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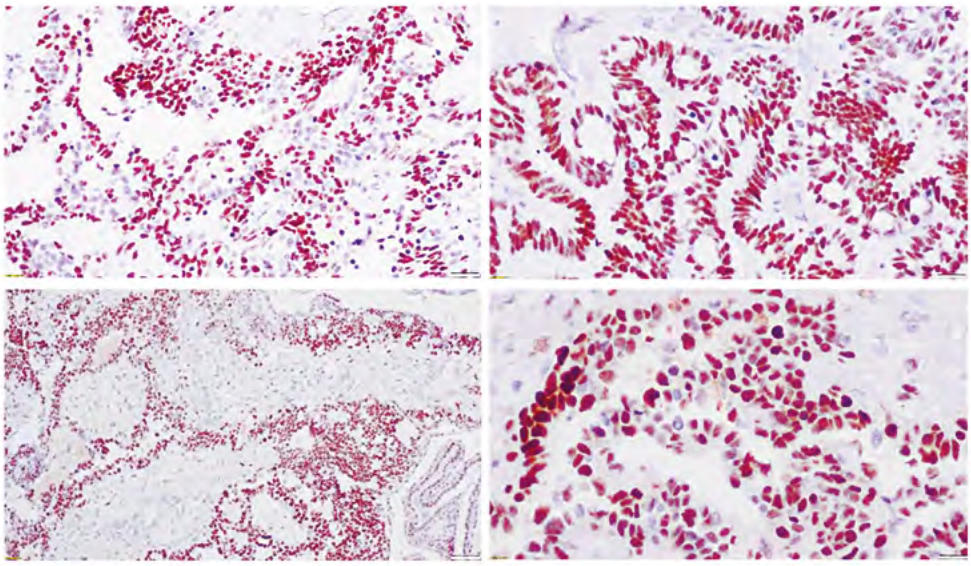
CONTENT HIGHLIGHTS:

Valeria Pînzaru, Tatiana Mărițoi, Valeriu David,
Ecaterina Foca, Lilian Șaptefrați
Expression of estrogen and progesterone receptors and
their clinicopathological correlations in serous ovarian carcinoma



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RESEARCH ARTICLES

Expression of estrogen and progesterone receptors and their clinicopathological correlations in serous ovarian carcinoma

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ABSTRACT

Introduction. Ovarian cancer continues to be the most lethal gynecologic malignancy, with high-grade serous ovarian carcinoma representing the most common and aggressive histological subtype. Although estrogen receptor and progesterone receptor have established prognostic and therapeutic relevance in other hormone-dependent cancers, such as breast carcinoma, their role in high-grade serous ovarian carcinoma remains insufficiently characterized and requires further elucidation.

Materials and methods. A retrospective study was conducted on 40 cases of high-grade serous ovarian carcinoma diagnosed between 2022 and 2024 at two medical institutions in Chișinău, Republic of Moldova. Estrogen receptor and progesterone receptor expression was evaluated by immunohistochemistry, employing the Allred scoring system (range: 0–8). Clinical and pathological data were collected, including patient age, menopausal status, tumor grade, FIGO stage, CA-125 serum levels, and tumor laterality. Statistical analysis comprised descriptive statistics, Student's t-test, Kruskal-Wallis test, Spearman's rank correlation, Mann-Whitney U test, and Chi-square test. A p-value < 0.05 was considered statistically significant.

Results. Estrogen receptor positivity was detected in 60% of cases (Allred scores 4-8), exhibiting a diffuse nuclear staining pattern, whereas progesterone receptor positivity was observed in 50% of cases (scores 2–6) with focal or mosaic staining. Four immunoprofiles were identified: ER+/PR+ (35%), ER+/PR− (25%), ER−/PR+ (15%), and ER−/PR− (25%). A significant positive correlation was found between ER and PR expression ($\rho = 0.472$, $p = 0.001$). Elevated CA-125 levels were significantly associated with advanced FIGO stage ($H = 15.52$, $p = 0.0014$), higher tumor grade ($H = 4.15$, $p = 0.041$), and PR negativity. Bilateral ovarian involvement showed a strong correlation with advanced disease ($\rho = 0.658$, $p < 0.00001$). The ER−/PR+ immunoprofile was predominantly associated with the most advanced FIGO stages. No significant differences in estrogen receptor or progesterone receptor expression were observed between premenopausal and postmenopausal patients.

Conclusions. Hormone receptor expression in high-grade serous ovarian carcinoma exhibits significant associations with key clinicopathological parameters, including CA-125 levels, tumor grade, FIGO stage, and tumor laterality. Notably, loss of progesterone receptor expression correlates with more aggressive tumor behavior. Hormonal receptor profiling using the Allred scoring system may provide valuable insights for prognostic stratification and aid in therapeutic decision-making in serous ovarian carcinoma. Further investigations involving larger patient cohorts and comprehensive molecular characterization are warranted to validate and expand upon these findings.

Keywords: serous ovarian carcinoma, estrogen receptor, progesterone receptor, CA-125 antigen.

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

There is currently no definitive evidence supporting the applicability of the Allred scoring system for evaluating hormonal receptor expression in serous ovarian carcinoma. Nonetheless, given the

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genetic and hormonal parallels between breast and ovarian cancers, as well as the hormone-dependent physiology of the ovaries, the use of this scoring method may have potential relevance in the context of gynecologic oncology.

The research hypothesis

The expression of estrogen (ER) and progesterone (PR) receptors in serous ovarian carcinoma has been shown to correlate with various clinicopathological parameters and may contribute to the identification of tumor subgroups characterized by distinct biological behavior and prognostic outcomes.

The novelty added by the manuscript to the already published scientific literature

This study proposes a standardized approach for the semi-quantitative immunohistochemical evaluation of estrogen receptor (ER) and progesterone receptor (PR) expression in serous ovarian carcinoma, employing the Allred scoring system – a model extensively validated in breast cancer but insufficiently investigated in ovarian neoplasms. The results underscore the potential utility of hormonal profiling in facilitating personalized therapeutic strategies and warrant further investigation in the field of gynecologic oncology.

Introduction

Ovarian cancer remains the most lethal gynecologic malignancy worldwide [1, 2]. In 2022, approximately 324,600 new cases were recorded globally, and the 5-year relative survival rate remains only 51.6% in the United States, despite the availability of multimodal therapies [3, 4]. Around 90% of ovarian cancer cases are of epithelial origin, while the remaining 10% include non-epithelial forms such as sex cord-stromal tumors, germ cell neoplasms, and other rare carcinoma types [5-7].

According to the 2020 World Health Organization classification, epithelial ovarian carcinoma is divided into five main histologic subtypes: serous, mucinous, endometrioid, clear cell, and NOS-type (not otherwise specified) adenocarcinoma [6, 7]. High-grade serous tubo-ovarian carcinoma represents the most frequent and extensively studied form of ovarian cancer, accounting for approximately 70% of all cases [8]. In contrast, low-grade serous carcinoma is considered a rare entity, representing 2–5% of all ovarian malignancies and approximately 5–10% of serous carcinoma cases [9, 10]. Each histologic subtype displays distinct biological characteristics, is associated with different cellular origins and specific immunohistochemical markers, and facilitates both diagnostic accuracy and prognostic assessment. Advances in the molecular understanding of these tumors have opened new directions for developing targeted therapies; nevertheless, further studies are required to adapt treatment strategies to the features of each subtype [11].

Mutations in the *BRCA1* and *BRCA2* genes, as well as Lynch syndrome, are involved in up to 25% of cases [13, 14]. The disease exhibits a hormone-dependent component, and an imbalance between estrogen receptors ERα and ERβ – particularly the overexpression of ERα – plays an important role in the carcinogenesis process [15]. Protective factors include oral contraceptive use, pregnancy, breastfeeding, and tubal ligation, whereas endometriosis, a vegetable-poor diet, and in vitro fertilization procedures may increase the risk of developing the disease [12, 15, 16].

In the effort to identify prognostic and therapeutic biomarkers in serous ovarian carcinoma, research has focused on the expression of estrogen (ER) and progesterone (PR) hormone receptors [17, 18]. Recent evidence suggests that tumor hormonal status is not merely an immunohistochemical artifact but rather reflects meaningful biological traits: elevated PR expression is frequently associated with improved survival, while the prognostic role of ER remains ambiguous and appears to be context-dependent [19]. However, most studies addressing hormone receptors have been conducted in breast cancer, where compelling results have led to the development of effective endocrine therapies, demonstrating the clinical value of these markers [20, 21]. In contrast, the literature on serous ovarian carcinoma continues to reveal contradictory results, driven by cohort heterogeneity and the lack of consensus on positivity thresholds – such as the use of the Allred scoring system. Within this framework, methodological standardization becomes essential for clinical validation of hormone receptors and for adapting them to the specific context of ovarian oncology [22, 23].

The study hypothesizes that the immunohistochemical expression of estrogen (ER) and progesterone (PR) receptors in serous ovarian carcinoma influences disease progression and holds prognostic relevance. It is presumed that increased receptor expression is associated with a more favorable prognosis. The objective of the study is to evaluate ER and PR receptor expression through immunohistochemistry in serous ovarian carcinoma and to correlate expres-

sion levels with clinicopathological parameters, aiming to identify potentially useful markers for personalized oncologic treatment.

Material and methods

The study material consisted of tissue samples morphopathologically diagnosed with high-grade and low-grade serous ovarian carcinoma, collected from patients aged between 18 and 85 years. These specimens were retrieved from the archives of the Oncology Institute and *Gheorghe Paladi* Municipal Clinical Hospital, Chișinău, Republic of Moldova, during the period 2022–2024 and included in the final analysis. All patients underwent surgical procedures, including laparotomy or exploratory laparoscopy with biopsy, adnexectomy, total hysterectomy with bilateral adnexectomy, and tumor cytoreduction.

For external control in immunohistochemistry using anti-ER and anti-PR antibodies, breast carcinoma samples with confirmed hormone receptor expression were employed. Negative controls were performed by omitting the primary antibody, ensuring the specificity of the reaction. Relevant clinical data were collected from the medical records of the patients included in the study.

Histological examination methodology included standard processing of tissue samples, hematoxylin-eosin (H&E) staining, and application of immunohistochemical techniques using specific anti-ER and anti-PR antibodies. Morphological evaluation of immunohistochemical reactions was performed via microscopic analysis, followed by rigorous statistical processing of the obtained data.

Primary processing. Selected tissue specimens were immediately fixed in a 10% buffered formalin solution with a pH between 7.2 and 7.4, to prevent autolysis and microbial proliferation. Fixation time did not exceed 24 hours.

Histological processing was performed using the Histo-Core PEARL tissue processor (Leica), followed by paraffin embedding with the Arcadia system (Leica). Tissue sectioning was carried out with the Biocut microtome (Leica), yielding 3–4 µm sections mounted on positively charged slides (Detalab, Spain), suitable for both histologic and immunohistochemical evaluation.

Histological technique. Slides were stained using the classical H&E method with the ST Infinity H&E Staining System (Leica). Automated staining was conducted using the ST5010 XL autostainer (Leica), and slide mounting was completed using the CV5030 system (Leica). Sections with adequate tissue material were selected for immunohistochemical staining.

Immunohistochemical technique. Immunostaining was performed using the BOND-MAX automated system (Leica), applying ready-to-use antibodies: BOND Estrogen Receptor (clone 6F11) and BOND Progesterone Receptor (clone 16). Detection was performed with the BOND Polymer Refine Detection system (Leica). The standard IHC-F protocol was applied as described in Table 1.

Deparaffinization of sections was achieved by exposure to DEWAX solution and Histanol 100 (H100-118/20). Epitope retrieval involved treating the slides with BOND ER Solution 1 (low pH) for ER and BOND ER Solution 2 (high pH) for PR antibodies, at 97–98°C for 20 minutes. Endogenous peroxidase neutralization was performed using BOND Peroxide Block for 5 minutes.

Table 1. Characteristics of primary antibodies used for immunohistochemistry.

Antibody/ clone	Source/incubation time/dilution	Retrieval system/ time	Detection/time
Er/6F11	BOND/15 min / ready-to-use	BOND Epitope Retrieval Solution 1 / 20 min	BOND Polymer Refine Detection, Leica / 8 min
Pr/16	BOND/15 min/ ready-to-use	BOND Epitope Retrieval Solution 2 / 20 min	BOND Polymer Refine Detection, Leica/ 8 min

Note: *ER – estrogen receptor; PR – progesterone receptor. All antibodies were ready-to-use and applied according to manufacturer protocols. Data presented are categorical (qualitative) descriptions of IHC protocol parameters.*

Primary antibody incubation lasted 15 minutes at room temperature for each antibody. BOND Post Primary and BOND Polymer reagents were applied for 8 minutes each. DAB (3,3′-diaminobenzidine) was used as the chromogen substrate, applied for 10 minutes. Nuclear counterstaining was performed using BOND Hematoxylin. The final immunohistochemical reaction was visualized by brown nuclear staining.

