibers cause adverse health effects in both humans and animals remain a subject of interdisciplinary research, with many aspects still unresolved. Diffraction and SEM analyses of fiber dissolution suggest

that chrysotile undergoes rapid amorphization, forming a nanophase, silica-rich metastable pseudomorph as an early stage of dissolution. In contrast, amphibole asbestos and fibrous erionite show little evidence of dissolution even after prolonged exposure (9–12 months), contributing to their persistence and long-term pathogenic effects [42].

The greatest carcinogenic risk of asbestos exposure lies in its role in inducing malignant pleural mesothelioma (MPM). Although pleural mesothelioma is a rare disease, accounting for less than 0.04% of all cancers and 0.2% of respiratory system tumors [43], it remains a significant health concern. Diffuse malignant mesothelioma arises from mesothelial cells and exhibits a characteristic pattern of diffuse growth along the pleural surface [44]. Epidemiological data indicate that mesothelioma most frequently affects the pleura (57.1%), followed by the peritoneum (39.1%) and, less commonly, the pericardium (1%) [45].

The primary etiological factor in MPM development is asbestos exposure, with incidence rates rising significantly since the 1960s due to increased industrial use [46]. However, chrysotile asbestos is not considered an obligate cause of the disease [47]. Long, thin asbestos fibers, particularly crocidolite (blue asbestos), are believed to be the most carcinogenic. Notably, the duration of asbestos exposure appears to be less critical than the intensity of exposure. In some cases, even a single high-dose exposure can trigger mesothelioma, though disease onset typically occurs 20–40 years later [48]. Consequently, many countries that experienced peak asbestos production and consumption in the mid-20th century, including the former USSR (which extracted 5 million tons of asbestos in 1975), anticipated a peak in mesothelioma incidence between 2010 and 2025. In Russia, MPM has been legally recognized as an occupational asbestos-related disease since 1996, and Kazakhstan followed suit in 2020.

Despite its rarity, MPM remains the most common primary malignant pleural tumor, with incidence rates continuing to rise [49]. In Germany, approximately 120 new cases are diagnosed annually, while in the United States, the figure is around 1,100 [50]. A review of the Belarusian Cancer Registry (2000–2019) revealed that mesothelioma incidence in Belarus is significantly lower than in Western, Central, and Eastern Europe, with a moderate downward trend over this period [51].

Asbestos-related malignancies, including mesothelioma and lung cancer, pose a global health challenge. Cellular and molecular research has explored the immunological effects of asbestos exposure, suggesting that prolonged exposure may impair immune responses, potentially contributing to cancer development. These studies could facilitate a deeper understanding of asbestos-induced biological effects and aid in identifying biomarkers for early detection of asbestos exposure and mesothelioma [52, 53].

Previously, mesothelioma was thought to result from the total burden of inhaled asbestos fibers in the pleura and/or peritoneum, similar to lung cancer. However, current evidence suggests that only an ultrafine fraction of fibers—measuring approximately 0.2 μm in diameter and only a few microns in length—can penetrate the pulmonary-pleural barrier. These fibers are now recognized as the primary cause of mesothelioma and other benign pleural conditions, such as pleural plaques [54, 55].

The Zhitikarinskoye chrysotile asbestos deposit in Kazakhstan has been in operation since 1965 and ranks fifth in the world in terms of chrysotile reserves. The chrysotile content in the mined ore has increased over time, from 3.88% in the early 1990s to 4.82%–5.74% in the early 2000s, with an average of 5.16% [56]. Ore extraction in the open pit mine involves drilling, blasting, excavation, and transportation. The enrichment complex processes chrysotile asbestos ore through crushing, beneficiation, and drying to produce commercial-grade chrysotile asbestos [57].

Internationally, Convention No. 162 of the International Labour Organization (ILO), adopted in 1986, addresses safety in asbestos-related work. In Kazakhstan, this document acknowledges the need for asbestos substitution where feasible but permits its controlled use under strict compliance with sanitary regulations, as outlined in the "Code of Practice for Safe Work with Asbestos" [58]. Preventive measures against asbestos-related diseases (ARD) primarily include pre-employment and periodic medical examinations, along with sanitary and hygienic interventions. A set of recommendations has been developed for preventing and managing ARD among workers in asbestos industries, with a focus on integrating medical and technical safety measures. The system for preventing occupational lung diseases in workers exposed to chrysotile asbestos includes diagnostic criteria for bronchopulmonary conditions and methods for identifying high-risk groups for occupational diseases [59].

A retrospective cohort study was conducted to assess the mortality of workers exposed to chrysotile asbestos-containing dust. Unlike other asbestos types, chrysotile has distinct chemical and physical properties and remains the only form of asbestos still permitted for use in many countries. The cohort included current and former employees who worked for at least one year at chrysotile asbestos mining and processing enterprises between January 1, 1975, and December 31, 2010. A comparative analysis of mortality causes among cohort members revealed that, of the 35,837 workers studied, 12,729 (35.5%) had died by the end of the follow-up period in 2015. Among these, 2,373 deaths were due to malignant neoplasms of various types, including 10 cases of mesothelioma. At the end of the study, 18,799 individuals (52.5%) were still alive, while 4,309 (12.0%) were lost to follow-up before 2015 [60].

An important question in asbestos-related cancer research is whether the risk of mesothelioma decreases after exposure ceases, as has been observed with some other carcinogens. A meta-regression analysis of nine studies examining mesothelioma risk post-exposure found that the relative risk (RR) over a 10-year interval after asbestos exposure cessation was 1.02 (95% CI: 0.87–1.19; $p = 0.01$), indicating no significant risk reduction. In contrast, analysis of four studies on lung cancer showed a decrease in risk, with an RR of 0.91 (95% CI: 0.84–0.98). These findings suggest that, unlike lung cancer, the risk of mesothelioma does not decline even after asbestos exposure ends [61].

Mortality from asbestos-related diseases and the incidence of mesothelioma have been extensively studied. A cohort study of 3,434 Italian asbestos workers who ceased employment between 1950 and 1986 examined the long-term risks of asbestos exposure [62]. According to regression analysis, the odds ratio (OR) of death from pleural neoplasms increased linearly with exposure duration and nonlinearly with latency period and time since cessation of exposure. The OR for peritoneal neoplasms continued to rise with increasing latency, duration of exposure, and time after exposure cessation. The study also confirmed a decline in the OR for lung cancer mortality following asbestos exposure cessation. While a reduction in pleural mesothelioma

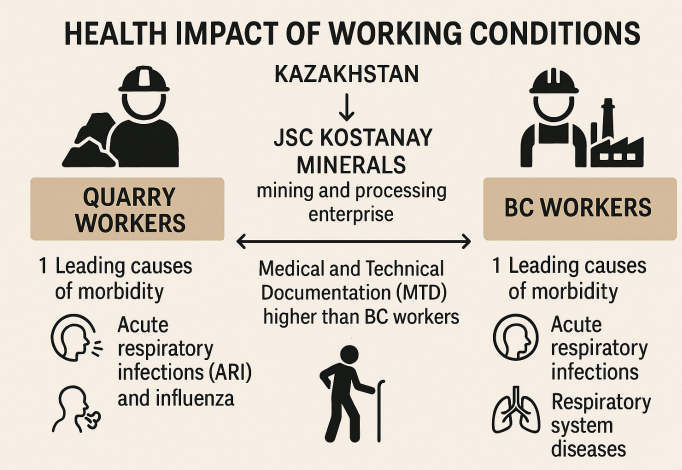

Figure 3 – Leading causes of morbidity in quarry workers and beneficiation complex workers

risk was anticipated after a latency of more than 40 years, the risk of peritoneal mesothelioma continued to increase.

Similarly, the risk of lung cancer was analyzed in a cohort of 189,896 Swedish construction workers who had experienced significant asbestos exposure. Among them, 2,835 cases of lung cancer were recorded, corresponding to an increased risk (OR = 1.74; 95% CI: 1.25–2.41). However, for those with low asbestos exposure 20 years after cessation, the OR was 0.94 (95% CI: 0.77–1.15), indicating no increased risk [63].

In Kazakhstan, studies on the health impact of working conditions at JSC Kostanay Minerals, a mining and processing enterprise, revealed variations in health risks between quarry workers and beneficiation complex (BC) workers. The Medical and Technical Documentation (MTD) and hygienic assessments indicated that quarry workers exhibited higher MTD values than BC workers [64]. The leading causes of morbidity in both groups were: acute respiratory infections (ARI) and influenza, musculoskeletal disorders, respiratory system diseases.

From 2011 to 2017, the number of workers diagnosed with occupational diseases sharply declined to just two cases due to the dismissal of probationary employees. The predominant occupational diseases were respiratory conditions, with asbestosis stage 1 (13.3–50%), chronic bronchitis (14.3–26.7%), and chronic obstructive bronchitis (14.3–26.7%) being the most common. The degree of loss of professional working capacity ranged from 10% to 60% [65]. However, the low reported prevalence of occupational diseases in asbestos industry workers may be due to the use of insufficiently sensitive diagnostic methods [66]. These findings emphasize that occupational bronchopulmonary diseases remain a significant issue in modern asbestos production [67].

The severe health consequences of asbestos exposure, particularly its role in lung cancer and mesothelioma, have prompted researchers at the Institute of Occupational Medicine, Department of Environmental Epidemiology in Łódź, Poland, to implement regular spirometry screenings (every three years) for former asbestos industry workers to assess lung cancer risk [68]. Additionally, they identified a potential link between occupational asbestos exposure and esophageal cancer in men [69].

Animal studies have also demonstrated that asbestos contributes to atherogenesis [70]. The impact of occupational hazards on human health is often associated with cumulative effects [71], which peak in old age when protective physiological mechanisms weaken, leading to increased morbidity and premature mortality [72]. However, the delayed effects of exposure are particularly relevant for oncological diseases, where latency periods can range from 15 to 25 years for moderate-strength carcinogens. This underscores the importance of long-term health monitoring for individuals at high risk of developing late-onset occupational diseases.

Notably, global burden of disease analyses from 1990 to 2015 revealed an increase in mortality due to occupational carcinogens [73]. In some countries, cancer-related mortality has surpassed cardiovascular mortality. Moreover, some studies suggest a possible association between circulatory system diseases and cancer, further emphasizing the need for comprehensive health surveillance of workers exposed to harmful occupational factors.

Establishing a causal relationship between a disease and a carcinogen involves several criteria, which can sometimes be difficult to identify. The presence of a hazardous agent may not always be confirmed retrospectively through ecological and hygienic assessments of past occupational and environmental conditions, nor through clinical and chemical analyses of biological material. However, identifying acute effects is crucial, as it can serve as the basis for developing preventive measures to protect the health and quality of life of former employees exposed to occupational hazards.

In the European Union, occupational diseases account for an estimated 2.6% to 3.8% of GDP losses. While their social significance appears to decrease among older age groups, the medical and social burden—expressed in lost years through the epidemiological integral indicator DALY (Disability-Adjusted Life Years)—continues to rise. This growing burden underscores the need for increased healthcare investments and highlights the importance of preventive measures. In Poland, for example, the Department of Health Policy has proposed adjusting retirement age based on lost years calculated for various occupational cohorts, including mining, industrial processing, construction, agriculture, fisheries, healthcare, and education. This approach aims to address social inequality in predicted healthy life expectancy [74, 75].

A 17-year study (1995–2011) on cancer incidence among workers in asbestos-cement enterprises (ACE) in Ukraine identified malignant neoplasms (MN) in 72 workers, representing 2.3% of the workforce. The average annual incidence rate of MN at ACE was 138.2 per 100,000 workers, which was lower than that of the general population ($p<0.01$). When calculating the population carcinogenic risk (PCR) from exposure to chrysotile asbestos dust and silicon dioxide (quartz), it was estimated that the expected number of additional cancer cases in target organs (larynx, bronchi, lungs, and pleural mesothelioma) would be 0.34 cases over 35 years or 1.34 cases per 100,000 exposed individuals per year. The distribution of expected cancer cases was attributed to chrysotile asbestos (32.3%) and silicon dioxide (67.7%). Accordingly, over the 17-year observation period, the expected number of cancer cases in target organs should be 0.16 cases, including 0.05 cases due to chrysotile asbestos exposure [76].

A study on cancer mortality among workers at a chrysotile asbestos mining and processing enterprise (OJSC Uralasbest, Asbest, Sverdlovsk Region) raised several unresolved issues regarding the quantitative assessment of carcinogenic risks from chrysotile asbestos exposure. While the carcinogenic hazard of chrysotile asbestos has been established, more precise quantification of the dose-response relationship and

the latency period for malignant neoplasm development remains necessary. The mineral composition of asbestos fibers and the extent of chrysotile contamination with amphibole asbestos are also critical factors. Nearly 75% of prior cohort studies on asbestos-related cancer primarily examined exposure to amphibole fibers or a combination of serpentine and amphibole fibers. This highlights the need for further research to specifically quantify the relationship between chrysotile exposure and cancer while addressing existing gaps in knowledge [77].