Subsequently, slides underwent dehydration and clearing through two immersions in Histanol 100, one mixed immersion in Histanol 100 and Bioclear, and three Bioclear immersions, each lasting 5 minutes. The procedure concluded with coverslipping using BMC-100 mounting medium.

Microscopic Evaluation. Positive expression of estrogen (anti-ER) and progesterone (anti-PR) receptors within intra- and peritumoral compartments was determined based on nuclear immunoreactivity. Immunopositivity was considered satisfactory when cells exhibited a clear nuclear pattern, highlighted by intense brown staining. Expression quantification was performed using the semi-automated protocol proposed by Pathology Outlines [24, 25].

Cell counting was conducted using an Olympus BX53 optical microscope equipped with an Olympus DP28 digital camera. ER+ and PR+ expression was quantitatively assessed in all cases, on a representative section from the paraffin block. Initially, at 100× magnification, areas of highest cellular expression ("hotspots") were identified, followed by quantification at 400× magnification.

Quantification methods. Quantification of ER-positive cells followed the semi-automated method described by Pathology Outlines [24, 25]. This approach involves assessing at least five microscopic fields at 40× objective magnification, selected from regions with the highest number of immunolabeled tumor cells, and calculating the mean percentage. To facilitate numeric evaluation, the tumor fields were photographed, and the images were contrast-inverted using Olympus cellSens Entry software.

Final assessment of ER and PR hormone marker expression was performed using the Allred scoring system, which

combines the percentage of immunolabeled cells with the intensity of nuclear staining [26-28].

Ethical Considerations. Favorable approvals were obtained from the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy for two research projects: the first, titled "Molecular status of the tumor microenvironment in serous ovarian carcinoma," approved by decision no. 8 on October 1, 2024; and the second, "Expression of growth and proliferation factors in high- and low-grade serous ovarian carcinoma," approved by decision no. 2 on December 13, 2024.

Data analysis. Statistical analysis was performed using Winstat 2012.1 software (R. Fitch Software, Bad Krozingen, Germany), integrated into Microsoft Excel 2010, for preliminary descriptive and inferential data processing. To validate results and ensure comprehensive analysis, SPSS Statistics software version 23.0 (IBM, Chicago, IL, USA) was also employed.

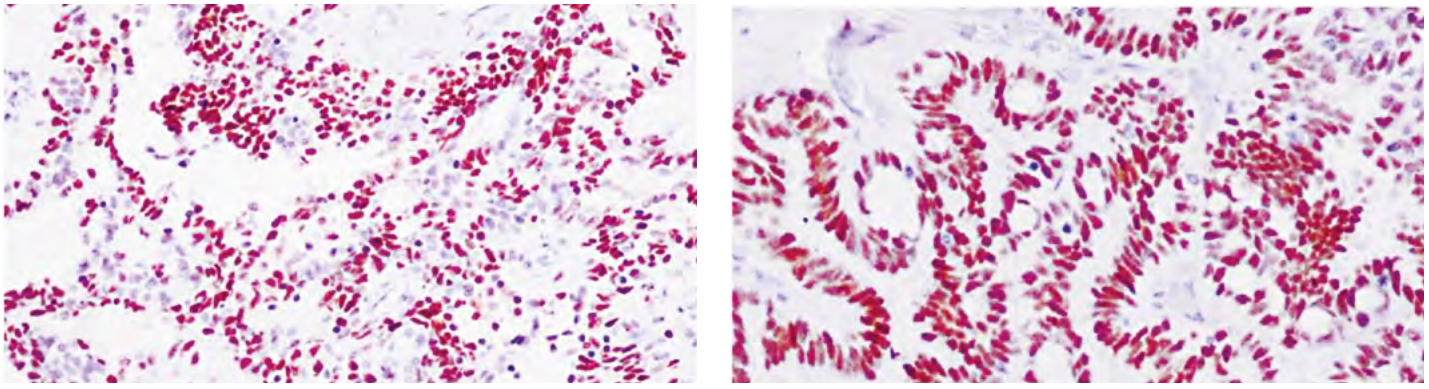
Descriptive statistics included calculation of arithmetic mean (M), standard deviation (SD), and standard error of the mean (SE) for Allred scores of ER and PR hormone receptors, as well as for patients' age distribution.

Comparison of quantitative variables between two groups – such as premenopausal versus postmenopausal patients – was performed using the Student's t-test for normally distributed data and the Mann–Whitney U test for non-parametric distributions. Differences among multiple groups (e.g., CA-125 levels stratified by FIGO stage and tumor grade) were evaluated using the Kruskal–Wallis test.

Correlations between hormone scores (Allred ER and PR) and clinicopathological parameters were examined using Spearman's correlation coefficient (ρ). The association between ER/PR hormone profiles and FIGO stage distribution was assessed using the Chi-square test (χ^2). All statistical analyses were interpreted at a significance level of $p < 0.05$.

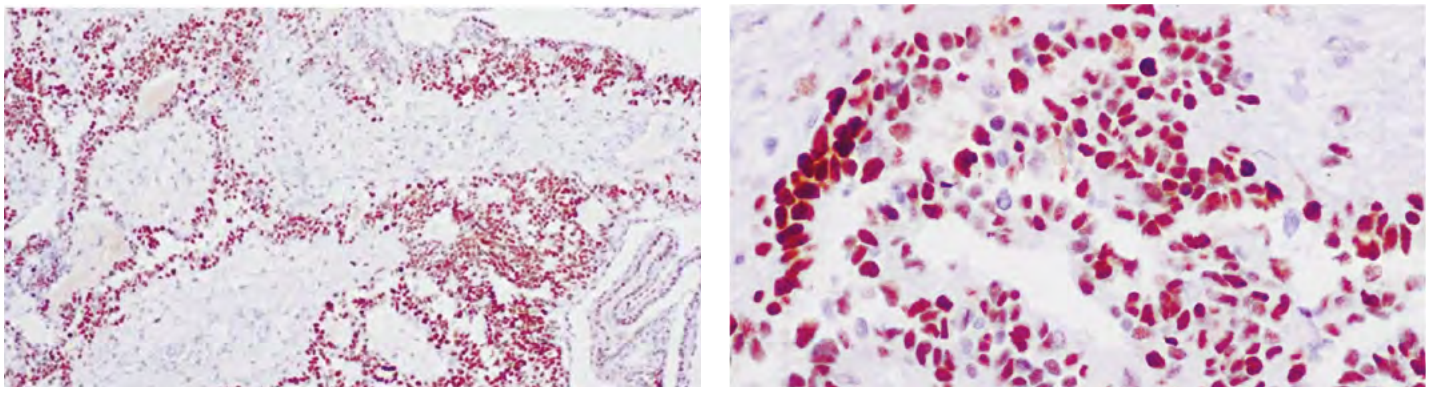
Results

A retrospective analysis was performed on 40 cases of serous ovarian carcinoma, involving patients aged between 18 and 85 years. Age group distribution revealed a predominance in the 52-67 years range (42.5%) and the 35-51 years range (35%), with the majority of patients (75%) being postmenopausal.



a b

Fig. 1 Heterogeneous nuclear immunoexpression of estrogen receptor (ER) in serous ovarian carcinoma.
(a) Low-magnification view (×20) showing patchy ER positivity within tumor cell nuclei.
(b) High-magnification view (×40) highlighting variable staining intensity among tumor cells.
Immunohistochemical staining with ER (clone 6F11); detection performed using BOND Polymer Refine Detection system.



a b

Fig. 2 Diffuse nuclear immunoexpression of progesterone receptor (PR) in serous ovarian carcinoma.
Note: *(a) Low-magnification view (×10) showing widespread nuclear positivity for PR in tumor cells.*
(b) High-magnification view (×40) confirming diffuse and strong nuclear staining pattern.
Immunohistochemical staining with PR (clone 16); detection performed using BOND Polymer Refine Detection system.

Tumor localization was relatively evenly distributed, with 35% of cases involving the left ovary, 32.5% the right ovary, and 32.5% showing bilateral involvement. Histopathologically, 85% of the tumors were classified as high-grade serous carcinoma (HGSC), and 75% of patients were diagnosed at advanced stages according to the FIGO classification system (stage III-IV).

Estrogen receptor (ER) and progesterone receptor (PR) expression was evaluated using the Allred scoring system, which integrates the percentage of immunopositive cells with the staining intensity on a scale from 0 to 8 (Fig. 1 and 2).

ER expression was predominantly nuclear, with a diffuse pattern and variable intensity, yielding Allred scores ranging from 4 to 8 and an overall positivity rate of 60%. PR expression was more heterogeneous, with focal or mosaic patterns and weak to moderate intensity. The Allred scores for PR ranged from 2 to 6, with a total positivity rate of 50%.

Four immunohistochemical profiles were defined:

- **ER+/PR−** – 25% of cases (10/40);
- **ER+/PR+** – 35% of cases (14/40);
- **ER−/PR+** – 15% of cases (6/40);
- **ER−/PR−** – 25% of cases (10/40);

A moderate and statistically significant correlation was observed between Allred scores for ER and PR (ρ = 0.472, p = 0.001), indicating a partially synchronized hormonal expression pattern (Fig. 3).

The distribution of Allred ER and PR scores according to CA-125 categories revealed a statistically significant association (p = 0.001), with a predominance of positive hormone profiles (ER+/PR+) observed in cases with CA-125 levels ≤200 U/mL. In contrast, low PR scores were frequently associated with CA-125 levels >1000 U/mL, suggesting a more aggressive tumor biology.

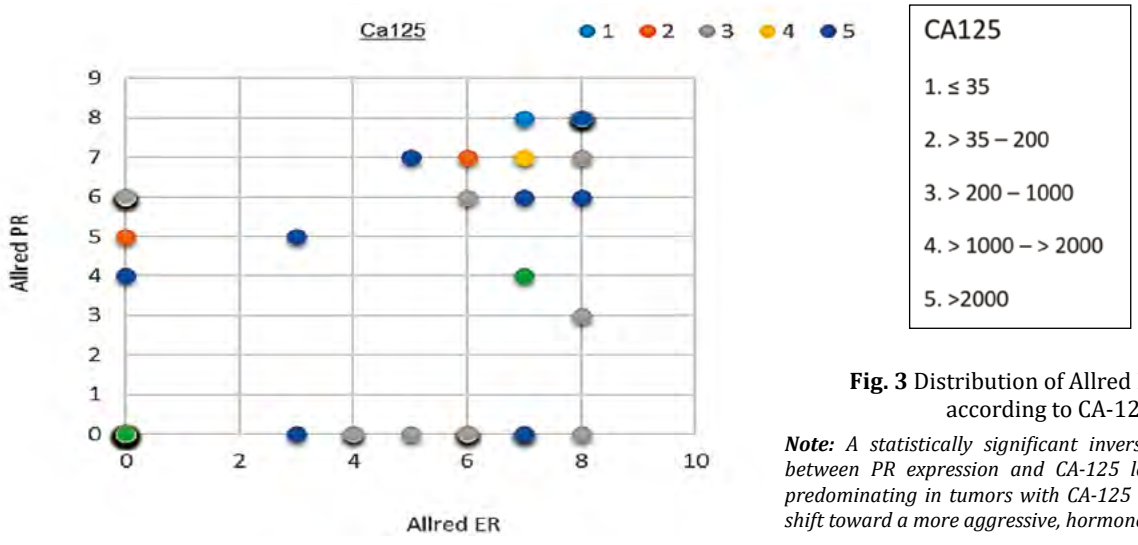


Fig. 3 Distribution of Allred ER and PR scores according to CA-125 levels

Note: *A statistically significant inverse relationship was noted between PR expression and CA-125 levels, with low PR scores predominating in tumors with CA-125 >1000 U/mL, suggesting a shift toward a more aggressive, hormone-resistant phenotype.*

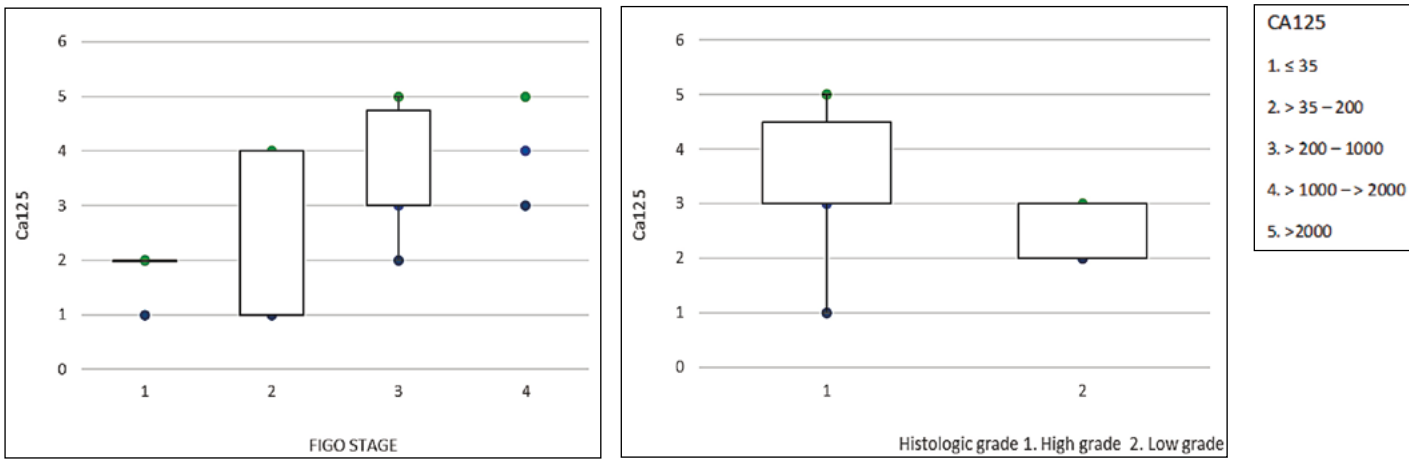


Fig. 4 Distribution of CA-125 levels according to FIGO stage and tumor grade, represented through comparative boxplots.