The widespread use of asbestos-cement materials raises concerns about the potential release of asbestos fibers due to mechanical stress and environmental factors. The concentration of respirable asbestos fibers in the atmosphere was measured using optical microscopy in accordance with the "Methodology for Measuring the Counting Concentration of Asbestos Fibers in Workplace Air." Findings showed that fiber concentrations in residential areas near asbestos-cement enterprises were 0.5–7.5 times lower than the maximum allowable concentration (MAC) for ambient air. At an asbestos-cement waste storage site, concentrations were 1.5 times lower than the MAC; at a thermal power plant (where asbestos is used as insulation), concentrations were 10 times lower; near highways, concentrations ranged from 1.5 to 8.6 times lower; and in public buildings, concentrations were 2.4 to 4.6 times lower than the MAC. These results suggest that emissions of chrysotile asbestos fibers from asbestos-cement materials due to natural and anthropogenic factors are relatively low [78].

Naturally occurring chrysotile fibers are considered the most optimal reinforcing agent for fiber concrete. They enhance tensile strength, fire resistance, durability, and electrical insulation properties while forming stable compositions and exhibiting chemical resistance in alkaline environments. Studies have even shown a decrease in the biological activity of chrysotile fibers due to environmental exposure and Portland cement hydration products—by factors of 10 and 30, respectively. This has led some researchers to question the necessity of banning chrysotile in production.

Dose-Dependence and Chemical Carcinogenicity

International Agency for Research on Cancer (IARC) has classified asbestos, including chrysotile, as a Group 1 carcinogen, indicating that it has proven carcinogenic properties for humans and increases the risk of developing malignant tumors [79–81]. However, it is noted that the risk of developing lung cancer due to asbestos exposure is associated with the duration of exposure and the cumulative dose [90–92]. It is well established that some of the chemical components of asbestos possess mutagenic and cytotoxic properties, which contribute to its carcinogenic effects [93–94]. Consequently, the use of chrysotile as an additive in building materials has become increasingly restricted.

It has been established that in cases of chrysotile exposure, lung cancer typically develops only in the presence of asbestosis or dust bronchitis—occupational diseases primarily caused by inadequate adherence to safety regulations, particularly the use of respiratory protection. Leading experts in the field generally agree that asbestos-related carcinogenesis, like other forms of carcinogenesis, follows a "dose-effect" relationship. However, there is still no consensus regarding a specific threshold for asbestos-related cancer risk [82–84].

Existing assessments of the carcinogenic risk of chrysotile and asbestos in general are based primarily on studies involving high exposure levels. Whether lower levels of dust exposure pose a significant risk of lung cancer remains under investigation, as their cumulative effect is still being studied [85].

Studies have shown that chrysotile asbestos is eliminated from the human body within approximately two weeks [86]. For comparison, ceramic fiber has a half-life of 60 days, aramid fiber (Kevlar) up to 90 days, and cellulose fiber more than 1,000 days. Research [87, 88] indicates that chrysotile lacks the strong biological activity and oncogenic potential of amphibole asbestos and is expelled from the lungs significantly faster.

Risk assessments suggest that mortality caused by chrysotile exposure is orders of magnitude lower than other common environmental hazards. For instance, the estimated risk of premature death per 1 million people due to asbestos-containing building materials is 0.04, whereas for radon exposure in residential air, the figure ranges from 2 to 5 [89].

Additionally, chrysotile exhibits environmental advantages. Unlike synthetic alternatives, it does not require energy-intensive melting processes or generate additional atmospheric pollutants during refinement. Due to these factors, Russia supports the controlled use of chrysotile-based building materials. The Russian Government's Resolution No. 869 (July 31, 1998) asserts that bans on asbestos in certain countries are primarily based on health data related to amphibole asbestos and fail to consider national socio-economic interests, recent scientific research, and technological advancements.

This narrative review reinforces the critical findings of our study by underscoring the persistent health risks faced by mineworkers, particularly due to exposure to hazardous airborne particles such as chrysotile asbestos. Despite significant advancements in occupational safety, mining remains a high-risk industry with elevated rates of respiratory illnesses, including pneumoconiosis, silicosis, and lung cancer [95].

Scientific Debate and Classification by IARC

Although some studies (e.g., Bernstein et al., 2022) suggest a relatively low carcinogenic potential of chrysotile asbestos, independent experts have criticized these findings for potential bias due to industry funding. For an objective interpretation, it is essential to consider independent evaluations, particularly those of the International Agency for Research on Cancer (IARC), which classifies chrysotile as a Group 1 human carcinogen based on a comprehensive analysis of animal and human studies.

In its recent monographs, IARC has reaffirmed that all forms of asbestos, including chrysotile, are carcinogenic to humans (Group 1). Despite historical controversies, IARC concluded in Monograph 100C (2012) that there is sufficient evidence linking chrysotile exposure to both lung cancer and mesothelioma. Recent reviews continue to support this classification, emphasizing that no safe exposure threshold has been identified.

Conclusion

Kazakhstan's development strategy prioritizes safety, public health, and preventive medicine, especially in high-risk industries. Special attention is given to vulnerable groups, including elderly workers and individuals exposed

to occupational hazards even after retirement. Dust-related diseases may manifest post-employment, highlighting the need for long-term monitoring and preventive health measures. A thorough analysis of occupational disease trends, periodic medical examinations, and advanced epidemiological, ecological, and clinical studies are essential for mitigating risks, reducing premature mortality, and improving life expectancy—particularly in industrial regions and among retired workers.

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Regenerative Medicine Unveiled: Principles, Technologies, and Clinical Breakthroughs in Tissue Regeneration

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Abstract

Regenerative medicine leads the way in healthcare innovation, harnessing the body's inherent regenerative abilities to transform treatment methods for tissue injuries. This review provides a thorough analysis of the core principles, historical development, and various elements that make up regenerative medicine. It explores crucial aspects like stem cells, biomaterials, tissue engineering techniques, gene therapy, small molecules, and biologics, clarifying their functions and possible uses in tissue repair and regeneration. This review examines the newest progress in regenerative medicine influenced by innovative technologies, highlighting their transformative effects on the discipline. It also examines the path of regenerative medicine from research findings to successful clinical applications, emphasizing accomplishments, obstacles, and current efforts. Regulatory structures overseeing regenerative therapies, ethical issues related to their application, and future possibilities are thoroughly examined to offer a complete insight into the field. Through the integration of these varied elements, this review aims to provide readers with a detailed understanding of the current environment and future possibilities in tissue regeneration and repair. It highlights the capacity of regenerative medicine to transform healthcare models, presenting encouraging opportunities for tackling unfulfilled medical requirements and improving patient outcomes.

Keywords: Regenerative medicine, stem cells, tissue engineering, gene therapy, biomaterials, small molecules, clinical translation.

1. Introduction

Regenerative medicine lies at the intersection of several scientific disciplines and involves different exogenous methods to undertake the repair or replacement of damaged tissues and organs by restoring both structure and function. Regenerative medicine is conceptualized as evolving principles from developmental biology, stem cell biology, tissue engineering, and molecular genetics [1]. Here, we take a comprehensive look at the history of regenerative medicine from ancestral wound healing modalities to today's science. Regenerative medicine is not a modern idea, but a practical based observation and natural treatment for enhancing tissue regeneration has probably been practiced by ancient cultures such as the Egyptian civilization. Various methods were documented by cultures, including ancient Egyptians, Greeks, and Chinese, as treatment for injuries in the form of herbal remedies, dressings for wounds, and surgical techniques. Nevertheless, these initial practices did not possess a systematic comprehension of the fundamental biological mechanisms [2].

The contemporary period of regenerative medicine started with important progress in cellular and molecular biology throughout the 20th century. The identification of stem cells, especially embryonic stem cells, by researchers like Ernest McCulloch and James Till in the mid-20th century established the groundwork for contemporary regenerative medicine. This finding revealed the presence of cells that possess the

exceptional capability to self-renew and develop into different cell types, opening new pathways for tissue regeneration. The conceptual foundation of regenerative medicine grew more comprehensive with the emergence of tissue engineering in the 1980s [3]. Groundbreaking research conducted by scientists such as Robert Langer and Joseph Vacanti showed the potential for cultivating tissues and organs in vitro through the use of biomaterial scaffolds and cellular techniques. This represented a significant change from conventional transplantation to engineered tissue designs for regenerative treatments [4].

21st century begins with the advancement in regenerative medicine was catalyzed by huge progress in the fields of genomics, proteomics, and bioinformatics. The Human Genome Project was completed creating possibilities of understanding the causative factors behind various diseases and came up with prospects of targeting them through gene therapy. At the same time, advances in stem cell research, notably the discovery of induced pluripotent stem cells (iPSCs) by Kentaro and Shinya, expanded the potential for cell-based therapies for tissue engineering [5].

Regenerative medicine today includes a broad spectrum of strategies to replenish loss of function tissue, encompassing stem cell therapy, tissue engineering and regeneration, gene therapy, and small molecule intervention. Today regenerative medicine encompasses diverse functional tissue restoration strategies with applications in stem cell-based therapy, tissue engineering, gene therapy and small molecule therapies[6]. This field is ever-evolving with ongoing research on novel biomaterials, novel fabrication techniques and individualized regenerative therapies. Regeneration combines fundamental science, translational research, and clinical application making it possible to impact the health care field by re-defining patient outcomes [7].

2. Tools utilized in regenerative medicine

(i) Stem Cells

In the growing discipline of regenerative medicine, stem cells can be said without any doubt to be the basic building blocks, because of their high self-renewal capacity and the ability to further specialize and help build different types of tissues [8]. The article provides information on stem cell biology, which is quite expansive in that it addresses different types of stem cells, how these cells undergo self-renewal as well as differentiation and their potential use in tissue regeneration. The categories of stem cells include embryonic stem, adult stem and induced stem cells, which are the most basic and which further reflect the general principles of their use in regenerative medicine. One of the arguments leading to the restrained use of ESCs, which are such cells derived from the inner mass of the blastocyst, is their ability to undergo modification into any cell type within the human body; such a possibility can raise ethical barriers of the usage of human embryos in clinical applications [9]. On the other hand, adult stem cells, such as somatic stem cells, also called tissue-specific stem cells are found in several sites in the body and are in active use for purposes of tissue homeostasis and healing. Hematopoietic stem cells (HSC) present in the bone marrow, mesenchymal stem cells (MSCs) from the stroma of several tissues, including umbilical cord tissue and neural stem cells (NSC) in the brain all depict adult stem cells [10]. Although the differentiation capacity of adult stem cells is considerably low than that of in embryo-derived stem cells, they have some distinct advantages such as access and immune tolerance, making them valuable counterparts in the task of effective regenerative medicine. Induced pluripotent stem cells (iPSCs) have been established as a promising source for future regenerative therapies due to their inherent plasticity. iPSCs can be generated by the targeted introduction of key transcription factors such as Oct4, Sox2, Klf4, and c-Myc into adult somatic cells, enabling cellular reprogramming and conferring pluripotency similar to that of embryonic stem cells (ESCs). This technique is free from ethical concerns and provides an opportunity to develop patient-specific regenerative medicine [10].

Table 1 provides a brief overview of stem cells' various types, sources, and therapeutic uses. Stem cells have distinct and appropriate application areas. With such diverse applications, stem cells resemble and are perfect candidates for future

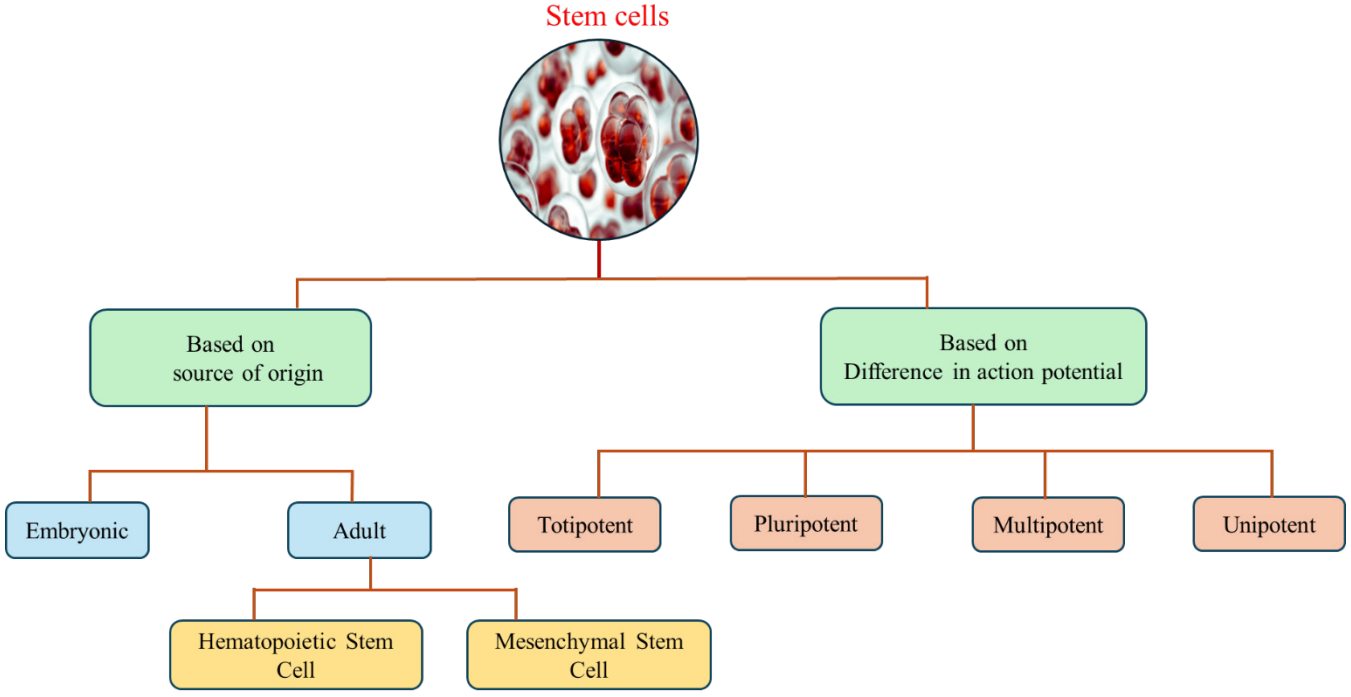

Figure 1 – Classification of stem cell

Table 1 Different types of stem cells, source and their applications

Stem Cell Type	Source	Key Characteristics	Applications in Tissue Engineering	Reference
Embryonic Stem Cells (ESCs)	Inner cell mass of blastocysts	Pluripotent, can differentiate into any cell type	Development of various tissues and organs, regenerative medicine	[13]
Mesenchymal Stem Cells (MSCs)	Bone marrow, adipose tissue, umbilical cord	Multipotent, differentiate into bone, cartilage, fat cells	Bone and cartilage repair, cardiovascular tissue engineering	[14]
Induced Pluripotent Stem Cells (iPSCs)	Somatic cells reprogrammed to a pluripotent state	Pluripotent, similar to ESCs	Personalized medicine, tissue regeneration, disease modeling	[15]
Hematopoietic Stem Cells (HSCs)	Bone marrow, peripheral blood, umbilical cord blood	Multipotent, give rise to blood cells	Blood disorders treatment, immune system reconstruction	[16]
Epithelial Stem Cells	Skin, lining of the gut	Multipotent, maintain and repair epithelial tissues	Skin regeneration, wound healing, gut lining repair	[17]
Neural Stem Cells (NSCs)	Brain and spinal cord	Multipotent, differentiate into neurons, astrocytes, oligodendrocytes	Neurodegenerative disease treatment, brain injury repair	[18]
Muscle Stem Cells (Satellite Cells)	Skeletal muscle tissue	Unipotent, differentiate into muscle cells	Muscle repair and regeneration, muscular dystrophy treatment	[19]

medical strategies such as tissue/organ reconstructive therapy, cell replacement therapy and even drug delivery. With their biologically-active components, stem cells have emerged as effective weapons against blood disorders, complex neurodegenerative diseases, and musculoskeletal injuries, accelerating the dynamics of the healing process. Although, the realistic and clinically relevant application of stem cells is accompanied by a number of challenges concerning safety, immunogenicity and ethical concerns. Some strategies directed at circumventing these problems are the improvement of cell and material purification, enhancement of transplantation protocols and development of appropriate materials for cells and tissue engineering [11]. Despite the hurdles that loom large, stem cell research perseveres at the forefront of regenerative medicine, propelling a relentless march of innovation and discovery aimed at unraveling the mysteries of stem cell biology, refining differentiation protocols, and ushering stem cell-based therapies from the realm of conjecture into the realm of clinical translation. It is through harnessing the remarkable versatility and plasticity of stem cells that regenerative medicine holds the promise of revolutionizing healthcare, transcending existing paradigms, and addressing the unmet medical needs of a burgeoning populace [12]. Broad classification of stem cell is illustrated by figure 1.

(ii) Biomaterials in Regenerative Medicine

Biomaterials have a significant function in the practice of regenerative medicine as they can be used as a scaffold for cell proliferation, act as carriers of bioactive molecules and modulate the microenvironment for tissue regeneration. In this subsection, the focus will be on the design principles, properties and propulsion of application of natural and synthetic materials as biomaterials, scaffolding and their functionalization strategies in regenerative medicine [20].

Biomaterials are defined as substances that may serve as a therapeutic or supportive medium in the bio-system, including structural and molecular signaling. They can be classified based on their origin (natural or synthetic), structure (polymeric, metallic, ceramic) and activity (structural, functional, bioactive). Such biomaterials intended to use in regenerative medicine should have certain features and properties like biocompatibility, biodegradability, mechanical strength and ability to provide anchored for cells, aid in their division and promote their growth and maturation [21].

Different types of biomaterials are summarized in table 2 and figure 2.

Collagen

It's a well-known tissue engineering biomaterial that has a structural function in the extracellular matrix of different tissues.

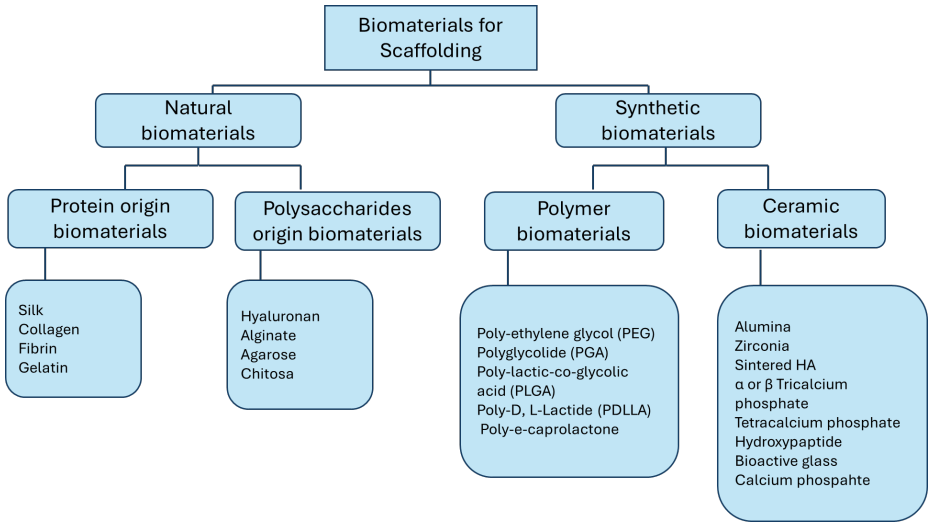

Figure 2 – Classification of biomaterials used in bioactive scaffold

Several types of collagen, including natural ones such as animal skin or tendons, possess the characteristics of biocompatible and bioactive, which makes collagen an ideal scaffold material for tissue regeneration tissues [22]. The mechanical stability is provided by its triple helical structure, allowing cells to adhere, proliferate and differentiate. Collagen-based biomaterials are widely used in wound healing, skin tissue engineering and cartilage repair treatments [23]. Colloquial terms incorporate the ability of collagen to modify properties during which its structure is changed, as its crosslinking for enhanced stability, or bioactive molecules are incorporated for selectively targeted effects. The variability and compatibility of collagen with cells prove its basic place in the development of regenerative medicine [24].

Poly(lactic-co-glycolic acid) (PLGA)

PLGA is a man-made polymer commonly used in tissue engineering because of its ability to biodegrade, adjustable mechanical characteristics, and superior processability. PLGA, made up of lactic acid and glycolic acid monomers, can be customized to break down at precise rates that align with tissue regeneration timelines. PLGA scaffolds facilitate cell adhesion and growth, proving especially beneficial in drug delivery mechanisms and tissue regeneration scenarios like the repair of bone and cartilage [25]. In addition to this, PLGA's compatibility with biological systems and capability to regulate drug release rates position it as a favorable option for targeted therapeutic delivery, improving treatment effectiveness while reducing side effects [26].

Polyethylene glycol (PEG)

It is a flexible synthetic polymer commonly utilized in biomedical fields because of its biocompatibility, hydrophilicity, and ability to resist protein adsorption. In tissue engineering, PEG hydrogels are utilized as injectable scaffolds for cell encapsulation, offering a three-dimensional setting that promotes cell growth and tissue development [46]. PEG's adjustable characteristics enable accurate regulation of gel rigidity, degradation speed, and bioactive molecule integration, rendering it appropriate for multiple tissue engineering applications such as cardiac tissue regeneration, neural tissue engineering, and drug delivery systems. In addition, PEG's inert properties reduce the risk of immunological reactions, making it an ideal biomaterial for therapeutic use [47].

Polycaprolactone (PCL)

It is a biodegradable polyester commonly utilized in tissue engineering due to its outstanding mechanical characteristics, biocompatibility, and gradual degradation rate. PCL scaffolds offer durable structural support for tissue regeneration and are especially important in uses that need ongoing mechanical stability, including bone tissue engineering and vascular grafts [48]. Its gradual degradation kinetics facilitate the slow replacement by newly developed tissue, enhancing integration and functional recovery [49]. Its adaptability allows for the creation of multiple forms, such as fibers, meshes, and porous scaffolds, supporting customized strategies for tissue regeneration and organ restoration [50].

Hyaluronic Acid (HA)

Hyaluronic acid belongs to the class of polysaccharides and is found within connective tissues, the synovial fluid and the extracellular matrix. It is known for its biocompatibility, viscoelasticity and its water retention ability. HA, due to its several applications, is being increasingly used in tissue engineering and regenerative medicine. HA-based hydrogels are synergistic scaffolds useful in the encapsulation of cells and the delivery of bioactive agents due to their high-water content and tunable mechanical properties [51]. Furthermore, HA in collaboration with cell surface receptor(s), enables the process of cell adhesion, migration and differentiation, which is useful in wound healing, cartilage regeneration and tissue augmentation. Moreover, the incorporation of several functional groups into the HA backbone may enhance the properties of HA-based biomaterials, caused by crosslinking for improved stability or conjugation with growth factors for tissue regeneration [52].

Alginate

It is a polysaccharide that occurs naturally and is derived from brown algae. It is famous for its compatibility with biological systems, gel-forming abilities, and simple gelation when divalent

Table 2 Different types of biomaterials and their applications

Biomaterial Type	Applications	Reference
Collagen	Skin grafts, bone grafts, cartilage repair	[27]
Chitosan	Wound dressings, drug delivery, nerve regeneration	[28]
Fibrin	Tissue adhesives, wound healing, cell encapsulation	[29]
Polyethylene Glycol (PEG)	Hydrogels for cell encapsulation, drug delivery systems	[30]
Polyurethane	Soft tissue repair, wound dressings, tissue scaffolds	[31]
Cellulose	Wound dressings, drug delivery, tissue scaffolds	[32]
Starch	Drug delivery, tissue engineering scaffolds	[33]
Dextran	Drug delivery, tissue scaffolds, wound healing	[34]
Chondroitin Sulfate	Cartilage repair, drug delivery, tissue scaffolds	[35]
Polyhydroxyalkanoates (PHA)	Biodegradable plastics, tissue scaffolds, drug delivery	[36]
Agarose	Cell encapsulation, tissue engineering scaffolds, drug delivery	[37]
Elastin	Vascular grafts, wound healing, tissue scaffolds	[27]
Poly(lactic-co-glycolic acid) (PLGA)	Drug delivery, tissue scaffolds, biodegradable sutures	[38]
Polyvinyl Alcohol (PVA)	Hydrogels for wound dressings, drug delivery	[39]
Silk Fibroin	Tissue scaffolds, bone regeneration, drug delivery	[40]
Gelatin	Coating capsules for delivering drugs orally and developing a substance to stop bleeding	[41]
Poly(ortho ester) (POE)	Drug delivery, and stents	[42]
Hyaluronic acid	Utilizing dressings for wound treatment, delivering drugs, advancing tissue engineering, creating artificial bone grafts, and developing substitutes for synovial fluid	[43]
Polydioxanone (PDS)	Fixing fractures in bones that don't bear weight, stitching materials, and clips for closing wounds	[44]
Poly(propylene fumarate) (PPF)	Orthopaedic applications	[45]

cations like calcium are present [53]. Alginate hydrogels are commonly employed as scaffolds for tissue engineering and cell encapsulation because of their gentle gelation conditions and capacity to maintain cell viability and function [54]. The porous nature of alginate facilitates the exchange of nutrients and waste, rendering it appropriate for uses like pancreatic islet transplantation, wound coverings, and cartilage regeneration [55]. It can be altered to improve its mechanical characteristics and cell adhesion, thereby increasing its applications in tissue engineering and regenerative medicine [56].