Note: *Higher CA-125 levels were significantly associated with advanced FIGO stages and high-grade serous carcinomas, supporting their role as surrogate markers of tumor aggressiveness.*

The Kruskal–Wallis test revealed statistically significant differences in the distribution of CA-125 levels both according to FIGO stage (H = 15.52, p = 0.0014) and tumor grade (H = 4.15, p = 0.041), with an ascending trend observed in serum marker levels among advanced stages and high-grade tumors (HGSC), suggesting prognostic relevance and an association with more aggressive tumor biology (Fig. 4).

The ER+/PR+ profile was predominant in patients with moderate CA-125 levels (≤200 U/mL), while profiles with reduced or absent PR expression (ER−/PR−) were frequently associated with elevated CA-125 levels (>1000 U/mL), suggesting a link between PR loss and tumor aggressiveness (Table 2).

Bilateral ovarian involvement was significantly associated with advanced FIGO stages (ρ = 0.657, p < 0.00001), highlighting the severity of neoplastic spread. Spearman correlation confirmed this association, indicating a clear trend toward tumor progression in cases with bilateral localization (ρ = 0.658, p < 0.00001), thus underscoring bilateral involvement as an unfavorable prognostic factor in serous ovarian carcinoma (Fig. 5).

Table 2. Distribution of patients according to ER/PR profile and serum CA-125 level

ER/PR profile vs CA125	≤35	36–200	201–1000	1001–2000	>2000
ER+/PR+	1	2	6	3	2
ER+/PR−	0	4	3	2	1
ER−/PR+	1	2	1	1	1
ER−/PR−	0	1	5	3	1

Note: *Positive ER/PR profiles were predominantly observed in patients with CA-125 levels ≤1000 U/mL, while double-negative (ER−/PR−) and ER−/PR+ phenotypes were more frequent at higher CA-125 levels, suggesting a potential link between hormonal receptor loss and elevated tumor burden.*

Immunohistochemical profiles defined by Allred ER/PR scores demonstrated significant differences in FIGO stage distribution. The most advanced tumor extension was observed in the ER−/PR+ group (mean = 3.33), suggesting a possible association between the absence of estrogen receptor expression and disease severity (Fig. 6). Chi-square analysis confirmed a statistically significant association between hormone profiles and FIGO stage (χ^2 = 59.95, df = 39, p = 0.017). Comparison of Allred scores for hormone receptors between postmenopausal and premenopausal patients revealed no statistically significant differences. The analysis of Allred ER scores indicated a similar distribution of estrogen receptor expression across both groups (Z = 0.36, p = 0.719), while Allred PR scores showed a comparable profile of progesterone receptor expression (Z = 0.326, p = 0.745). These findings suggest that physiological hormonal status does not significantly influence ER and PR receptor expression levels in serous ovarian carcinoma (Fig. 7).

Discussion

The results obtained in this study suggest a significant relationship between hormone receptor expression and clinicopathological parameters in serous ovarian carcinoma. The moderate correlation observed between Allred scores for ER and PR reflects a partially synchronized hormonal profile, indicating that these receptors may hold complementary prognostic value in assessing tumor behavior. This finding is consistent with certain studies emphasizing the role of hormone receptors as relevant markers in various hormone-dependent neoplasms [29].

The differential distribution of hormonal scores in relation to CA-125 tumor marker levels revealed an association between reduced PR expression and elevated CA-125 values. This observation suggests a more aggressive tumor biology and a potential loss of the protective influence of PR in serous ovarian carcinoma, a concept supported by previous data linking lower PR levels with poorer prognosis [29].

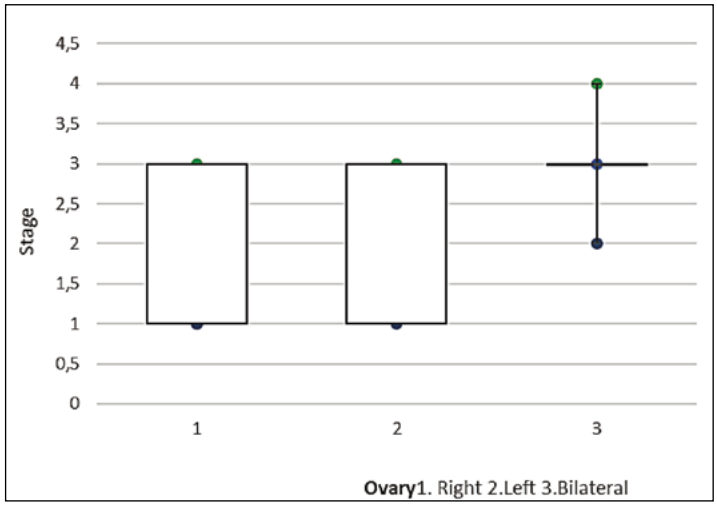


Fig. 5 FIGO stages according to ovarian localization (right, left, bilateral) – comparative boxplot.

Note: *Bilateral ovarian involvement was associated with significantly higher FIGO stages compared to unilateral cases, suggesting a more advanced tumor spread in bilaterally localized disease.*

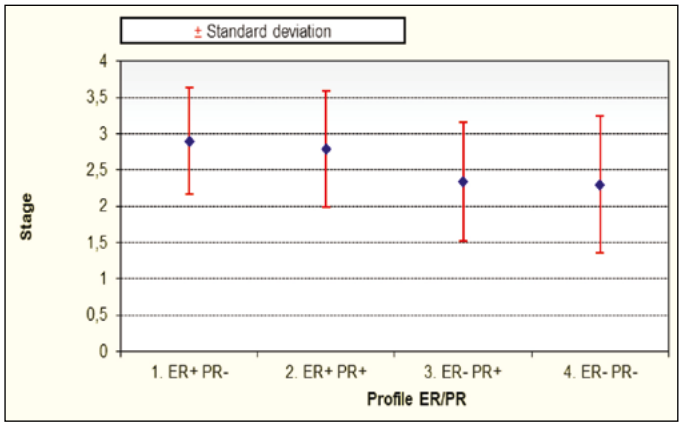
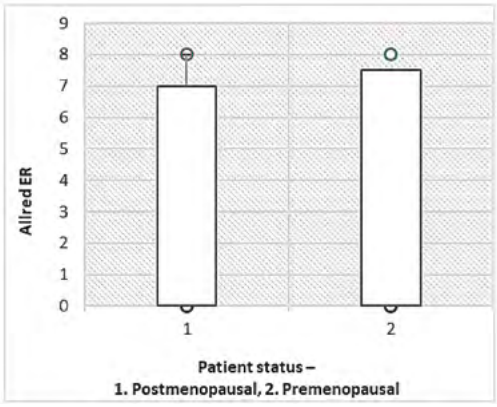


Fig. 6 Means and standard deviations of FIGO stage according to ER/PR hormonal profile

Note: *Lower FIGO stages were observed in ER+/PR+ tumors, while ER−/PR− profiles tended to associate with more advanced disease, supporting a link between hormone receptor negativity and tumor aggressiveness.*



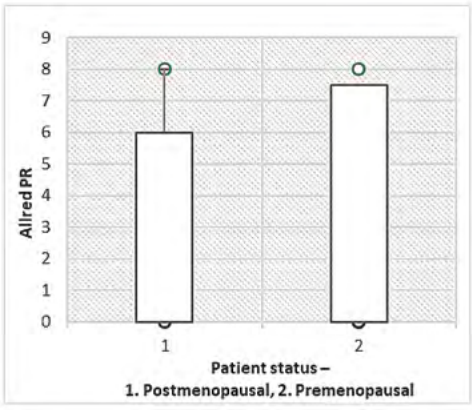


Fig. 7 Comparison of Allred ER and PR scores between postmenopausal and premenopausal patients
Note: *Premenopausal patients exhibited higher mean Allred scores for both ER and PR compared to postmenopausal counterparts, suggesting a more pronounced hormonal receptor expression profile in younger patients.*

Additionally, significant differences in CA-125 levels based on FIGO stage and tumor grade reaffirm the prognostic importance of this marker, with higher values characteristic of advanced stages and high-grade serous carcinoma (HGSC), reflecting greater disease extent and aggressiveness [30, 31].

The association between bilateral ovarian involvement and advanced stages of disease supports the hypothesis that bilateral extension indicates a more aggressive and unfavorable prognostic profile. This finding underscores the importance of evaluating bilaterality in morphopathological examination as a complementary prognostic factor [30, 31].

Chi-square analysis revealed a statistically significant association between ER/PR hormonal profiles and tumor extension stage, with an increased prevalence of advanced disease observed in the ER−/PR+ group. This suggests that the absence of estrogen receptor expression, coupled with progesterone receptor positivity, may represent a distinct biological phenotype with implications in neoplastic progression [32]. This observation opens avenues for future research exploring the molecular mechanisms underpinning these phenotypes and assessing their potential as therapeutic targets or specific prognostic markers.

Although comparative analysis of hormone receptor expression between postmenopausal and premenopausal patients did not yield significant differences, this finding should be interpreted with caution, considering the relatively small sample size and potential confounding variables that could not be fully controlled in this study [18, 33]. Physiological hormonal changes are known to influence receptor expression, but their relevance in the context of serous ovarian carcinoma requires further investigation in larger cohorts with detailed hormonal status analysis.

Major limitations of the study include the relatively small cohort size, which may affect statistical power and the generalizability of results. Additionally, the absence of detailed molecular data – such as *BRCA* mutational status or extended genomic profiling – represents an important gap that may influence both hormone receptor expression and patient prognosis. Integrating these molecular insights could provide a deeper understanding of the biological heterogeneity of serous ovarian carcinoma and facilitate the development of personalized therapeutic strategies based on individual tumor characteristics.

Conclusions

The data obtained in this study support the hypothesis that estrogen (ER) and progesterone (PR) hormone receptor expression has clinically and prognostically relevant significance in serous ovarian carcinoma, significantly influencing clinicopathological parameters and tumor behavior. In particular, the correlations identified between receptor expression and CA-125 tumor marker levels, as well as with disease progression stage, highlight the important role of ER and PR in defining the tumor's biological profile. Hormonal profiles established using the Allred scoring system provide valuable prognostic insights, and the association between low PR expression and more aggressive tumor forms reveals the potential of this marker as an indicator of disease severity. Additionally, the correlation between bilateral ovarian involvement and advanced cancer stages underscores the importance of morphopathological parameters in prognostic stratification. However, to validate and further explore these findings, additional studies are required, incorporating larger cohorts and detailed molecular analyses, thereby facilitating the development of optimized therapeutic strategies tailored to the tumor's molecular profile.

Competing interests

None declared.

Authors' contributions

VP conceived the study, contributed to the design and case selection, participated in data analysis, and drafted the initial version of the manuscript. TM carried out the histopathological evaluation of all cases, conducted detailed microscopic analyses, and was responsible for the quantification of immunopositive cells, playing a crucial role in ensuring the accuracy of the morphological assessment. VD performed all laboratory procedures, including immunohistochemical staining, protocol optimization, and preparation of histological sections. EF contributed to the histological interpretation and provided partial supervision of the scientific content of the project. LȘ served as the scientific coordinator of the study, critically contributed to data interpretation, ensured scientific validation, and participated in drawing the final conclusions. All authors were involved in the writing and revision of the manuscript, critically reviewed the content, and approved the final version of the article.

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Ethics approval

Favorable approvals were obtained from the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy for two research projects: "Molecular status of the tumor microenvironment in serous ovarian carcinoma," approved by decision no. 8 on October 1, 2024; and the second, "Expression of growth and proliferation factors in high- and low-grade serous ovarian carcinoma," approved by decision no. 2 on December 13, 2024.

Provenance and peer review

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RESEARCH ARTICLES

Chromosomal variations in infertile men diagnosed by cytogenetic analysis

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ABSTRACT

Introduction. Male infertility has a heterogeneous etiology, most commonly caused by disorders of spermatogenesis, clinically manifested as azoospermia or severe oligospermia. Genetic factors account for approximately 30% of male infertility cases associated with azoospermia. This high frequency is due to the involvement of numerous genes in the regulation of sexual development and reproduction. Among the various genetic causes of spermatogenic failure, chromosomal abnormalities are among the most clinically significant. The objective of the study was to evaluate the profile of chromosomal variations in infertile men with azoospermia, to optimize assisted reproductive strategies in infertile couples.

Material and methods. A group of 96 azoospermic men underwent karyotype analysis. The diagnosis of azoospermia was established based on at least two consecutive semen analyses performed according to the guidelines of the World Health Organization (WHO). Cytogenetic analysis was carried out on peripheral blood lymphocytes, with results interpreted according to the 2016 International System for Human Cytogenetic Nomenclature (ISCN). Hormonal profiles (FSH, LH, prolactin, testosterone) were correlated with chromosomal findings. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences), version 22.0.

Results. Cytogenetic investigations in azoospermic patients (n = 96) revealed karyotype variations in 25.0% of cases, including sex chromosome abnormalities in 16.7%: 47,XXY – Klinefelter syndrome (11.5%); microscopic structural variations of the Y chromosome (2.1%); and single cases of 47,XYY – Jacobs syndrome; 46,XX male – sex reversal; and 45,X/46,XY – mixed gonadal dysgenesis. Autosomal abnormalities were found in 8.3% of cases: translocations (3.1%), inversions (2.1%), chromosomal polymorphisms (2.1%), and one case with 46,XY,fra(17)(p12). Patients with sex chromosome abnormalities exhibited significantly higher FSH and LH levels compared to those with autosomal abnormalities ($p < 0.05$), whereas prolactin and testosterone levels did not differ significantly between the groups.

Conclusions. The high prevalence of chromosomal abnormalities in azoospermic men supports the inclusion of cytogenetic testing in the routine evaluation of male infertility. Identifying the type of chromosomal defect allows for appropriate genetic counseling and aids in decision-making regarding assisted reproductive options.

Keywords: azoospermia, male infertility, chromosomal abnormalities, Klinefelter syndrome, cytogenetic analysis, sex chromosomes, autosomal translocations.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

While the prevalence of chromosomal abnormalities in male infer-

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tility is well documented, the specific distribution and clinical correlations of sex chromosome and autosomal variations among azoospermic men in the Moldovan population remain underexplored. There is limited regional data on how these abnormalities influence hormonal profiles and diagnostic decision-making in assisted reproductive practices.

The research hypothesis

Chromosomal abnormalities are significantly associated with male infertility, and identifying the specific type of chromosomal anomaly through karyotyping and genetic testing enables precise genetic counseling, contributing to optimized decision-making regarding assisted reproductive technologies and reducing the genetic risk for offspring.

The novelty added by the manuscript to the already published scientific literature

This study represents a comprehensive cytogenetic analysis of azoospermic men. The research demonstrated a significant genetic contribution in patients with severely impaired semen parameters – azoospermia – allowing the identification of chromosomal abnormalities and polymorphisms, as well as the classification of cytogenetic forms correlated with the phenotypic manifestations of these patients.

Introduction

Male infertility refers to a man's inability to achieve pregnancy within a couple, despite the presence of a fertile female partner. It has been well established that male fertility is primarily dependent on both the quantity and quality of semen. When the volume of ejaculated semen is reduced or of poor quality, the likelihood of natural conception is significantly diminished. According to the World Health Organization (WHO), male-factor infertility is defined as an abnormality in sperm concentration, motility, and/or morphology in at least one of two semen samples, collected at intervals of one to four weeks. Therefore, men whose semen parameters fall below the WHO-established reference values are considered to have impaired fertility [1-3].

The most severe form of male infertility is azoospermia, which refers to the complete absence of spermatozoa in the collected or ejaculated semen. Azoospermia affects approximately 1% of the general male population, while its prevalence among infertile men ranges from 10% to 15% [4].

Historically, men diagnosed with azoospermia were classified as having irreversible infertility, and the use of donor sperm was considered one of the most viable reproductive options. However, with the advent of advanced assisted reproductive technologies (ART) such as intracytoplasmic sperm injection (ICSI), testicular sperm extraction (TESE), and microsurgical TESE (micro-TESE), even men with the most severe forms of azoospermia may now achieve biological parenthood [5].

The frequency of chromosomal abnormalities is inversely correlated with sperm concentration. Among patients with moderate oligozoospermia, chromosomal anomalies are detected in approximately 4% of cases [6]. In contrast, among azoospermic patients, the prevalence of chromosomal alterations ranges from 15% to 25%, depending on the specific subgroups studied [7]. Given the high incidence of chromosomal abnormalities among men with non-obstructive azoospermia and moderate oligozoospermia, karyotype analysis is recommended as the first-line genetic test for patients with quantitative spermatogenesis disorders. Furthermore, this genetic test is also indicated in men with a family history of recurrent miscarriage, congenital malformations, cognitive developmental disorders, or infertility, regardless of semen concentration [6].

The identification of an abnormal karyotype is of great importance for comprehensive and effective genetic counseling, which should provide detailed information regarding the type of chromosomal anomaly or polymorphism, its clinical significance, inheritance pattern, genetic risk to offspring, and available options for prenatal diagnosis.

Genetic counseling plays a critical role in guiding infertile couples to make informed decisions regarding assisted reproductive choices. Therefore, cytogenetic screening remains a key component in the accurate diagnosis, evaluation, prognostic assessment, and successful treatment of male infertility. The objective of the study was to evaluate the profile of chromosomal variations in infertile men with azoospermia, in order to optimize assisted reproductive strategies in infertile couples.

Material and methods

The study was conducted on a cohort of 96 male patients diagnosed with azoospermia, who presented for infertility evaluation. The diagnosis of azoospermia was established based on at least two consecutive semen analyses confirming the complete absence of spermatozoa in the ejaculate. Semen samples were collected in accordance

with WHO guidelines, following a recommended abstinence period of 3-7 days. The mean age of the men with azoospermia in the entire sample (n = 96) was 33.8 ± 5.3 years (95% CI: 32.7 – 34.9; median: 33.0). The mean duration of infertility across the cohort was 6.5 ± 4.6 years (95% CI: 5.6 – 7.5).

The cytogenetic analysis was performed at the Cytogenetics Laboratory of the Mother and Child Institute, Center for Reproductive Health and Medical Genetics, Republic of Moldova. All patients underwent classical cytogenetic analysis using peripheral blood lymphocytes. Blood samples were cultured and enriched with phytohemagglutinin to stimulate cell division. After 72 hours of incubation, metaphase was arrested with colchicine, followed by hypotonic shock and fixation with methanol-acetic acid. Karyotyping was performed and interpreted according to the 2016 International System for Human Cytogenetic Nomenclature (ISCN). Between 10 and 30 metaphases were analyzed per patient, with 5 to 10 karyotyped, increasing accordingly in the presence of abnormal cells. In cases with atypical findings, such as suspected sex reversal (46,XX with male phenotype), complementary FISH (Fluorescent In Situ Hybridization) analyses were performed for the SRY and DXZ1 (X) and DYZ1 (Y) markers, along with molecular PCR testing for Y chromosome-specific markers (AZFa, AZFb, and AZFc regions).

All patients underwent endocrine evaluation by measuring serum levels of the following hormones: follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, total testosterone, and free testosterone.

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) version 22.0. Comparison of mean parameters between patient groups (with sex chromosome vs. autosomal abnormalities) was carried out using the ANOVA (analysis of variance) test. Differences were considered statistically significant at a p-value < 0.05.

Results

Cytogenetic analysis performed on 96 infertile men with azoospermia revealed a normal karyotype 46,XY in 75% of cases (n = 72), while 25% (95% CI: 16.3–33.7; n = 24) exhibited chromosomal numerical or structural abnormalities. The prevalence of sex chromosome abnormalities was identified in 16.7% of cases (n = 16), whereas 8.3% of cases (n = 8) presented autosomal abnormalities (Fig. 1).

A comparative analysis of patient age revealed a statistically significant difference between the two groups. The mean age in the group with sex chromosome abnormalities (n = 16) was 36.2 ± 4.9 years (IQR: 32.5–39.0; median: 35.5), compared to 32.0 ± 2.9 years (IQR: 29.5–34.5; median: 32.0) in the group with autosomal chromosomal abnormalities (n = 8) (F = 4.917; p = 0.037). No statistically significant difference was found in semen volume between the two groups (F = 0.601; p = 0.447). The sex chromosome group had a mean volume of 2.2 ± 0.7 mL (IQR: 1.5–2.5; median: 2.2), while the autosomal group had a mean of 2.4 ± 0.8 mL (IQR: 2.0–2.9; median: 2.4). In

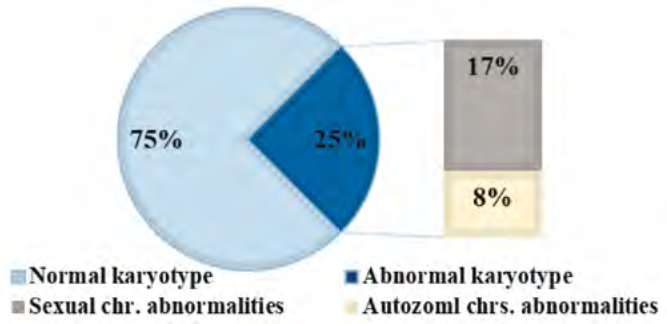

Fig. 1 Proportion of chromosomal abnormalities in men with azoospermia (n = 96)

Note: *The figure illustrates the distribution of chromosomal findings among azoospermic men. A normal karyotype was identified in 75% of cases. Chromosomal abnormalities were found in 25% of the cohort, with 17% involving sex chromosomes and 8% involving autosomal chromosomes. The inner bar chart expands the abnormal karyotype group, distinguishing between sex chromosome abnormalities and autosomal abnormalities. Data are expressed as percentages of the total number of patients.*

contrast, FSH levels differed significantly between groups (F = 7.406; p = 0.012). Patients with sex chromosome abnormalities showed a mean FSH level of 15.4 ± 10.3 IU/L (IQR: 7.5–20.6; median: 15.5), whereas those with autosomal abnormalities had a mean of 4.9 ± 4.8 mIU/mL (IQRR: 1.8–6.7; median: 2.9). Similarly, LH levels were significantly higher in the sex chromosome group (F = 6.576; p = 0.018), with a mean of 15.3 ± 8.9 mIU/mL (IQR: 8.1–23.3; median: 13.0) compared to 6.7 ± 4.0 mIU/mL (IQR: 3.9–9.2; median: 5.8) in the autosomal group. Prolactin levels did not differ significantly between groups (F = 1.158; p = 0.294). The mean prolactin concentration was 12.9 ± 6.5 ng/mL (IQR: 7.0–19.1; median: 11.3) in the sex chromosome group and 15.9 ± 5.6 ng/mL (IQR: 11.3–19.6; median: 18.0) in the autosomal group. Testosterone levels also showed no statistically significant differences (F = 1.224; p = 0.281), with a mean of 3.4 ± 0.9 ng/mL (IQR: 2.9–3.8; median: 3.4) in the sex chromosome group and 3.9 ± 1.2 ng/mL (IQR: 2.8–4.9; median: 3.7) in the autosomal group (Table 1).

From the total cohort of azoospermic men who underwent cytogenetic investigation (n = 96), 11 patients (11.5%) were diagnosed with X disomy. According to cytogenetic results, the most frequently identified chromosomal variant among these 11 infertile patients with Klinefelter syndrome (KS) was the homogeneous form of trisomy 47,XXY (10 cases – 90.9%), followed by the mosaic form of the same classical variant 47,XXY/46,XY (1 case – 9.1%) (Table 2, Fig. 2 and 3).

Additionally, one case of each of the following rare sex chromosome abnormalities were identified (1.04% per abnormality): Y disomy (47,XYY), sex reversal (46,XX + SRY), mixed gonadal dysgenesis in a male (45,X/46,XY), and two structural Y chromosome variation, 46,X,del(Y)(q11.21) (Y≤21) and 46,XYqh+ (Y ≥ 18) (Table 2, Fig. 4-7).