Silk Fibroin

The main source of silk fibroin is the cocoons of silkworms. It is a natural protein known for its exceptional mechanical characteristics, compatibility with biological systems, and capacity for biodegradation [57]. Biomaterials derived from silk have attracted interest in tissue engineering because of their adaptability and capacity to enhance cell adhesion, growth, and differentiation. Silk fibroin scaffolds can be produced in different configurations, such as films, fibers, and porous structures, ideal for uses like skin tissue engineering, nerve healing, and bone restoration [58]. Aside from this, the regulated degradation rates of silk fibroin and its low inflammatory response render it a promising option for long-term implants and managed drug delivery systems, aiding progress in regenerative medicine and biomedical engineering [59].

Calcium Phosphate Ceramics

Widely used inorganic biomaterials in bone tissue engineering and dental applications is calcium phosphate ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP). Due to their similar mineral composition to natural bone, calcium phosphate ceramics are also extensively used in clinical applications, exhibiting superior biocompatibility, osteoconductivity and bioactivity with excellent potential for bone regeneration and integration [60]. Such biomaterials can be processed into porous scaffolds or coatings to support the structure and facilitate osteogenic differentiation of progenitor cells. They can be used in bone graft substitutes, dental implants and orthopedic implants where osseointegration takes place restoring the skeletal function [61]. It can be paired up with the organic polymers or biological growth factors themself, thus having its tailoring in the scope of plasticity enhancement to foster a bio–mechanical niche, breaking through boundaries for bone tissue engineering and regenerative medicine [62].

3. Tissue Engineering Strategies

Tissue engineering combines principles of engineering and life sciences to make practical alternatives for injured, diseased or amputated tissues or organs [63]. In this section, we explore various tissue engineering strategies such as cell-based approaches, acellular scaffolds, and organ-on-a-chip technologies, that might be helpful for tissue regeneration. We also discuss the recent advancements in the field of tissue engineering, various challenges and future directions as well for enhancing in vivo tissue-specific functionality and integration. To develop a biomimetic scaffold for the structural fulfillment of the organ, with the desired mechanical, and biological properties, is the main objective of tissue engineering [64]. The "regenerative triad," (Figure 3) is the central model for tissue regeneration, which consist of scaffolds, cells, and signaling molecules. All the components act synergistically to promote the development and growth of tissue for successful regeneration. It is broadly classified into two categories the one is cell based and scaffold-based approach is the second. Among both categories, each has unique advantages and challenges as well [65]. In the cell-based approaches, in vitro culture and manipulation of cells are involved to generate the functional tissues for transplantation. For the growth of scaffolds and tissue constructs, various cell sources including stem cells, progenitor cells, and differentiated cells are used alone or in combination. Cell-based approaches offer the advantage of cellular diversity and functionality, enabling the recreation of complex tissue architectures and functions [66]. Cell-based tissue engineering faces major key challenges in finding a suitable source for the expansion and differentiation of cells for tissue regeneration. Mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) show an accessible and versatile characteristic for tissue regeneration applications [67]. Although, some issues like heterogeneity, immunogenicity,

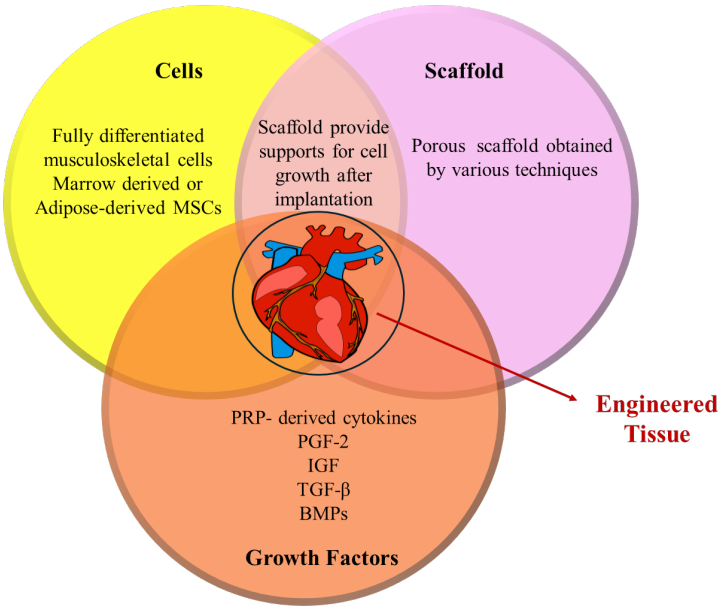

Figure 3 – Tissue engineering triad showing essential components for tissue regeneration

and tumorigenicity must be addressed to ensure the safety and efficacy of stem cell-based therapies [68].

On the other hand, the design and fabrication of biomaterial scaffolds are parts of Scaffold-based tissue engineering. Scaffolds provide the support for the attachment, proliferation and differentiation of the cells [64]. There are variety of techniques available, such as 3D printing, electrospinning, and microfluidics to fabricate the bioactive scaffold of complex architectures with controlled porosity, mechanical properties, and degradation kinetics. There are various advantages offered by scaffold-based approaches like unable properties, scalability, and adaptability to various tissue types and clinical applications [69].

Tissue engineering success is dependent on the cellular integration, scaffold, and signaling molecules create functional tissue constructs with appropriate biomechanical and biochemical properties [70].

Advances in biomaterial design, stem cell biology, and tissue engineering techniques have facilitated the construction of tissue-specific constructs for various biomedical applications like bone tissue regeneration, cartilage repair, and cardiac tissue engineering [71]. Although, various challenges are also there that cannot be ignored they include vascularization, innervation, and immune responses [72].

Organ-on-a-chip technologies offer a promising method for mimicking tissue and organ functions in vitro, applicable in drug screening, disease modeling, and regenerative medicine [73]. These microfluidic systems include living cells, biomaterial frameworks, and microengineering methods to replicate the physiological microenvironment of tissues and organs in a consistent and controllable way. Organ-on-a-chip systems provide benefits like high-throughput screening, real-time observation, and possibilities for personalized medicine uses [74].

Other recent methods being used in tissue engineering studies involve biofabrication techniques, including bioprinting and organoid culture, to develop intricate tissue structures with spatial arrangement and cellular diversity [75]. Progress in biomaterials, stem cell technology, and microfabrication methods shows potential for creating functional tissue replacements for clinical application. Utilizing interdisciplinary methods and cutting-edge technologies, tissue engineering progresses toward the aim of regenerating tissues and organs for therapeutic use.

4. Gene Therapy and Genetic Engineering in Tissue Repair

Gene therapy and genetic engineering in tissue regeneration present hopeful approaches for addressing genetic disorders, facilitating tissue restoration, and improving regenerative mechanisms [76]. This part outlines methods for gene delivery, gene editing technologies, and the uses of gene therapy in regenerative medicine. We examine recent developments, obstacles, and ethical issues related to gene-oriented methods for tissue restoration and regeneration.

The main objective of gene therapy is to modulate gene expression, genetic defect correction or therapeutic gene introduction for therapeutic purposes [77]. The delivery of genes can be achieved via two different techniques, that is viral and non-viral vectors, with unique advantages and limitations of each. Viral vectors include adenovirus, adeno-associated virus (AAV), and lentivirus, which are efficient in gene transfer and long-term transgene expression, although they may elicit immune responses and pose safety concerns. On the other hand, non-viral vectors, such as lipid nanoparticles, polymer-based carriers, and naked DNA, offer advantages like safety, versatility, and ease of manufacturing, but often exhibit lower transfection efficiency and transient gene expression [78].

Accurate alteration to the genome can be facilitated by various gene editing approaches. These approaches include CRISPR-Cas9, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs). They act by creating specific DNA double-strand breaks (DSBs) and aiding in gene correction, insertion, deletion, or replacement. Due to having good effectiveness, ease of use and adaptability, CRISPR-Cas9 has a vital role in the area of genetic engineering [79]. CRISPR-based approaches hold promise for treating genetic diseases, engineering cells for regenerative medicine applications, and modulating gene expression to enhance tissue repair and regeneration [80].

Along the newly found and paved roads of regenerative gene therapy applications, one can find the treatment of monogenic diseases, or cardiovascular, neurological, and musculoskeletal pathologies. Among them are gene therapies, such as gene therapy for cystic fibrosis and x-linked disorders, which utilize gene editing and gene therapy to fix mutations in the cells harvested from the patient, or to enhance the healing processes in tissue [81]. This gene therapy strategy offers a number of options, including targeting stem cells, somatic or immune cells, thus allowing for personalized medicine [77].

Restorative medicine, in turn, is based on the use of gene therapy, which allows for establishing new relationships in healthy organisms, however, such technology also has limitations and problems. The first group of factors affecting the use of gene therapy approaches can be classified as safety issues like unwanted off-target effects, immunogenicity or insertional mutagenesis [82]. The second group of factors can be described in regard to technical aspects like the issues of delivery efficacy with frequency, its broad application, and cost-effective aspects, and more. Other risk mitigation measures include safeguarding the preventative and ethical issues, such as preparing consent, addressing privacy concerns, and affording equity in implementation practices: all such factors intertwine in a comprehensive fabric to construct a unified approach to the application of gene-targeted therapy [81].

5. Small Molecules and Biologics

It consist of growth factors, cytokines, and signaling molecules whose collective functions are important in the regulation of cells and tissue reconstruction [83]. This section focuses on the use of small molecules and biologics as repair agents for the damaged tissue, to alter immune reactions, and accelerate regeneration processes. We present innovations in the delivery of small and large biologics as well as their engineering and clinical applications in regenerative medicine.

Small molecules are organic substances with a low molecular weight (less than 900 Daltons), which can control cellular signaling pathways and biochemical events. Small molecules act through binding their specific targets, such as receptors, enzymes, or ion channels and modifying their functions or expression levels [84]. In this regard, small molecules have a great deal of different ways of action which include stimulation of cell proliferation and differentiation, and migration, and enhancing cell survival as well as regulating immune and inflammatory responses [85].

On the other hand, biologics are large molecules obtained from living organisms. The molecules include proteins, peptides, nucleic acids and antibodies. Their therapeutic effects is produced by interacting with specific molecular targets [86]. Biologics are produced using recombinant DNA technology, cell culture systems, or transgenic organisms. They have high specificity, potency, and selectivity for their targets [87]. Biologics encompasses a wide range of therapeutic modalities, including growth factors, cytokines, monoclonal antibodies, and nucleic acid-based therapeutics [88].

A thorough study of small molecules and biologics supports the utilization for promoting tissue repair and regeneration of tissues [89]. There are various examples of growth factors, including bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and vascular endothelial growth factor (VEGF), which are extensively used for angiogenesis, osteogenesis and wound healing stimulation. Various cytokines like interleukins (ILs) and tumor necrosis factor (TNF) inhibitors have been used to modulate immune responses and inflammation in autoimmune diseases and tissue injuries [90]. In addition to their direct effects on cellular behavior and tissue regeneration, small molecules and biologics can also be used as carriers or vehicles for delivering therapeutic payloads to target tissues or cells. Drug delivery systems, such as liposomes, nanoparticles, and hydrogels, can encapsulate small molecules or biologics and facilitate their controlled release at the site of injury or disease. Moreover, bioengineering approaches, such as scaffold functionalization and surface modification, can enhance the retention, stability, and bioactivity of small molecules and biologics for prolonged therapeutic effects [91].

Although they hold therapeutic promise, small molecules and biologics encounter issues like restricted stability, inadequate pharmacokinetics, and off-target effects, which may undermine their effectiveness and safety. Approaches to tackle these issues consist of optimizing formulations, designing drug delivery systems, and employing targeted delivery methods to enhance tissue specificity and reduce systemic toxicity [92]. Progress in high-throughput screening, combinatorial chemistry, and computational modeling shows potential for identifying new small molecules and biologics that exhibit improved therapeutic effects and fewer side effects [93].

6. Cutting-edge Technologies Driving Regenerative Medicine Advancements

Recent technological progress has transformed regenerative medicine by allowing accurate management of cellular behavior, tissue engineering, and gene editing [115]. This part emphasizes innovative technologies, including 3D bioprinting, CRISPR/Cas9 gene editing, and single-cell omics, propelling progress in regenerative medicine. We examine their uses, constraints, and possibilities for converting laboratory findings into clinical treatments.