Table 1. Characteristics of sex chromosome abnormalities compared to autosomal abnormalities in patients with azoospermia

Parameters	sex chromosem ab.	Autosomal chrs. ab.	Total	p
No. of patients	n = 16	n = 8	n = 24	
Age (years)	36.2 ± 4.9 35.5 (32.5-39.0)	32.0 ± 2.9 32.0 (29.5-34.5)	34.8 ± 4.8 34.5 (31.0-36.0)	p=0.037
Volume (mL)	2.2 ± 0,7 2.2 (1.5-2.5)	2.4 ± 0.8 2.4 (2.0-2.9)	2.2 ± 0.7 2.2 (1.8-2.6)	p=0.447
FSH (mIU/mL)	15.4 ± 10.3 15.5 (7.5-20.6)	4.9 ± 4.8 2.9 (1.8-6.7)	11.9 ± 10.1 9.2 (2.9-19.1)	p=0.012
LH (mIU/mL)	15.3 ± 8.9 13.0 (8.1-23.3)	6.7 ± 4.0 5.8 (3.9-9.2)	12.4 ± 8.6 9.9 (5.9-19.5)	p=0.018
Prolactin (ng/mL)	12.9 ± 6.5 11.3 (7.0-19.1)	15.9 ± 5.6 18.0 (11.3-19.6)	13.9 ± 6.2 11.8 (7.9-19.5)	p=0.294
Testosterone (ng/mL)	3.4 ± 0.9 3.4 (2.9-3.8)	3.9 ± 1.2 3.7 (2.8-4.9)	3.6 ± 1.0 3.4 (2.9-4.1)	p=0.281
Free testosterone (ng/mL)	7.1 ± 6.8 2.8 (2.8-8.4)	15.9 ± 10.5 15.9 (8.4-23.3)	9.6 ± 8.3 8.4 (2.8-18.5)	p=0.231

Note: *Chrs. ab.- chromosomal abnormalities; FSH - follicle-stimulating hormone; LH - luteinizing hormone; mL – milliliters, n - number of cases. Statistical analysis: The Student's t-test was used for comparison of normally distributed continuous variables Mean ± SD (Standard Deviation). The Mann–Whitney U test was applied for non-normally distributed variables Median, IQR (Interquartile Range). A p-value < 0.05 was considered statistically significant.*

Table 2. Sex chromosomal abnormalities categorized by FSH levels

Sex chromosome abnormalities	FSH (mIU/ml)							
	Decreased <2		Normal 2–10		Increased >10		Total	
	n = 10	%	n = 58	%	n = 28	%	n = 96	%
Total	1	10.0	5	8.6	10	35.7	16	16.7
SK 47,XXY			1	1.7	9	32.1	10	10.4
SK 46,XY/47,XXY					1	3.6	1	1.0
47,XYY			1	1.7			1	1.0
46,XX +SRY			1	1.7			1	1.0
45,X/46,XY	1	10.0					1	1.0
46,Xdel(Y) (q11.21)			1	1.7			1	1.0
46,XYqh+			1	1.7			1	1.0

Note: *FSH – follicle-stimulating hormone, measured in mIU/mL; n - number of cases; % - percentages correspond to the proportion within each FSH group. Statistical analysis: Categorical variables were compared using the Chi-square test or Fisher's exact test when appropriate. A p-value < 0.05 was considered statistically significant.*

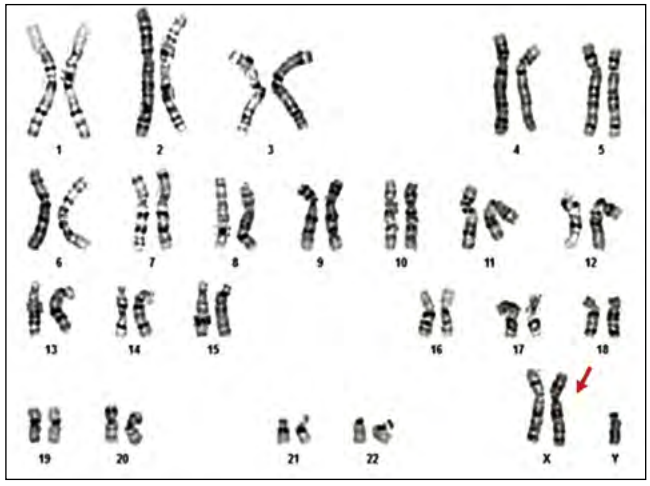

Fig. 2 Cytogenetic variant – classical form of Klinefelter syndrome, 47,XXY in a 35-year-old patient

Note: *The image presents a G-banded karyotype demonstrating the presence of an extra X chromosome, consistent with Klinefelter syndrome. A total of 47 chromosomes are identified, including two X chromosomes and one Y chromosome. The arrow indicates the additional X chromosome.*

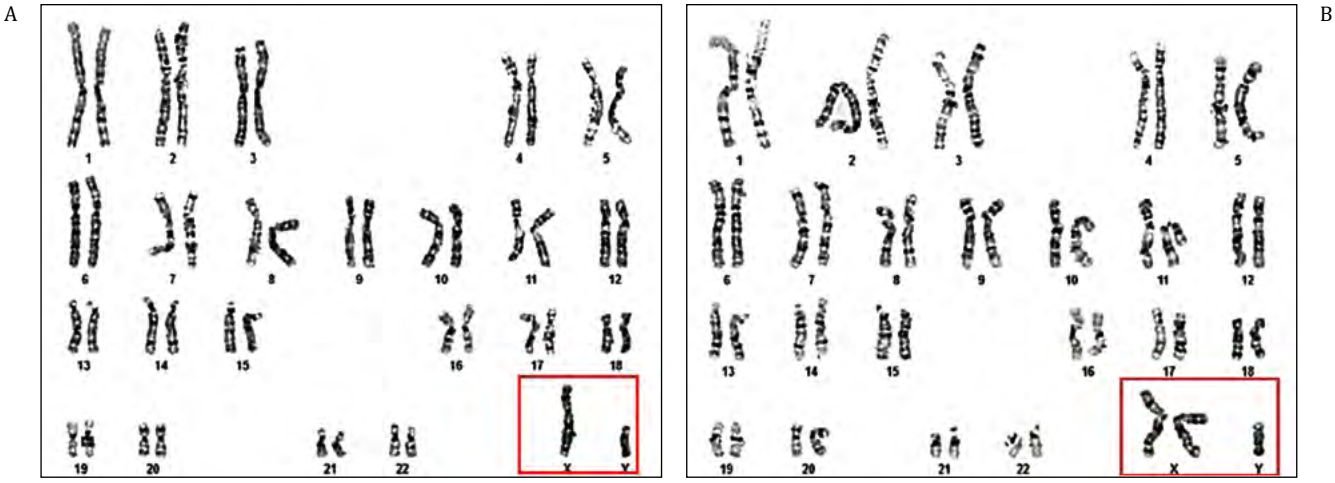

Fig. 3 Cytogenetic variant – mosaic form of Klinefelter syndrome 46,XY/47,XXY in a 31-year-old patient

Note: *The figure shows two karyotypes (A and B) illustrating the mosaicism detected in a male patient. Image A displays a normal male karyotype (46,XY), while image B shows a cell line with an additional X chromosome (47,XXY). This cytogenetic mosaicism indicates the coexistence of two distinct cell lines within the same individual.*

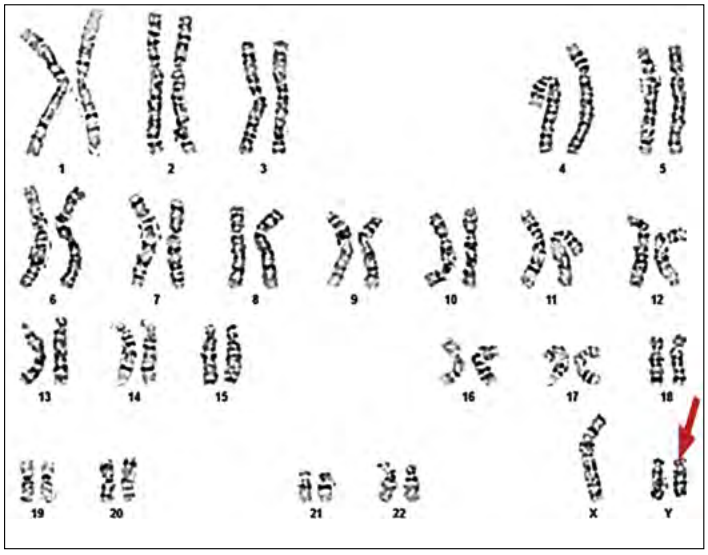

Fig. 4 Karyotype 47,XYY of a 36-year-old patient [8]

***Note**: The karyotype reveals the presence of an extra Y chromosome, resulting in a 47,XYY karyotype, known as Jacobs syndrome. The red arrow indicates the second Y chromosome.*

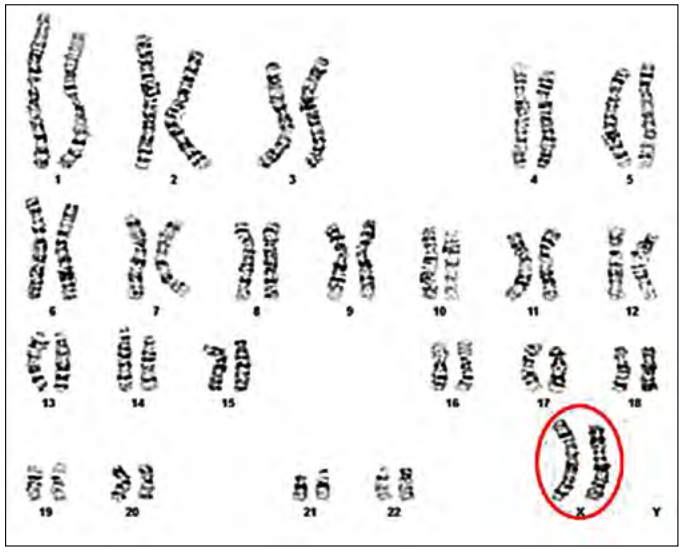

Fig. 5 Karyotype *46,XX* in all cells analyzed in a 31-year-old man [9]

***Note:** The patient, exhibiting a male phenotype, presented with a 46,XX karyotype – comprising 46 chromosomes, including two X chromosomes in the sex chromosome pair – consistent with a female chromosomal pattern in all analyzed cells.*

(Metaphases counted: 33; Karyotyped metaphases: 10; Band resolution level: 575-700)

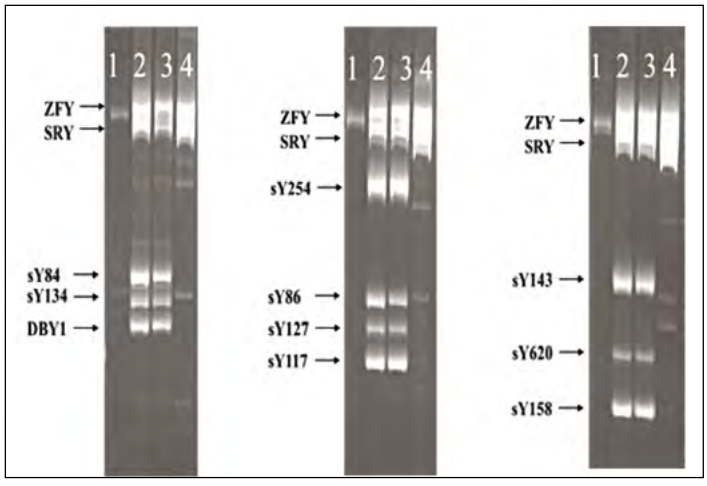

Fig. 6 The result of electrophoresis for detection of Y markers in the man with 46,XX [9]

Note: 1 – Female control; 2, 3 – Normal male; 4 – Male with deletions of the AZF (a, b, c) region

The patient presented with a male phenotype, but had a 46,XX karyotype characteristic of a female chromosomal pattern (Fig. 5). A total of 33 metaphases were analyzed, of which 10 were karyotyped, with a banding resolution level of 575-700, which revealed no evidence of mosaicism or other structural or numerical abnormality.

In addition, a FISH (Fluorescence in situ Hybridization) test was performed using probes for the X chromosome (DXZ1, Xp11.1–q11.1) and the Y chromosome (SRY, Yp11.32, and DYZ1, Yq12). The FISH analysis revealed two signals for the X chromosome (DXZ1) and one signal for the SRY gene (Yp11.32).

Molecular analysis of Y chromosome-specific markers – SY81, SY84, sDBY1, and sY620 from the AZFa region; SY127, SY134, sY117, and sY143 from the AZFb region; SY254, SY255, sY153, and sY158 from the AZFc region – showed their absence in the patient's DNA. However, the presence of ZFX and SRY was confirmed. These results support the diagnosis of an XX male with the presence of the SRY gene, but without other Y-specific markers (Fig. 6).

In the patient with a 45,X/46,XY karyotype, two distinct cell lines were identified (chromosomal mosaicism): one cell line with 45 chromosomes, containing a single X chromosome in the sex chromosome pair (X monosomy – 45,X), observed in 3 analyzed cells (20%); and an apparently normal male cell line with 46 chromosomes (46,XY), observed in 12 analyzed cells (80%). The cytogenetic result, according to the 2016 International System for Human Cytogenetic Nomenclature, was 45,X/46,XY (Fig. 7).

Cytogenetic testing of azoospermic patients identified structural variations in autosomal chromosomes in 8.3% of cases (n = 8). These included a variety of chromosomal rearrangements such as translocations 46,XY,t(1;19)(23.2q:q12.4); 46,XY,der(5),t(9;5); 45,XY,rob(13/14); 46,XY,inv(9)(p11q12); 46,XY,inv(9)(p13.21); 46,XY,15ps+; 46,XY,22sts; 46,XY,fra(17)(p12) (Table 3).

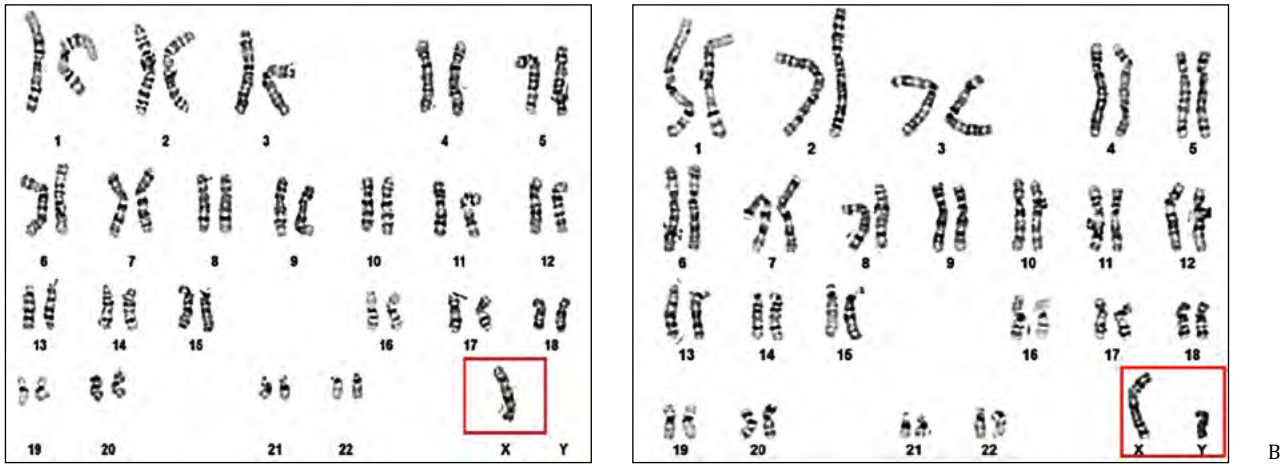

Fig. 7 Mosaic karyotype 45,X(3)/46,XY(12) of a 46-year-old patient [10]

Note: *The mosaic karyotype have two different cell lines: A: 45,X, characterized by monosomy X, which is commonly associated with features of Turner syndrome, was observed in 3 analyzed cells (20%). B: 46,XY, representing a normal male karyotype, cell line was observed in 12 cells (80%). The presence of this mosaicism can result in a wide spectrum of clinical presentations, depending on the distribution and proportion of each cell line in various tissues. In this case, the patient presents an apparently normal male phenotype, likely due to the predominance of the 46,XY cell line, which accounts for 80% of the analyzed cells.*

Table 3. Distribution of autosomal chromosomal abnormalities in men with azoospermia according to FSH levels

Autosomal abnormalities	FSH (mIU/ml)							
	Decreased <2		Normal 2–10		Increased >10		Total	
	n = 10	%	n = 58	%	n = 28	%	n = 96	%
Total	2	20.0	5	8.6	1	3.6	8	8.3
Reciprocal translocation							2	2.1
46,XY, t(1;19)(23.2q:q12.4)	1	10.0						
46,XY,der(5),t(9;5)			1	1.7				
Robertsonian translocation							1	1.0
45,XY,rob(13/14)			1	1.7				
Chromosomal inversions							2	2.1
46,XY,inv(9)(p11q12)			1	1.7				
46,XY,inv(9)(p13.21)	1	10.0						
Chromosomal polymorphisms							2	2.1
46,XY,15ps+					1	3.6		
46,XY,22sts			1	1.7				
Fragile site							1	1.0
46,XY,fra(17)(p12)			1	1.7				

Note*: FSH – follicle-stimulating hormone, measured in mIU/mL; n - number of cases; % - percentages correspond to the proportion within each FSH group. Statistical analysis: Categorical variables were compared using the Chi-square test or Fisher's exact test when appropriate. A p-value < 0.05 was considered statistically significant.*

The most frequent autosomal chromosomal abnormalities detected were balanced chromosomal rearrangements, identified in 5 cases. Translocations were the most common balanced chromosomal abnormalities, found in 3.1% (n = 3) of the total cohort (n = 96). In the present study, among 96 azoospermic men, simple reciprocal translocations were detected in 2.1% (n = 2) of cases – specifically t(1;19) and t(9;5). In one case, a Robertsonian translocation involving

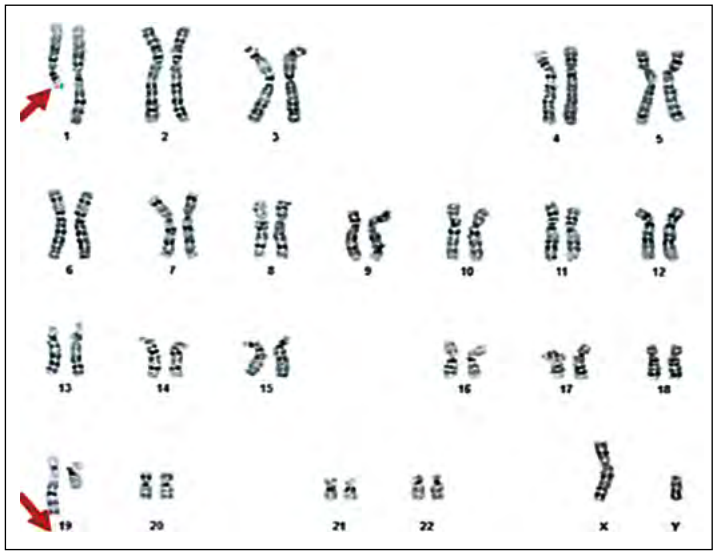

Fig. 8 Karyotype 46,XY,t(1;19)(q23.2;q13.4) of a 35-year-old patient

Note: *The karyotype 46,XY,t(1;19)(q23.2;q13.4) indicates the presence of a balanced translocation between the long arm (q) of chromosome 1 at region 23.2 and the long arm (q) of chromosome 19 at region 13.4, in a male with a normal chromosome number (46,XY). The chromosomes involved in the translocation are indicated by arrows. Metaphases counted: 33; Metaphases karyotyped: 10; Band resolution level: 650*

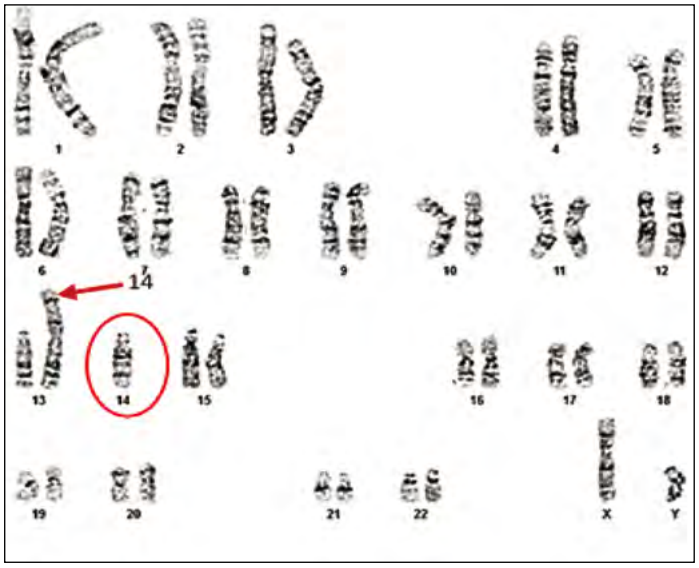

Fig. 9 Karyotype 45,XY,rob(13;14)(q10;q10) of a 31-year-old patient

Note: *The karyotype 45,XY,rob(13;14)(q10;q10) indicates a Robertsonian translocation between chromosomes 13 and 14 at the centromeric regions (q10;q10) in a male with a total of 45 chromosomes instead of the normal 46. Chromosome banding technique: GTG; Metaphases counted: 33; Metaphases karyotyped: 10; Band resolution level: 600*

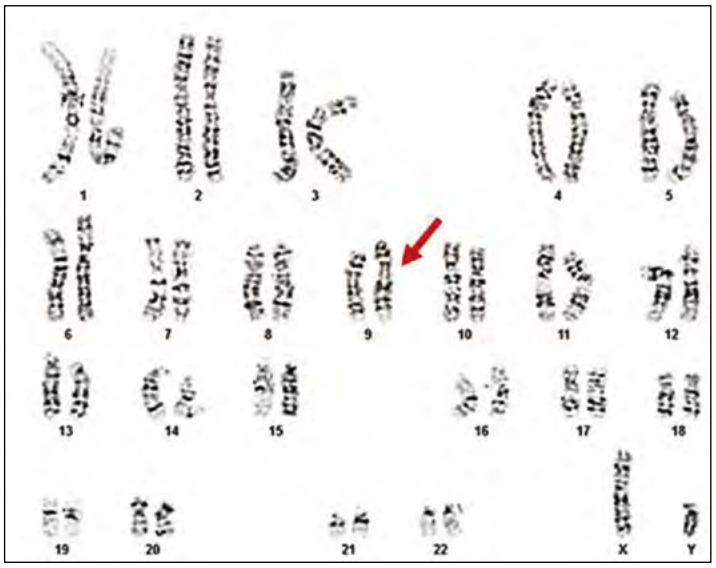

Fig. 10 Karyotype 46,XY,inv(9)(p11q12) of a 35-year-old patient

Note: *The karyotype 46,XY,inv(9)(p11q12) represents a male with a normal chromosome number (46 chromosomes) who carries a pericentric inversion on chromosome 9. This inversion involves a segment between the short arm region p11 and the long arm region q12. Metaphases counted: 15; Metaphases karyotyped: 10; Band resolution level: 550-570*

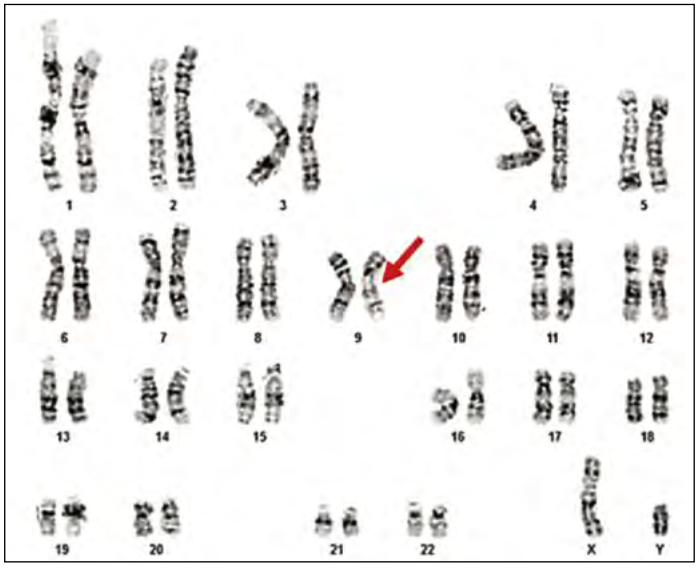

Fig. 11 Karyotype 46,XY,inv(9)(p13q21) of a 31-year-old patient

Note: *The karyotype 46,XY,inv(9)(p13q21) indicates a male with a normal chromosome number (46 chromosomes) who carries a pericentric inversion on chromosome 9. This inversion involves a segment between the short arm region p13 and the long arm region q21.*

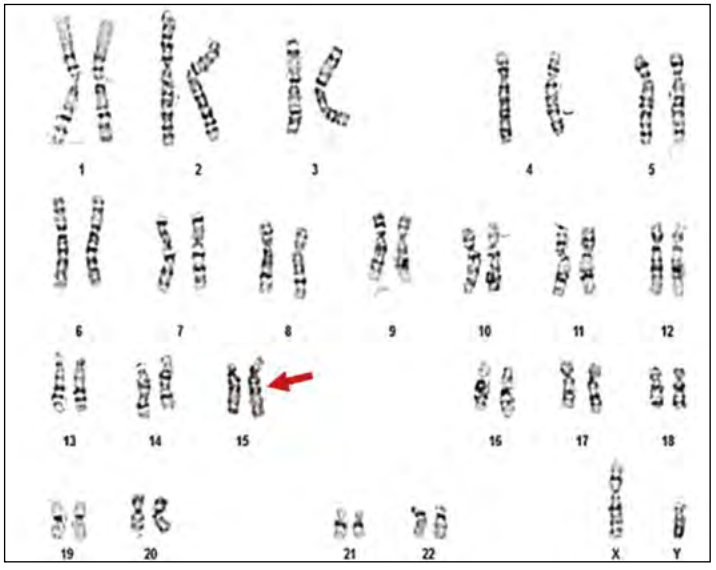

Fig. 12 Karyotype 46,XY,15ps+ of a 34-year-old patient

Note: *The image shows a standard human male karyotype with all 46 chromosomes. A chromosomal polymorphism is observed on chromosome 15, specifically on the short arm (p arm), and is noted as 15ps+. This indicates an enlargement of the satellite region of chromosome 15. The variation is considered benign and classified as a normal chromosomal polymorphism.*

the long arms of chromosomes 13 and 14 was identified, resulting in a karyotype with 45 chromosomes, present in all analyzed cells: rob(13;14) (Table 3, Fig. 8 and 9).