6.1. 3D bioprinting: It is an innovative technology that facilitates the accurate placement of living cells, biomaterials, and bioactive molecules to fabricate three-dimensional tissue structures featuring intricate designs and functions [116]. 3D bioprinters employ computer-aided design (CAD) tools to create tissue scaffolds and manage the arrangement of cells and biomaterials in layers. By replicating the structural and functional intricacies of natural tissues, 3D bioprinted structures present potential uses in tissue engineering, organ transplant, and drug testing [117].

Table 3 Different types of growth factors and their applications

Type	Applications	Reference
Bone Morphogenetic Proteins (BMPs)	Bone regeneration, cartilage repair	[94]
Transforming Growth Factor-beta (TGF-β)	Cartilage repair, fibrosis treatment	[95]
Vascular Endothelial Growth Factor (VEGF)	Angiogenesis, tissue regeneration	[96]
Platelet-Derived Growth Factor (PDGF)	Wound healing, angiogenesis	[97]
Fibroblast Growth Factor (FGF)	Wound healing, tissue regeneration	[98]
Insulin-like Growth Factor (IGF)	Bone regeneration, wound healing	[99]
Hepatocyte Growth Factor (HGF)	Liver regeneration, wound healing	[100]
Interleukin-1 Receptor Antagonist (IL-1Ra)	Reduces inflammation in tissue engineering applications	[101]
Sonic Hedgehog (Shh)	Neurogenesis, tissue patterning	[102]
Retinoic Acid	Neurogenesis, bone regeneration	[103]
Thrombin	Wound healing, tissue sealing	[104]
Angiopoietin	Angiogenesis, vascular tissue engineering	[105]
Stromal-Derived Factor-1 (SDF-1)	Cell migration, tissue repair	[106]
Prostaglandin E2 (PGE2)	Bone regeneration, anti-inflammatory effects	[107]
Hydroxyurea	Induces erythropoiesis, tissue engineering applications	[108]
Nicotinamide	Neurogenesis, cell proliferation	[109]
Tacrolimus (FK506)	Immunosuppression in transplantation, tissue engineering	[110]
Cyclosporin A	Immunosuppression in transplantation, tissue engineering	[111]
Erythropoietin (EPO)	Erythropoiesis, wound healing	[43]
Granulocyte-Colony Stimulating Factor (G-CSF)	Stimulates hematopoiesis, wound healing	[112]
Interferon-gamma (IFN-γ)	Modulates immune response, tissue regeneration	[113]
Interleukin-4 (IL-4)	Anti-inflammatory, promotes tissue repair	[114]
Tumor Necrosis Factor-alpha (TNF-α)	Pro-inflammatory, tissue remodeling	[99]

6.2. CRISPR/Cas9 gene editing: It has surfaced as an effective instrument for accurate genome alteration, allowing focused modification of DNA sequences to fix mutations, control gene expression, and adjust cellular activities. CRISPR-based methods provide benefits like ease of use, effectiveness, and the ability to edit multiple genomic sites at the same time. CRISPR/Cas9 has been utilized for gene knockout, knock-in, and base editing across various cell types and organisms, creating new opportunities for addressing

genetic disorders and designing cells for regenerative medicine uses [118].

6.3. Single-cell omics technologies: These techniques, such as single-cell RNA sequencing (scRNA-seq) and single-cell proteomics, enable the characterization and profiling of individual cells at the molecular level, providing insights into cellular heterogeneity, lineage dynamics, and functional states. Single-cell omics approaches have revolutionized our understanding of cell types, cell-cell interactions, and developmental processes in health and disease [119]. By dissecting the cellular and molecular mechanisms underlying tissue regeneration and repair, single-cell omics technologies hold promise for identifying therapeutic targets and biomarkers for regenerative medicine [120].

Other cutting-edge technologies driving advancements in regenerative medicine include organoid culture, microfluidics, and tissue-on-a-chip platforms for modeling complex tissues and organs in vitro [121]. Organoids are tissue-derived miniaturized 3D organ models that have potential in disease modeling, drug screening and regenerative medicine. Microfluidic devices provide the ability to directly manipulate microenvironments surrounding cells, making it possible to investigate cell behaviors, tissue morphogenesis as well as drug responses in vitro [122]. Tissue-on-a-chip platforms co-cultured several cell types in multiple microenvironments to replicate the physiological functions of tissue and disease states, allowing high-throughput screening of drugs and therapeutics [123].

Although highly innovative with the potential for revolutionizing regenerative medicine, various modern technologies remain limited and face major issues of scalability, reproducibility and clinical translation [121]. Standardization of protocols, validation of assays and optimization of culture conditions are necessary steps for the accurate reproduction and robustness of experimental results. Controlling these variables reduces variability and increases the validity of the results. In addition, regulatory consideration, ethical issues and acceptance by the society need to be addressed in order for laboratory findings to ultimately advance into the practice (clinical application or commercial product). Such things help maintain legal compliance, moral responsibilities and public trust respectively to facilitate the transition of scientific innovations from the lab-bench to SC.

7. Clinical Translation

Family caregivers can be viewed as a disadvantage of treatment due to the differences in recent development versus future clinical promises, such as safety, regulation, and scale up; all discourage successful translation of regenerative medicine therapies from bench sid to bedside. We also evaluate important benchmarks in clinical applications of regenerative medicine, including licensure of stem cell therapies for hematological neoplasms and successful implementation of tissue-engineered skin grafts for burn injuries, followed by emerging clinical trials and the challenge of broad clinical translation.

Translating regenerative medicine therapies to the clinic represents a critical milestone and is necessary for realizing their potential to benefit patient populations and meet unmet medical needs. Stem cell therapy for hematologic disorders (for example, hemopoietic stem cell transplantation [HSCT] for leukaemia and lymphoma) and tissue-engineered products for wound healing (for example, dermal substitutes for burn injuries and diabetic ulcers) have been accepted as successful clinical applications of regenerative medicine. [124]. These therapies are promising for the treatment of numerous diseases and injuries as they have proven effective in clinical trials, and are approved for use by health professionals [125].

However, despite these success stories, major challenges remain to the application of regenerative medicine therapies in clinical practice, including safety issues, regulation and reimbursement. Safety issues like tumorigenicity, immunogenicity, and off-target effects may be harmful to the patient and should be cautiously investigated during preclinical and clinical studies [126]. The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), among others, support both existing products as well as facilitate the approval of new regenerative medicine by evaluating their safety, efficacy, and quality with regulatory requirements for marketing approval [127].

The complexity, variability and novel mechanism of action (MoA) presented by this class of therapies pose particular regulatory challenges for regenerative medicine. Unlike traditional drug or device approvals, regulatory pathways for regenerative medicine products can be more nuanced and require novel approaches to addressing safety and efficacy [128]. Market Access and Commercialization, driven by reluctance of use reimbursement policy and healthcare economics as they pertain to the adoption and utilization of regenerative medicine therapies [129].

8. Regulatory Landscape and Ethical Considerations in Regenerative Medicine

Regulatory frameworks and ethical considerations are critical components of the development, governance, and implementation of regenerative medicine therapies [130]. This chapter presents an overview of regulatory agencies, guidelines and policies regulating regenerative medicine products development, manufacture and clinical application. We highlight examples of ethical challenges such as informed consent, patient safety and equitable access to therapies before proposing approaches for all regenerative medicine research and practice that is in need of solutions.

The development and ensuing approval of such regenerative medicine products for clinical use is governed by the regulations of various authorities, including but not limited to the US Food and Drug Administration (FDA), European Medicines Agency (EMA) and Pharmaceuticals and Medical Devices Agency (PMDA) Japan [131]. This varies from the expected uses or characteristics of the product or the extent of risk associated with the product. In the US, regenerative medicine products are regulated under the Federal Food, Drug, and Cosmetic Act (FD&C Act) and may use different regulatory pathways, including Investigational New Drug (IND) application, Biologics License Application (BLA) or Device Premarket Approval(PMA) [132].

Within the European Union (EU), the medicinal products and devices have been defined within legislation regulating advanced therapy medicinal products (ATMPs), including regenerative medicine products. ATMPs consist of gene therapy, cell therapy, as well as tissue-engineered products and are to be approved by the EMA. The united Kingdom regulatory frameworks for advanced medicinal products are provided by the European Medicines Agency for the CAT, which assesses any given ATMP applications as well as provides scientific opinions and probably approves regulatory

agencies' recommendations for promoting ATMP regulatory events [131].

Regulatory authorities require evidence of safety, efficacy and quality following the appropriate clinical trials and preclinical studies of lots of potential regenerative medicine before the issuance of standards for commercial lots. Such preclinical studies investigate biological, pharmacokinetic and toxicologic properties of the product in animals. Clinical trials, on the other hand, investigate the safety and efficacy of the product in people. Additional risk management plans, post-marketing surveillance, and pharmacovigilance activities may also be required to ensure the safety and effectiveness of regenerative medicine products over time [133].

Several ethical aspects that need to be observed in the understanding of regenerative medicine regard respect for persons (informed choice), patient and research subject autonomy, beneficence, nonmaleficence, and justice [133]. Informed consent implies giving patients adequate information concerning risks, benefits and available alternatives to taking part in research or receiving treatment to make a choice about their health care. Patient autonomy means in this context that individuals should be able to decide how their bodies and health care, including the use of unapproved therapies or participation in clinical trials, should be administered [132].

Healthcare providers and researchers must act in the best interest of patients, making sure to do them no harm (i.e. benefit and nonmaleficence). This also consists of duty to patient safety, handling privacy and confidentiality, and avoiding conflict of interest. Justice involves equal access to healthcare resources and opportunities [134]. Challenges in access to investigational therapies, allocation of scarce resources for repair efforts and the conflict between individual and societal interests may give rise to ethical dilemmas in regenerative medicine research and practice [135]. Ethical considerations in regenerative medicine research and practice can be dealt with through transparent communication, multidisciplinary collaboration among scientists and ethicists, and adherence to certain ethical principles and guidelines. The development of ethical review boards, institutional review boards (IRBs), and ethics committees has been a critical step in evaluating the broader implications of research protocols beyond compliance with regulatory and ethical standards [136]. Beyond this, public engagement, stakeholder involvement and other community outreach efforts are also integral to trust, transparency and accountability in regenerative medicine research and practice [137].

Conclusion

Regenerative medicine is an exciting area of research that seeks to harness the body's own healing mechanisms in order to grow or replace tissue that has been damaged. And this strategic one holds the promise to realize the maximum benefit for patients, fill the gap of missing clinical needs, and reverse the course of interventions. Due to the personalized concept according to the genetic, molecular, and clinical profile of individual patients, regenerative therapies offer the possibility of treatments to be more effective in therapy and less toxic in treatment. They are directed therapeutics and targeted at specific molecular pathways, and they can modulate tissue repair and regeneration better.

The global population is ageing and as a result diseases like osteoarthritis, cardiovascular disease and neurodegenerative diseases are becoming more prevalent. Regenerative medicine offers new potentials not only for treating symptoms, but for repairing lost function and improving quality of life for those with age-related diseases. The faculty to reconstitute complex tissues and diseases taught more about disease processes and regeneration. In recent years, the regenerative landscape is being transformed, including stem cell therapy, imaging and gene editing, and 3D bioprinting, by the translation of interdisciplinary research by biologists, engineers, and data scientists.

Changing public attitudes toward health care such as increased health awareness, patient-focused care delivery, and demand for less invasive alternatives are driving the faster adoption and implementation of regenerative therapies into mainstream medical acceptance. But what is a fast-developing field also raises what are often referred to as "broader" questions of ethical and social issues that demand careful attention. But, regenerative medicine is still a major powerhouse industry that has the potential to seriously impact health and wellness across the globe. Now, with over 80 Billion adult stem cells circulating in your body, and combining that with the 21st century technology, we know as regenerative medicine, we are giving hope to a lot of people, who in their minds, had no choices and therefore no hope.

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Primary Biliary Cholangitis with Associated Raynaud Syndrome Treatment Outcome: a Case Report

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Abstract

Primary biliary cholangitis is an autoimmune liver disease, which is less common than viral hepatitis and fatty liver disease, but in recent decades there has been a steady increase in the PBC occurrence. The development of severe complications, many concomitant pathologies, and frequent lack of response to basic ursodeoxycholic acid (UDCA) therapy requires timely dynamic monitoring of patients with PBC for effective treatment correction.