Chromosomal inversions were the most frequent balanced chromosomal rearrangements after translocations, identified in 2.1% (n = 2) of the total azoospermic men. Both patients exhibited pericentric inversions involving both arms of a chromosome from pair 9, detected in all analyzed cells: 46,XY,inv(9)(p11q12) and 46,XY,inv(9)(p13q21) (Table 3, Fig. 10, 11).

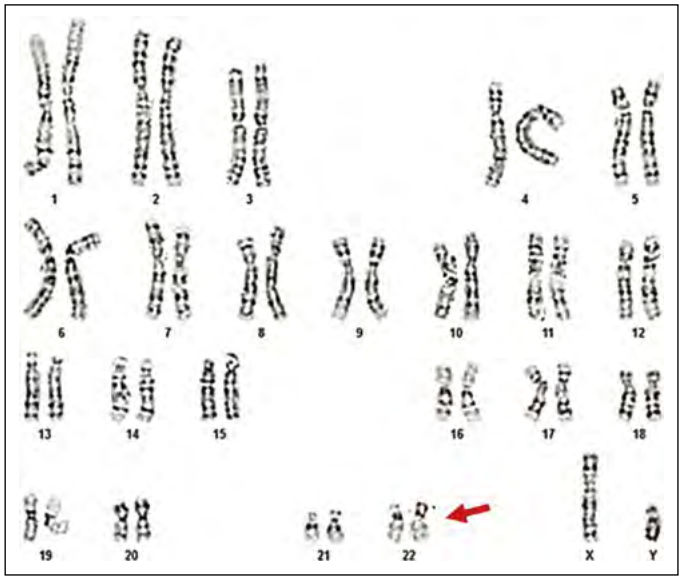

Fig. 13 Karyotype 46,XY,22ps+ of a 32-year-old patient

Note: *The karyotype shows a standard male chromosomal complement with a total of 46 chromosomes, including one X and one Y chromosome (46,XY). A chromosomal polymorphism is observed on chromosome 22, specifically on the short arm (p arm), and is designated as 22ps+. This indicates an enlargement of the satellite region on the short arm of chromosome 22. The polymorphism is indicated in the image with a red arrow.*

Chromosomal polymorphisms in the present study were identified in 2.1% (n = 2) of the 96 azoospermic men. One case exhibited an enlarged satellite region on the short arm of a chromosome from pair 15 (15ps+), and another on a chromosome from pair 21 (21ps+), observed in all analyzed cells (Table 3, Fig. 12, 13).

Discussion

Men with azoospermia have the highest risk of being carriers of genetic abnormalities. The prevalence of chromosomal variations in men with azoospermia has been reported in the literature to range between 15% and 25% [11]. According to the cytogenetic analysis results from the present study, out of a total of 96 infertile men with azoospermia, 75% (n = 72) exhibited a normal 46,XY karyotype, while 25% (95% CI: 24.1 – 25.9; n = 24) showed numerical or structural chromosomal variations (Table 1, Fig. 1). It is worth noting that the prevalence of chromosomal abnormalities identified in this study (25.0%, 95% CI: 24.1 – 25.9) among azoospermic men is consistent with findings reported in other countries from the same geographical region. For example, similar or slightly higher rates have been observed in Ukraine (35.0%, 95% CI: 32.7 – 37.3), Romania (30.0%, 95% CI: 29.1 – 30.9), and Turkey (19.2%, range 18.2 – 20.2), while slightly lower frequencies were reported in Bulgaria (20.7%, range 19.3 – 22.1), Morocco (13.1%, 95% CI: 12.9 – 13.2), and China (14.7%, 95% CI: 14.6 – 14.8). Other studies from Western countries reported comparable or lower prevalence, such as the Netherlands (14.5%, 95% CI: 14.4 – 14.5), the USA (13.3%, 95% CI: 13.0 – 13.5), Italy (10.5%, 95% CI: 9.8 – 12.0), and India (11.0%, 95% CI: 10.8 – 11.3) [11-16].

It is well known that sex chromosome abnormalities are the most common chromosomal causes of infertility [17]. In our study, among the total number (n = 96) of patients with azoospermia, the prevalence of sex chromosome abnormalities was identified in 16.7% of cases (n = 16), while autosomal abnormalities were observed in 8.3% of cases (n = 8). Among the total cohort (n = 96) of azoospermic men investigated cytogenetically, 11 presented X disomy, representing 11.5% (Fig. 1, Tables 1, 2). The results of our research are consistent with data from the literature, which report a similarly high frequency of KS among azoospermic men, ranging from 10% to 15%.

The phenotype of patients with KS is highly heterogeneous, being influenced by both the karyotype type and the age of the patient. During childhood, KS often remains undiagnosed due to its non-specific clinical presentation. Clinical manifestations typically become apparent in adulthood, with the onset of gynecomastia, hypogonadism, and infertility [18]. All patients in the present study were diagnosed in adulthood due to infertility. The cytogenetic variants identified in our study support the data in the literature, which report a high incidence (80-90%) of the classical 47,XXY form of KS, while mosaic forms are described in approximately 20% of cases. The 47,XXY aneuploidy is the most common sex chromosomal abnormality, with an incidence ranging from 1 in 500 to 1 in 1,000 live male births. Other cytogenetic variants involving X polysomies are rare: 48,XXXY (1 in 50,000) and 49,XXXXY (1 in 85,000) [19-20].

The identification of the cytogenetic variant in KS has significant clinical importance, as the severity of the clinical phenotype is directly proportional to the number of supernumerary X chromosomes. The phenotype depends on the extent of genetic imbalance caused by genes that escape X inactivation, androgen deficiency, and the sensitivity of androgen receptors. A greater degree of genetic imbalance, androgen deficiency, and impaired androgen receptor sensitivity correlates with a more severe phenotype. The clinical presentation of KS progressively worsens with each additional X chromosome [19, 21-23].

Among the total cohort (n = 96) of azoospermic men who underwent cytogenetic evaluation, one case was diagnosed with Y chromosome disomy, with a frequency of 1.04% (Table 2). Cytogenetic analysis revealed the presence of an extra Y chromosome in all analyzed cells, with a karyotype of 47,XYY (Fig. 4).

The 47,XYY syndrome is relatively common, occurring in approximately 1 in 1,000 live male births, making it the second most frequent sex chromosome abnormality after Klinefelter syndrome. However, up to 85% of XYY males remain undiagnosed. This was consistent with our case, as the 35-year-old male showed no clinical signs suggestive of a chromosomal abnormality, and the cytogenetic investigation was prompted solely by infertility due to azoospermia [24, 25].

According to the literature, most individuals with a 47,XYY karyotype exhibit normal spermatogenesis, while a minority may present varying degrees of spermatogenic impairment, ranging from normal to azoospermia [8, 25]. In the presented case, the patient had azoospermia, which may be explained by abnormal chromosomal pairing during spermatogenesis due to the presence of an additional Y chromosome.

FSH levels are typically elevated in such patients as a response to inadequate spermatogenesis, while testosterone levels are usually within normal limits or slightly increased. In this patient, both FSH and testosterone levels were within normal ranges.

Studies comparing the semen parameters of fertile and infertile men with XYY syndrome have shown that the majority of spermatozoa in these individuals have a normal karyotype.

In one azoospermic male from the total cohort (n = 96), a 46,XX karyotype was identified (1.04%) (Table 3, Fig. 5). The occurrence of 46,XX males in the general population is extremely rare (1 in 20,000), with a reported prevalence of approximately 0.9% among azoospermic men and 1–3% among normozoospermic men [9].

Following cytogenetic evaluation (46,XX karyotype), FISH analysis revealed two signals for the X chromosome and one signal for the Y chromosome (SRY, Yp11.32), and molecular genetic testing confirmed the presence of the SRY gene. Based on these findings, the diagnosis of testicular disorder of sex development (DSD), sex reversal, 46,XX male, also known as de la Chapelle syndrome, was established.

De la Chapelle syndrome is a form of sex reversal characterized by a female karyotype discordant with a male phenotype. Two categories have been described in the literature: SRY-positive cases, accounting for approximately 80% of patients, and SRY-negative cases, representing the remaining 20%. The present case falls into the SRY-positive category, as confirmed by multiplex PCR molecular testing and FISH analysis, which demonstrated the presence of the SRY gene translocated onto one of the X chromosomes [26, 27].

In one azoospermic patient, a 45,X/46,XY mosaic karyotype was identified (Table 3), a rare condition with a reported frequency of approximately 1 in 15,000 live births [10]. The clinical significance of 45,X/46,XY mosaicism is controversial in the literature and presents a major clinical challenge, as it is associated with a broad spectrum of manifestations ranging from infertility and ambiguous genitalia to phenotypically normal males [28-31].

According to the cytogenetic analysis, the patient with 45,X/46,XY mosaicism exhibited two distinct cell lines: 45,X (X monosomy) in 20% of the cells and a normal male 46,XY karyotype in 80% of the cells. This distribution explains the phenotypically male appearance and the absence of any suggestive clinical signs. The indication for karyotyping was a severely impaired semen analysis and infertility, which were diagnosed at a late age (46 years).

Y chromosome microdeletions represent the second most common genetic cause of impaired spermatogenesis in infertile men, following Klinefelter syndrome. In this study, two cases with structural variations of the Y chromosome were identified: 46,X,del(Y)(q11.21) (Y ≤ 21) and 46,XYqh+ (Y ≥ 18).

The prevalence of Y chromosome microdeletions is estimated to be approximately 1 in 2,000 to 1 in 3,000 men [32]. In this study, such a deletion was detected in 1 out of 96 azoospermic men, with a prevalence of 1.04% (Table 2). In a 36-year-old patient, an abnormal male karyotype was identified: 46,X,del(Y)(q11.21), indicating an unbalanced structural alteration (deletion) involving a portion of the long arm of the Y chromosome (region Yq11.21–qter) in all analyzed cells.

Polymorphic variants of the Y chromosome (Yqh+) have been reported in several studies concerning male infertility, particularly among men with azoospermia and severe oligozoospermia. In the present study, a chromosomal Y polymorphism was detected in one case with 46,XYqh+(Y ≥ 18), corresponding to a prevalence of 1.04% in the total sample (n = 96) (Table 2). Yqh+ represents a variation in the constitutive heterochromatin region of the Y chromosome, which cannot directly explain spermatogenic disorders. However, this topic remains controversial due to the potential role of heterochromatin, whose clinical significance has not yet been fully elucidated. The Y(≥18) variant results from excessive duplication of the DY21 sequence typical of the heterochromatic region of the Y chromosome. This may lead to mitotic errors, gene expression dysregulation, and impaired cellular differentiation, potentially resulting in gestational issues [27].

Autosomal chromosome variations have also been described in the literature among infertile patients, often without phenotypic manifestations. In the current study, among 96 azoospermic men, such variations were found in 8.3% (n = 8) of cases (Table 3).