Here we describe a case presentation of liver cirrhosis development in patient with PBC combined with Raynaud syndrome. Upon disease development, the patient refused to undergo liver biopsy. She was treated with non-conventional treatment regime. Our clinical case showed that the use of budesonide in PBC treatment did not show any promising result and led to rapid liver cirrhosis development.

Keywords: autoimmune hepatitis; liver cirrhosis; primary biliary cholangitis.

Introduction

Primary biliary cholangitis (PBC) is a chronic autoimmune cholestatic disease, predominantly occurring in middle-aged female population. PBC was first reported by Addison and Gull and named as primary biliary cirrhosis [1]. Considering recent research data and understanding of the underlying pathogenetic mechanisms of the disease, EASL (2014) and AASLD (2015) accepted the new definition of Primary biliary cirrhosis as primary biliary cholangitis . Incidence of PBC increases with age with the peak in the individuals between 60 and 79 years [2]. According to the latest systematic review, the pooled global incidence and prevalence of PBC were 1.76 and 14.60 per 100 000 persons, respectively [3]. Unlike majority of autoimmune diseases, PBC do not occur in pediatric patients [4]. The main pathogenetic mechanisms of the PBC are environmental factors, hereditary genetic predisposition and loss of tolerance [5]. Diagnosis of the disease at a young age (less than 45 years) and male gender are predictors of poorer prognosis [6]. The disease progression to biliary cirrhosis varies according to liver biochemistry and histology. PBC progresses for 1 histological stage every 1,5-2 year, almost half of patients with PBC develop cirrhosis after 4 years [7]. Average survival rate of patients with PBC is 7.5 years in symptomatic patients and 16 years in asymptomatic patients [8]. According to Tanaka A. et al., in Japanese population approximately 70–80% of patients are asymptomatic [9] . Patients with PBC are at higher risk of HCC development. The cumulative five-year incidence of decompensation, hepatocellular carcinoma, liver transplantation or death in patients with PBC was 6.95% (2.07–11.83%), 1.54% (0.9–2.19%), and 4.02 % (2.49–5.54%), respectively [10]. Most studies in recent years have reported a significant increase in the incidence and prevalence of PBC. According to Shimoda S et al., the increase in the number of patients may be associated with increased exposure to a currently unknown etiological environmental agent or demographic changes, increase in the number of elderly people at a risk. It is obvious that the increase in the prevalence of PBC most likely due to increased life expectancy and improved care and earlier diagnosis of the disease [11]. The coexistence of either antimitochondrial antibody (AMA) or antinuclear antibodies (ANA) with elevated liver functional tests

(LFTs) are considered as specific diagnostic hallmark of PBC [12-14]. Implementation of ANA, AMA screening in diagnostic algorithm significantly improved early detection of PBC.UDCA as the first-line treatment option revolutionized the management of PBC and inhibited the progress of the disease to cirrhotic stage in up to 60% of patients [15]. PBC is an autoimmune liver disease, which is less common than viral hepatitis and fatty liver disease, but in recent decades there has been a steady increase in the PBC occurrence [16]. The development of severe complications, many concomitant pathologies, and frequent lack of response to basic ursodeoxycholic acid (UDCA) therapy requires timely dynamic monitoring of patients with PBC for effective treatment correction. Here we report a case presentation of liver cirrhosis development in patient with PBC in combination with other autoimmune disease.

Case presentation

A36-year-old Asian female was referred to our hospital with complaints of pruritus, dry skin, insomnia, weight loss and severe fatigue in November 2022. The intensity of pruritus worsened in cold temperature. On physical examination, the patient had nutritional deficiency, body mass index- 20 kg/m 2 with generalized hyperpigmentation, pale conjunctivae with icteric sclera, dry mucosal membrane, mild epigastric abdominal tenderness, firm liver without hepatosplenomegaly. The results of her LFTs were as follows: alkaline phosphatase 15,44 μkkat/l (normal range- 0,58-1,75), gamma glutamyl transpeptidase 7,79 μkkat/l normal range 0-0,67), alanine aminotransferase (ALT),26 μkkat/l (normal range 0,0-0,52), aspartate aminotransferase (AST) 1,60 μkkat/l (normal range 0,0-0,53),total bilirubin 122,29 μmol/L and albumin 36 g/L.

Her past medical history stated that clinical manifestation of the disease started in 2019 with complaints of pruritus. Abovementioned complaints developed during pregnancy, no treatment was prescribed until December 2020, when abdominal pain and insomnia occurred. In December 2020 ALT and AST were > 2 times normal limit, GGTP and ALP > 6 times normal limit, total bilirubin- 68,5 μmol/L. The patient was prescribed with UDCA 1000 mg per day and referred for liver biopsy. However, the patient rejected the liver biopsy procedure.

In April 2021 LFT moderately decreased (total bilirubin-32 μmol/L), however GGTP, ALP, AST and ALT remained at the same level. The clinical manifestation was treated with UDCA without any specific tests to reach diagnostic conclusion. Eventually, in April 2021 the patient underwent liver biopsy and autoantibody testing. Antibody testing showed positive antibodies to AMA-M2, gp210, sp100 and F-actin. Immunoglobulin G level was 11.66 g/l (normal range 7.00-16.00).

Liver biopsy showed microscopic description of chronic destructive cholangitis with moderate fibrosis. Pathomorphological signs of liver cirrhosis were not detected. PBC was diagnosed on the basis of serological and morphological criteria.

The patient was recommended to continue UDCA (1000 mg/day) with budenoside (9 mg/day) added to the treatment. 15 month follow up showed poor response. In august 2022, laboratory testing showed increase in total bilirubin to 434 μmol/l, direct bilirubin to 113 μmol/l, AST up to 5 times. The Patient added to the treatment supplements, which were not improved or listed in any guideline.

The patient was admitted to our hospital with complaints of pruritus, dry skin, insomnia, weight loss and severe fatigue in November 2022. The results of her LFTs were as follows: alkaline phosphatase 15,44 μkkat/l (normal range- 0,58-1,75), gamma glutamyl transpeptidase 7,79 μkkat/l (normal range 0-0,67), alanine aminotransferase1,26 μkkat/l (normal range 0,0-0,52), aspartate aminotransferase 1,60 μkkat/l (normal range 0,0-0,53), total bilirubin 122,29 μmol/L and albumin 36 g/L.

Considering her history and physical examination findings, a connective tissue disorder diagnostic panel was performed. Autoantibody testing was repeated and showed positive ANF (anti-smooth muscle antibody) (at the level 1:320) and antibodies to CENP-A/B.AMA, IgM were also found to be elevated. However, ANA and immunoglobulin G4 were within normal range.

Skin and muscle biopsy from the forearm revealed scleroderma in the sclerotic stage and minimal muscle atrophy. MRCP showed mild dilatation of intrahepatic ducts.

The patient was diagnosed with primary biliary cholangitis and coexistent limited cutaneous systemic sclerosis (CREST syndrome), also known as Raynaud syndrome.

Liver biopsy was repeated in January 2023.Microscopic picture showed portal tract lymphocytic infiltration and loss of medium sized interlobular bile ducts. Hepatocytes with feathery degeneration, with intracellular cholestasis, and extracellular cholestasis located closer to the foci of fibrosis, with the formation of preductules. Masson-Trichrome staining showed focal periportal proliferation of connective tissue, in areas of capillarization of sinusoids, connective fibrous tissue is stained bright blue. Follow up period duration was 18 months.

During the observation period, while taking UDCA at a dose of 1000 mg/day + budesonide 9 mg/day, applying the Paris I criteria for 1.5 years, the patient did not respond to therapy. Considering abovementioned data, budesonide treatment was discontinued.

Taking into account resistant cholestasis, increase in total bilirubin up to 122 μmol/l, direct bilirubin to 118 μmol/l, decompensation of liver cirrhosis, the patient was referred to liver transplant center.

Discussion

Primary biliary cholangitis may ultimately progress to cirrhosis. The intensity of disease progression depends on plenty of factors, including treatment approaches and response to treatment [17].

The combination or transition of one autoimmune liver disease, characterized by immune-mediated injury to hepatocytes, has been described in the literature [18]. Differentiating between a true transition and the initial coexistence of multiple liver diseases is critical for determining the appropriate therapeutic approach. In the case described, the diagnosis of PBC was confirmed through histological examination. A liver biopsy revealed histological histological features characteristic of PBC, including the loss of medium-sized interlobular bile ducts, intracellular cholestasis and extracellular cholestasis localized near fibrotic areas, with the formation of preductules. Consequently, the combination of serological markers and histological findings allowed us to the exclude AIH as the component of overlap syndrome in this case.

Patients with PBC may be associated with various other autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome or CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly and telangiectasis) syndrome [19]. The association between PBC and CREST syndrome was first described in 1970 by Murray-Lyon et al. [20]. Since then, numerous cases have been reported; however, the prognosis of combined PBC and

CREST remains largely unknown. Anti-centromere antibody (ACA) is considered a serological marker of CREST syndrome [21]. In the case described, ACA was not analyzed. However a histological examination of skin tissue, which served as the basis for scleroderma diagnosis- as a component of CREST syndrome. Abe et al. published a study analyzing the long-term outcomes of patients with PBC alone versus those with a PBC-CREST combination. They found that liver transplantation-free survival was comparable between the two groups. Interestingly, the presence of CREST syndrome was identified as a protective factor against the development of cirrhosis [22].There is no consensus on the impact on patient survival when PBC is combined with other systemic diseases. Some authors have reported that overall patient survival is significantly lower with this combination than with PBC alone, while others have found no such difference [23,24].

Currently, ursodeoxycholic acid (UDCA) is the first-line therapy for patients with PBC. The European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) recommend oral UDCA 13-15 mg/kg per day [18].UDCA treatment demonstrated improved 5-, 10-, and 15-year transplant-free survival in treated patients (90%, 78%, and 66%, respectively) compared with untreated patients (70%, 59%, and 32%, respectively), and transplant-free survival was significantly higher in patients with moderate to severe disease. However, 40% patients of patients with PBC do not respond to the UDCA treatment [25].

Obeticholic Acid is accepted as the second-line treatment option, in patients who do not achieve adequate response to UDCA [26]. Nevertheless, its use requires careful patient selection. According to current FDA and EMA recommendations, OCA is contraindicated in patients with advanced liver cirrhosis (Child–Pugh B/C) and in those with complete biliary obstruction [31,32]. Postmarketing data have shown that treatment in patients with significant hyperbilirubinemia (>50 μmol/L) is associated with higher risk of decompensation and adverse outcomes [33,34]. In the POISE trial, the efficacy of OCA was demonstrated only in compensated cases of PBC []. Considering our patient's persistently elevated bilirubin levels above 50 μmol/L and signs of progressive fibrosis, OCA was not prescribed due to the high risk of hepatic decompensation. Instead, management was focused on symptomatic therapy, close monitoring, and referral for liver transplantation evaluation.

Budesonide is referred as a off-label treatment approach for patients with PBC. In some studies, the combination of UDCA and Budesonide showed encouraging results in patients with overlap syndrome (PBC-AIH) [27]. Budesonide is a synthetic corticosteroid that is extensively metabolized before entering the systemic circulation during its initial passage through the liver, and therefore has minimal side effects compared to prednisolone. With advanced liver damage, the metabolism of budesonide is reduced, which can lead to adverse consequences in cirrhosis and portal hypertension [28]. The basis for the use of budesonide was the association of increased levels of serum aminotransferases with "borderline" hepatitis and progression of PBC. However, increased levels of aminotransferases may also be a consequence of cholestatic damage to hepatocytes, which differs from parenchymal inflammation. Bile acids at nontoxic, nondetergent concentrations induce the expression and secretion of inflammatory mediators, while at high concentrations they induce apoptosis, and at very high concentrations even necrosis. From this point of view, immunosuppression may not have a beneficial effect [29]. According to an American open study in 22 patients with a persistent increase in ALP levels > 2 times the upper limit of normal that did not respond to UDCA, the beneficial effect of adding budesonide to therapy was minimal, the Mayo prognostic index significantly increased, and bone mineral density sharply decreased [30].

This case also underscores the importance of an individualized therapeutic strategy. Clinical outcomes in PBC, particularly when associated with other autoimmune conditions such as CREST syndrome, may vary widely. Therefore, conclusions drawn from a single case should be interpreted with caution, and treatment decisions should always be tailored to the individual patient's disease profile, comorbidities, and therapeutic response.

Conclusion

In summary, patients with PBC associated with other autoimmune diseases can develop faster progression to liver failure. Obeticholic acid was not used in this patient, therefore the classical treatment regimen was not followed in this clinical case. Our clinical case showed that the use of budesonide in PBC treatment did not show any promising result and was associated with progression to cirrhosis despite therapy.

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Transcervical Carotid Artery Stenting via Cut-down Access in Multifocal Atherosclerosis: Technical Note

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Abstract

Carotid atherosclerotic diseases are managed with carotid endarterectomy or carotid artery stenting, both associated with low risks of stroke and mortality during the procedure. However, in cases of severe carotid artery disease where the vessels are tortuous or the femoral or radial arteries are occluded, conventional access methods can be challenging. In such situations, direct puncture via the cut-down technique of the carotid artery offers an alternative approach for stenting severe stenosis.

Two patients (75 and 57 years old) presented with a right-sided hemiparesis and a history of transient ischemic attacks, strokes, or amaurosis fugax corresponding to a carotid stenosis of ≥50% and of ≥90%. Femoral angiography of both patients showed occlusion of the common and internal iliac arteries. The surgical procedure entailed a direct carotid artery puncture with a microsurgical dissection technique to expose the carotid artery within a neuroendovascular operating room under general anesthesia. All subsequent endovascular procedures were conducted through the sheath. Following the completion of the endovascular intervention, the sheath was removed, and closure of the puncture sites was directly achieved using either Prolene sutures or a purse-string suture technique. The patients had a smooth postoperative recovery devoid of neurological deficits.

Direct carotid artery puncture via the cut-down method for carotid artery stenting promises procedural safety, precision, and good clinical outcomes, despite potential cost increases and specialized personnel needs.

Keywords: multifocal atherosclerosis, carotid endarterectomy, carotid artery stenting, direct carotid puncture

Introduction

Multifocal atherosclerosis (MA) is a chronic, progressive systemic disease that affects major blood vessels across various anatomical regions, including the brain, heart, kidneys, and limbs. Its high prevalence highlights its importance as a major public health concern, particularly due to the substantial disability it causes among individuals of working age [1]. One of its primary consequences is ischemic stroke, most commonly occurring in the carotid artery territory and accounting for approximately 87% of all stroke cases [2].

In clinical practice, carotid atherosclerotic diseases have traditionally been addressed through two principal interventions: carotid endarterectomy (CEA) and carotid artery stenting (CAS). Comparative studies have shown no significant difference between the two in terms of stroke or mortality risk [3, 4]. Additionally, CAS has proven to be both safe and effective, particularly with the use of advanced technologies and embolic protection devices that help reduce the risk of microembolic events [5].

The standard approach for endovascular interventions typically involves access via the femoral or radial arteries [6,7]. However, despite technological advancements in catheters and guidewires, there are situations where a transfemoral or transradial approach is not feasible due to challenges such as anatomical nuances like vessel tortuosity or occlusion of the femoral/radial artery [8]. Additionally, variations in the

anatomy of the aortic arch and supra-aortic vessels, particularly in instances like the bovine arch, where the left common carotid artery (CCA) shares its origin or directly arises from the right brachiocephalic artery, further complicate the conventional access to carotid vessels [9].

Moreover, the complex manipulations of the catheter in the aortic arch provide an increased risk of transient ischemic attacks (TIAs) [10], which calls for an alternative method of accessing the carotid artery.

Direct carotid puncture (DAP) serves as an alternative approach for stenting severe carotid artery stenosis when conventional access to carotid vessels is impeded. This method offers a viable solution in complex scenarios where standard access techniques are rendered impracticable. However, achieving successful access through this approach necessitates the application of a nuanced combination of microsurgical and endovascular interventions.

Despite its potential utility, the application of DAP via a cut-down technique for stenting severe carotid artery stenosis remains relatively infrequent and less extensively documented in the existing literature. This study describes the technique and outcomes of direct CAS using a transcervical approach performed in a hybrid angiography suite.

Methods

2.1. Patient Selection and Preoperative Evaluation

The study entailed a retrospective analysis of patients who underwent CAS via DAP through a cut-down method within a hybrid angiography suite, spanning the period from 2018 to 2022 at the National Centre for Neurosurgery, Astana, Kazakhstan. The surgical procedures were performed by neurosurgeons possessing expertise in both endovascular interventions and microsurgical techniques.

The procedure involved symptomatic patients, with no specific exclusion criteria applied. Patients presented with a history of TIAs, strokes, or amaurosis fugax corresponding to a carotid stenosis of ≥50% and of ≥90% based on the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria. Before the intervention, patients underwent computed tomography angiography (CTA) imaging to evaluate the extent of the disease, supplemented by a neurological assessment conducted by an independent neurologist upon hospital admission. Written consent was obtained from each patient. Detailed patient demographics and indications for stenting are outlined in Table 1.

2.2. Surgical Technique

The surgical procedure entailed a direct dissection technique to expose the carotid artery within a neuroendovascular operating room under general anesthesia. It commenced with a 2 to 5-cm skin incision, followed by precise dissection along the front-inner edge of the sternocleidomastoid muscle to fully expose the carotid sheath. Identification of the CCA and internal carotid artery (ICA) was facilitated using vessel loops.

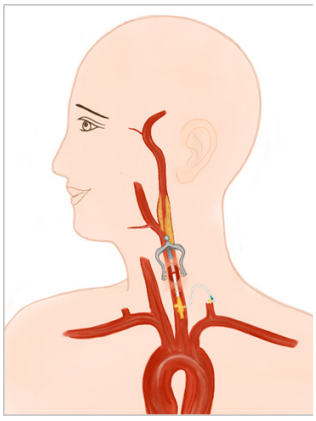

Figure 1 – Schematic illustration of the common carotid artery puncture technique using the cut-down method

After proximal puncture of the CCA with an 18-gauge needle via contrapertures, a 7-Fr sheath was advanced over a 0.035-inch guidewire into the vessel, as illustrated in Figure 1. All subsequent endovascular procedures were conducted through this sheath. After completion of the endovascular procedure, the sheath was removed, and the carotid puncture site was closed using either Prolene sutures or a purse-string technique. The skin was then meticulously sutured without necessitating the placement of an indwelling drain.

Patients received clopidogrel (75 mg orally) for 5 days preceding CAS. Dual antiplatelet therapy with aspirin and clopidogrel was maintained for the initial 6 months, followed by lifelong administration of either aspirin or clopidogrel unless contraindications or hemorrhagic complications emerged. Following arterial access, patients received intravenous heparin sodium (5,000 units, weight-adjusted) for anticoagulation. CAS involved using a self-expanding bare stent, with the stent size determined based on the characteristics of the stenosis and vessel diameter. A 0.014-inch guidewire was placed across the stenosis, and a cerebral protection device chosen by the operator was deployed. Stent placement and dilation, pre/post at the discretion of the operator, were followed by completion angiography at both bifurcation and brain levels. Subsequently, the protection device was retrieved.

Case presentation

3.1. Illustrative case 1

A 75-year-old male patient presented with complaints of headaches and vertigo. His medical history included an ischemic stroke in the left ICA territory three months prior. Neurological examination revealed right-side hemiparesis, graded as 4 on manual muscle testing. Preoperative CT angiography revealed critical stenosis of the left ICA, as demonstrated in Figures 2A and 2B. Femoral angiography demonstrated occlusion of both the common and internal iliac arteries, as shown in Figures 3A and 3B. Allen's test was positive bilaterally, indicating inadequate collateral circulation through the ulnar arteries

Table 1 Patient demographics and indications for stenting

Case	Age, years/ Gender	Symptoms/Signs	Internal carotid artery Stenosis, %(NASCET)	Side	Indications for direct carotid puncture	Hemodynamic instability	Type of stent	Residual stenosis, %	Complications
1	75/M	Right-sided hemiparesis	95	left	Both iliac arteries occlusive disease	none	Casper 7x30 mm.	20	none
2	57/M	Right-sided hemiparesis	55	left	Both iliac arteries have occlusive disease	none	Protege 8-6x30 mm.	20	none

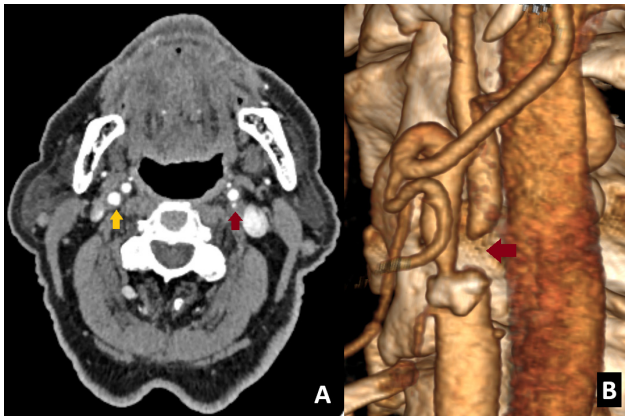

Figure 2 – Axial CT angiography (A) and 3D reconstruction (B) demonstrate critical stenosis of the left in-ternal carotid artery (red arrow – left internal carotid artery, yellow arrow – right internal ca-rotid artery).

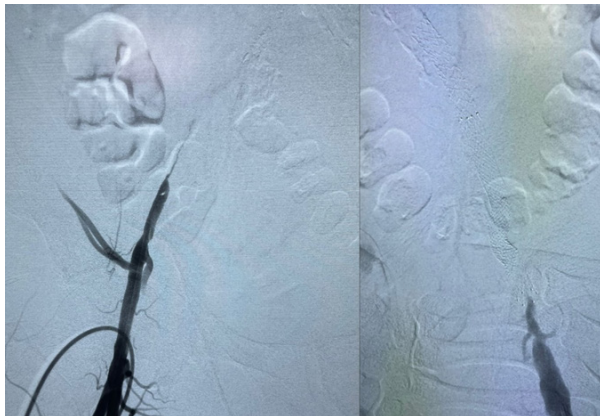

Figure 3 – Right-sided (A) and left-sided (B) femoral angiography demonstrating occlusion of both common iliac arteries

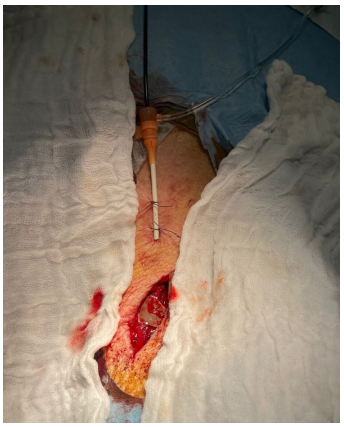

Figure 4 – Intraoperative view of left common carotid artery direct puncture and sheath placement

and representing a contraindication to using the radial artery for angiography. The patient had previously undergone left iliac artery stenting, prompting the decision for direct carotid access in this case. Following preparation and draping of the left neck, a suitable puncture site was confirmed, and DAP via the cut-down method of the left CCA was performed. A 7-Fr sheath was then placed directly into the CCA, as illustrated in Figure 4. Cerebral angiography from the left CCA revealed significant stenosis of the left ICA (95%, as per the NASCET criteria). The angiographic appearance of the stenotic segment is shown in Figures 5A–B. Considering the patient's symptoms and the high risk of ischemic stroke recurrence, it was decided

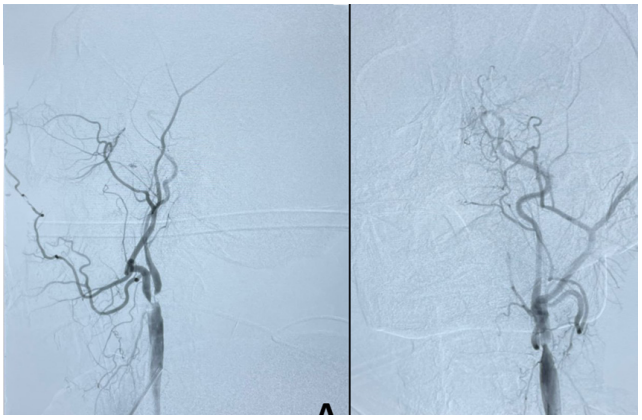

Figure 5 – Cerebral angiography from the left common carotid artery showing stenosis of the left internal carotid artery (A – frontal view, B – lateral view)

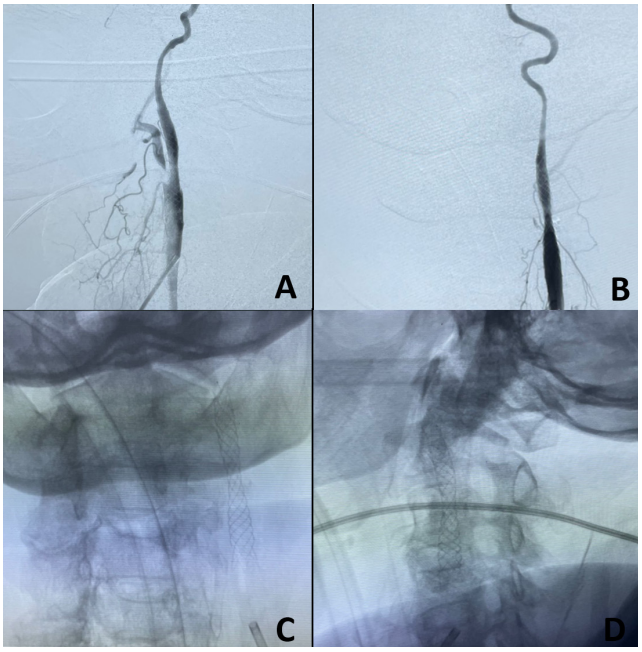

Figure 6 – Angiographic images showing successful stent deployment (A – frontal view, B – lateral view) and post-deployment confirmation of adequate stent expansion and apposition to the vessel wall (C – frontal view, D – lateral view)

to proceed with stenting of the left ICA. CAS was performed using a Casper 7x30mm stent and SpiderFX 6.0 mm Embolic Protection Device directly through the 7-Fr sheath without a guide catheter. Immediate post-stenting carotid angiography demonstrated significant improvement in the stenotic lesion, with residual stenosis reduced to 20%, as shown in Figures 6A–D. The sheath was removed with the preliminary application of a vascular temporary "bulldog" clip, and the defect of the left CCA was sealed using interrupted Prolene 7/0 and Ethilon 6/0 sutures. The patient experienced a favorable postoperative course without neurological deficits and was discharged on the fifth day following surgery. The patient remained neurologically intact at the 6-month follow-up, and CTA demonstrated patency of the stented segment without evidence of restenosis.

3.2. Illustrative case 2

A 57-year-old male patient presented with headaches and right-side hemiparesis. He had experienced an ischemic stroke in the left ICA territory just one month earlier. Neurological examination revealed right-side hemiparesis graded as 4

on manual muscle testing. The patient also had a history of aortoiliac occlusive disease (Leriche syndrome). Initial attempts to catheterize the right femoral artery were unsuccessful. Subsequently, left femoral artery catheterization and angiography revealed occlusion of the left common iliac artery, as shown in Figures 7A-B. Efforts to resolve thrombosis proved futile and posed a heightened risk of vessel dissection. Due to bilateral positive Allen's tests, the radial artery is contraindicated for use in angiography. Consequently, it was decided to proceed with a direct puncture of the CCA. After preparing and draping the left neck, and confirming a suitable puncture site, direct puncturing via cut-down method of the left CCA was carried out, as illustrated in Figure 8. Subsequently, the 7-Fr sheath was inserted directly into the CCA. Cerebral angiography conducted from the left CCA unveiled notable stenosis of the left ICA, estimated at 55% according to the NASCET criteria, as demonstrated in Figures 9A–B. Considering the patient's symptoms and the elevated risk of recurrent ischemic stroke, the decision was made to advance with stenting for the left ICA. CAS was conducted utilizing a Protege 8-6x30 mm stent. Follow-up carotid angiography demonstrated significant improvement in the previously stenotic segment, with residual narrowing reduced to 20%, as depicted in Figures 10A–D. The sheath was extracted, and the defect in the left CCA was closed using interrupted Prolene 7/0 sutures. The patient had a smooth postoperative recovery devoid of neurological deficits and was discharged on the sixth day post-surgery. At the 6-month follow-up, the patient showed no neurological deficits, and CTA confirmed that the stented segment remained patent with no signs of restenosis.

Discussion

The foundational technique of initial cerebral angiography, credited to Nobel laureate Egas Moniz in 1927, primarily involved the direct puncture of the CCA [11]. Over time, the technique was refined, and transfemoral catheter placement became the preferred approach in modern endovascular practice, gradually replacing direct puncture of the common carotid and brachial arteries. Moreover, the advent of novel methodologies, notably transradial access, has demonstrated a reduction in both the frequency and severity of procedural complications [12].

However, the intricate anatomical complexities of the aortic arch and supra-aortic vessels frequently present formidable challenges for endovascular interventions, irrespective of access route. Variations in anatomy and significant tortuosity in these vessels can complicate procedures conducted via both transfemoral and transradial accesses [8,13]. Particularly in cases characterized by severe peripheral arterial disease, such as aortic arch atherosclerosis, marked bilateral aortoiliac stenosis, abdominal aortic aneurysm, or aortobifemoral prosthesis, attempts to access the aortic arch via transfemoral catheterization may prove unfeasible [14].

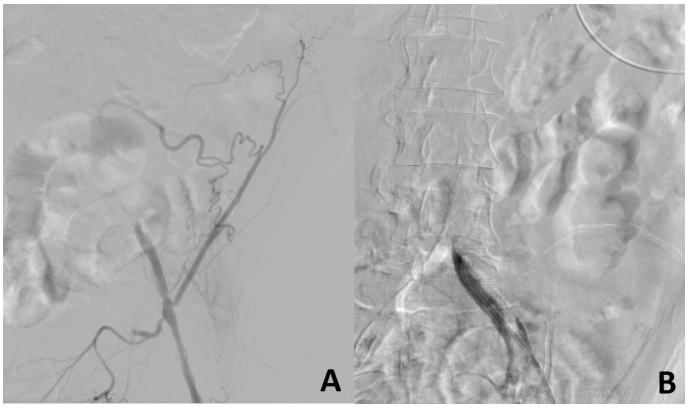

Figure 7 – Angiography via left femoral artery catheterization showing complete occlusion of the left common iliac artery

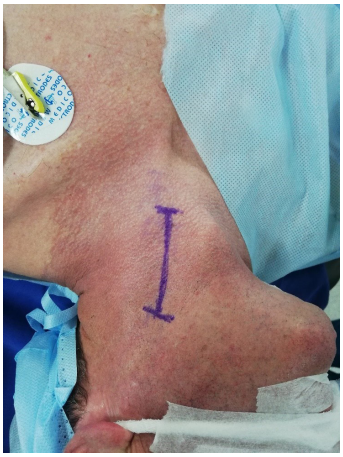

Figure 8 – Skin markings indicating the planned incision site for direct access to the left common carotid artery

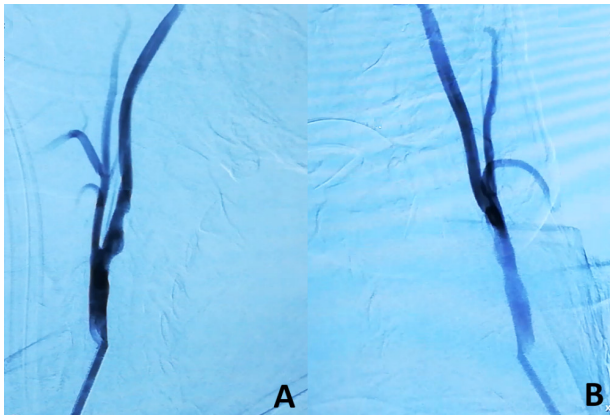

Figure 9 – Cerebral angiography from the left common carotid artery demonstrating stenosis of the left internal carotid artery

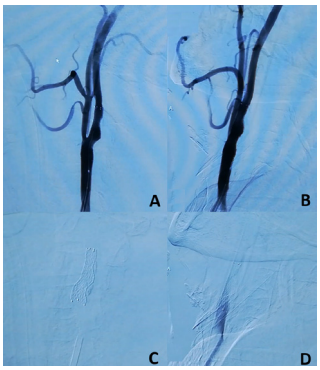

Figure 10 – Post-stenting angiography demonstrating successful stent deployment with restored luminal patency (A – frontal view, B – lateral view). Post-deployment images confirm appropriate stent expansion and positioning (C – frontal view, D – lateral view)

In such cases, navigating catheters through the complex anatomy of the aortic arch not only increases the risk of transient ischemic attacks (TIAs) [10], but also prolongs procedure time and raises radiation exposure for both patients and operators. Moreover, previous studies have shown a higher incidence of ischemic lesions detected by diffusion-weighted MRI (DW-MRI) with the transfemoral approach compared to direct cervical puncture (36.8% vs. 14.3%) [15].

In such challenging scenarios, DAP serves as a viable alternative, providing reliable access to the aortic arch and supra-aortic vessels. Importantly, its application extends beyond CAS; recent advancements have broadened its use to include aneurysm embolization and mechanical thrombectomy [6,16]. This expansion of indications highlights the versatility and growing value of DAP within the neuroendovascular field.

Another advantage of DAP over CEA is the ability to maintain continuous carotid blood flow, eliminating the need for shunt devices. Moreover, a meta-analysis comparing transfemoral and direct carotid access reported similar rates of local complications, suggesting that direct puncture does not increase the risk of access-site hematomas.

These findings support the safety profile of DAP, indicating a comparable risk of access-site hematomas to that of transfemoral approaches [15]. However, some reports note a 4%–7% incidence of neck hematomas following DAP, typically occurring after sheath removal [17]. Such hematomas may cause significant tracheal compression, potentially compromising airway patency. In contrast, open carotid access via the cut-down method provides better hemostatic control, thereby reducing the risk of hematoma formation [18].

Several studies have examined the use of percutaneous closure devices following DAP [9, 17]. While successful closures have been reported using these devices [19], it is important to acknowledge the associated risks. Deployment may lead to hematoma formation or ischemic complications, primarily due to potential displacement of the anchor within the vessel lumen. In light of these considerations, we propose that direct puncture via a cut-down technique emerges as a safer and preferable option. This approach eliminates the necessity for employing closure devices, mitigating the associated risks of hematoma formation and potential anchor migration.

The incorporation of DAP puncture via an open carotid access approach within a hybrid methodology ensures a well-defined and expansive operative field for puncture, thereby aiding in the achievement of hemostasis and diminishing the risk of severe complications, including pseudoaneurysm, dissection, and hematoma formation[20,21]. These factors collectively contribute to direct visualization at the puncture site of the sheath, thereby facilitating the suturing of this region. Further research has revealed additional advantages, such as the ability to perform repeat punctures and a decrease in bleeding even in patients taking antiplatelet medications. [22].

Although there is a theoretical risk of cranial nerve injuries during DAP via a cut-down method similar to those observed in CEA, none of the studies included in the analysis reported such occurrences [16]. This underscores the safety of the small incision required for Direct transcervical access in the CAS procedure, in contrast to the larger incision typically employed in CEA. In the reported cases, postoperative monitoring involved routine clinical evaluation and duplex ultrasonography of the puncture site. No access-related complications, including hematoma or neurological deficits, were observed.

In the described cases, the use of special holes through contrapertures in surrounding soft tissues to guide sheath insertion at a sharper angle and stabilize it presents a distinctive advantage. This advantage has demonstrated efficacy in overcoming common challenges encountered by certain authors, who have reported issues such as sheath deformations or vessel dissections during analogous procedures [23].

Crucially, all procedures involving direct puncture via the cut-down method are conducted under general anesthesia. This practice not only ensures maximum comfort for both the patient and the operator but also proves highly advantageous when implementing the roadmapping technique. Furthermore, it contributes to reduced contrast usage and minimizes the risk of embolization originating from the aortic arch. Significantly, this method is especially valuable for individuals with chronic kidney disease, as it aids in reducing the amount of contrast material.

It is important to acknowledge the limitations associated with this technique. While DAP puncture via the cut-down technique offers a valuable alternative in cases where transfemoral or transradial access is not feasible, it does carry certain logistical and economic considerations. The procedure may be associated with increased costs due to the need for an operating room, surgical instruments, and the involvement of a trained surgical team. Furthermore, successful execution requires familiarity with cervical anatomy and proficiency in microsurgical dissection techniques. Additionally, a minor drawback is the occurrence of bradycardia during the procedure, which can be effectively managed through the administration of atropine. Literature reports indicate an incidence of this clinical phenomenon ranging from 12.2% to 12.5% [24]. Executing this technique in the hybrid operations suite not only improves outcomes through clearer visualization, successful hemostasis, and a reduction in severe complications but also contributes to reduced radiation exposure. Using these benefits, the DAP puncture via the cut-down technique emerges as a promising approach for carotid interventions, offering comprehensive and efficient options.

Given the limited sample size of this technical note, the generalizability of the findings remains restricted. Although both procedures were successful and uneventful, further studies with larger cohorts are required to validate the reproducibility, safety, and clinical applicability of this approach.

Conclusion

The DAP via a cut-down method for CAS presents a significant avenue of promise. It markedly enhances procedural safety, precision, and patient outcomes by mitigating complications while ensuring uninterrupted blood flow. Despite potential increases in procedural costs and the requisite for specialized personnel, the overarching benefits in safety and outcomes are considerable. Further research and clinical application stand to refine the utilization of DAP in CAS, potentially optimizing its efficacy and addressing pertinent considerations regarding carotid artery interventions. Embracing DAP within clinical settings offers a safer and more precise alternative for carotid interventions, heralding advancements in patient care.

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