The most frequent autosomal findings were balanced chromosomal rearrangements, found in 5 cases. Among these, translocations were the most common type of balanced rearrangement. In the current study, they were detected in 3.1% (n = 3) of the total cohort (n = 96). Balanced chromosomal translocations involve breaks in two chromosomes and the abnormal rearrangement of chromosomal fragments, resulting in the exchange of genetic material without any loss. This typically explains why most translocation carriers have a normal phenotype. Azoospermia is largely attributed to the possibility that one of the breakpoints disrupts a gene directly involved in spermatogenesis, potentially leading to its arrest or incomplete development.

In this study, among 96 azoospermic men, simple balanced translocations were detected in 2.1% (n = 2) of cases – t(1;19) and t(9;5) (Fig. 8). In one case, a Robertsonian translocation involving 45 chromosomes was identified, affecting the long arms of chromosomes 13 and 14 in all analyzed cells: rob(13;14) (Fig. 9). According to the literature, this translocation between chromosomes 13 and 14 is the most common, followed by translocation between chromosomes 14 and 21 [33].

Chromosomal inversions are the second most common type of balanced chromosomal rearrangement after translocations. This finding is also supported by the current study, in which inversions were identified in 2.1% (n = 2) of cases among azoospermic men (Table 3, Fig. 10, 11). The probability that inversion carriers produce abnormal gametes due to meiotic crossover events ranges from 1% to 10%. As a result of homologous chromosome recombination within the inversion loop, four types of gametes can be produced: one normal, one with the inversion, and two partially duplicated or deleted [34].

Conclusions

The detection of an abnormal karyotype is highly important for comprehensive and effective genetic counseling. This process should include detailed information about the specific type of chromosomal abnormality or polymorphism, its clinical relevance, potential inheritance patterns, genetic risk to offspring, and available options for prenatal diagnosis.

Genetic counseling guides infertile couples in making informed decisions regarding medically assisted reproduction. Therefore, cytogenetic and molecular genetic screening remains a valuable practice for accurate diagnosis, assessment, prognosis, and successful treatment.

Competing interests

None declared.

Authors' contribution

SR conceived conceptualization, methodology, data collection, analysis and interpretation, writing – original draft preparation. SR, EC and VM analyzed the result. MS, VR and AM – supervision on differential diagnosis data. MS and SC – research coordinator, conceived writing review and editing, validation. The authors read and approved the final version of the manuscript.

Patient consent

Obtained

Ethics approval

This study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, Protocol No 48 of April 12, 2018.

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RESEARCH ARTICLES

Electrocardiographic changes in patients with acute pulmonary embolism

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ABSTRACT

Introduction. Pulmonary embolism is a life-threatening condition requiring prompt diagnosis. While contrast-enhanced computed tomography is the gold standard, its limited availability and associated risks necessitate complementary diagnostic tools. Recent studies suggest that combining multiple electrocardiographic abnormalities into a composite score may enhance diagnostic performance.

Material and methods. We conducted a prospective, cross-sectional study involving 200 patients with suspected pulmonary embolism admitted to two hospitals in the Republic of Moldova between 2022 and 2025. Among them, 168 had confirmed pulmonary embolism based on computed tomography pulmonary angiography, while 32 patients with similar symptoms but negative imaging served as the control group. All participants underwent a standard 12-lead electrocardiogram upon admission. A composite electrocardiographic score was applied, incorporating 10 criteria (e.g., sinus tachycardia, S1Q3T3 pattern, negative T waves in V1-V4, right bundle branch block, and right axis deviation), with a total score ranging from 0 to 12. Diagnostic thresholds were defined as follows: 0-3 low risk, 4-6 intermediate risk, and ≥7 high risk.

Results. Electrocardiographic abnormalities such as negative T waves in V1–V4 (42.9% vs. 6.2%), atrial fibrillation (28.0% vs. 6.2%), and S1Q3T3 pattern (21.4% vs. 3.1%) were significantly more frequent in pulmonary embolism patients. A composite electrocardiographic score of ≥5 demonstrated excellent diagnostic performance: sensitivity 89.9%, specificity 93.8%, positive predictive value 98.7%, and overall accuracy 90.5%. The area under the ROC curve was 0.92, indicating strong discriminative ability. Among combinations of electrocardiographic findings, the pairing of S1Q3T3 with negative T waves in V1-V4 showed a statistically significant association with confirmed pulmonary embolism.

Conclusions. Our study confirms that a composite electrocardiographic score ≥5 is a highly effective, rapid, and noninvasive tool for identifying pulmonary embolism, improving early triage, particularly in emergency settings where imaging may be delayed. The model significantly outperforms isolated electrocardiographic findings and should be interpreted within a broader clinical context, including symptomatology and imaging when available.

Keywords: pulmonary embolism, electrocardiographic changes.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Although multiple isolated electrocardiographic (ECG) findings for diagnosing acute pulmonary embolism have been described in the literature, their sensitivity is limited, and their practical utility remains low. It is still unclear how effective a composite ECG score, applied systematically, would be in the emergency setting for diagnosing acute pulmonary embolism compared to classical diagnostic methods.

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The research hypothesis

Applying a composite ECG score of ≥5 significantly improves diagnostic accuracy for acute pulmonary embolism and can serve as an efficient triage tool when computed tomography angiography is not immediately available.

The novelty added by the manuscript to the already published scientific literature

This manuscript prospectively validates a composite ECG score in a cohort of 200 patients and demonstrates high diagnostic accuracy (AUC = 0.92), superior to individual ECG signs. It is an innovative study to systematically evaluate the performance of an integrated ECG score for diagnosing acute pulmonary embolism.

Introduction

Pulmonary embolism (PE) is a frequent and potentially fatal cardiovascular emergency, especially in the absence of a rapid and accurate diagnosis. Contrast-enhanced CT imaging (CTPA) is the reference method, but it has important limitations related to availability, radiation exposure, and renal toxicity [1]. In this context, rapid and non-invasive tools for initial triage of patients remain essential.

The electrocardiogram (ECG), although present in all emergency services, is often underutilized due to the low sensitivity of isolated findings. However, recent literature shows that composite ECG models, which combine multiple abnormalities, can significantly increase diagnostic accuracy. Su et al. developed an ECG model based on 8 criteria, which showed a sensitivity of 79% and an area under the ROC curve of 0.87 (AUC) in identifying acute PE [2]. Other studies confirm these results, underlining the importance of the added value of right-sided leads and early T wave changes. In the same vein, combined ECG scores have shown superior performance compared to classic clinical scores (Wells, Geneva), especially when integrated with D-dimer [3, 4].

The purpose of this study was to evaluate the diagnostic value of electrocardiographic findings in acute pulmonary embolism by integrating multiple ECG abnormalities into a composite diagnostic score.

Material and methods

This prospective, cross-sectional study was conducted between January 2022 and February 2025 in two hospitals in Chișinău, Republic of Moldova: the *Holy Trinity* Municipal Clinical Hospital (163 patients) and the Institute of Cardiology (37 patients). The study protocol was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova (minutes No. 2/25, March 30, 2023).

Participant selection. A total of 200 adult patients with clinical suspicion of acute PE were evaluated, and all underwent CTPA for diagnostic confirmation. The diagnosis was based on PE guideline recommendations [1]. Inclusion criteria: age ≥18 years, clinical suspicion of acute PE, availability of ECG and CTPA results. Differential diagnoses included acute and chronic coronary syndrome, dissecting aneurysm of the aorta, acute abdomen, and acute heart and respiratory failure [5]. Based on CTPA results, acute PE was confirmed in 168 patients, and 32 patients with similar symptoms but no filling defects were assigned to the control group.

Electrocardiographic analysis. All participants underwent a standard 12-lead electrocardiogram upon admission. Based on literature evidence [1-4], a composite ECG score was developed, including 10 criteria known to correlate with PE (Table 1). Each ECG parameter was assigned a score of 1 or 2 points based on its diagnostic weight. The total score ranged from 0 to 12 points, similar in structure to the model proposed by Su et al. [2].

Table 1. Components and scoring of the ECG-based composite score for pulmonary embolism

Parameter	Score
Sinus tachycardia (>100 bpm)	1
Atrial fibrillation	2
T wave inversions in V1–V4	1
S1Q3T3 triad	2
Qr pattern in V1	1
Complete right bundle branch block	1
Right axis deviation	1
P pulmonale	1
QTc > 460 ms	1
ST depression in V1–V3	1

Note*: ECG = Electrocardiogram; PE = Pulmonary embolism; QTc = Corrected QT interval; bpm = Beats per minute; V1–V4 = Precordial leads V1 through V4; S1Q3T3 = S wave in lead I, Q wave in lead III, and inverted T wave in lead III; Qr = Q wave followed by a prominent R wave (suggestive of right ventricular conduction); T wave inversion = Negative T waves, typically indicating right ventricular strain.*
A 10-item ECG score (0–12) was used; ≥5 indicated high PE probability.

Electrocardiographic score interpretation. Patients were categorized into risk classes as follows: 0-3 points – low probability, 4-6 points – intermediate probability, and ≥7 points – high probability (recommendation for immediate imaging).

Statistical analysis. All statistical analyses were performed using IBM SPSS v26.0. Continuous variables were expressed as mean ± SD, and categorical variables as counts

and percentages. Comparisons between groups used the chi-square test. To assess the diagnostic performance of the ECG score (cutoff ≥5), the following metrics were calculated: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, positive and negative likelihood ratios (LR^+, LR^-), post-test probabilities, receiver operating characteristic (ROC) curve, and area under the curve (AUC).

Results

Analyzing the distribution of ECG abnormalities, such as T wave inversions in V1-V4, S1Q3T3 triad, atrial fibrillation, and right axis deviation, showed that they were much more frequent in the acute PE group, supporting their diagnostic value (Table 2).

Table 2. Frequency of individual ECG abnormalities in patients with and without pulmonary embolism

ECG Finding	PE (n = 168)	Non-PE (n = 32)	PE (%)	Non-PE (%)
Sinus rhythm	110	28	65.5%	87.5%
Atrial fibrillation	47	2	28.0%	6.2%
Bundle branch block	19	2	11.3%	6.2%
S1Q3T3 triad	36	1	21.4%	3.1%
P pulmonale	32	1	19.0%	3.1%
Right axis deviation	31	1	18.5%	3.1%
Qr in V1	37	2	22.0%	6.2%
R/S ratio > 1 in V1	26	1	15.5%	3.1%
Negative T waves in V1–V4	72	2	42.9%	6.2%
ST depression in V1–V3	44	1	26.2%	3.1%
QTc > 440 ms	33	1	19.6%	3.1%

***Note**: Data are presented as absolute frequencies and percentages. ECG = Electrocardiogram; PE = pulmonary embolism; QTc = Corrected QT interval; n = number of patients with the corresponding finding.*
% = proportion relative to the total number of patients in each group (PE: n = 168; Non-PE: n = 32).

The comparative analysis of ECG criteria between patients with and without acute PE revealed significant differences in the frequency of electrocardiographic abnormalities. Classic changes associated with PE, such as negative T waves in leads V1-V4 (42.9% vs. 6.2%), the S1Q3T3 triad (21.4% vs. 3.1%), and right axis deviation (18.5% vs. 3.1%), were considerably more frequent in the acute PE group, suggesting important predictive value. Atrial fibrillation was also almost five times more prevalent in PE patients (28% vs. 6.2%). Sinus rhythm was predominant in the non-PE group (87.5%), which supports the idea that the presence of arrhythmias or significant conduction deviations may raise suspicion of PE. Other signs, such as ST segment depression in V1-V3, Qr in V1, and prolonged QTc, were also more frequent in the acute PE group, but with relatively lower specificity. These findings confirm that certain combinations of ECG signs can add clinical value in supporting the diagnosis of acute PE and may guide prioritization of imaging studies, especially in resource-limited settings.

Table 3. Frequency of combined ECG abnormalities in patients with and without pulmonary embolism

ECG Combination	Patients with PE (n = 168)	Patients without PE (n = 32)	PE %	Non-PE %
S1Q3T3 + negative T waves in V1–V4	34	1	20,2%	3,1%
Atrial fibrillation + negative T in V1–V4	27	2	16,1%	6,2%
Bundle branch block + negative T in V1–V4	20	1	11,9%	3,1%
Qr in V1 + negative T in V1–V4	24	2	14,3%	6,2%
S1Q3T3 + right axis deviation	17	1	10,1%	3,1%

***Note**: ECG = Electrocardiogram; PE = pulmonary embolism; ECG Combination refers to the simultaneous presence of two electrocardiographic abnormalities observed in the same patient, used to assess their joint diagnostic value for pulmonary embolism.*

The analysis of associations between two ECG criteria (Table 3) showed that certain combinations are significantly more frequent among patients with acute PE than among those without PE, suggesting increased diagnostic value in the concurrent presence of multiple electrocardiographic signs. The most prevalent combination was S1Q3T3 + negative T waves in V1–V4, found in 20.2% of patients with acute PE, compared to only 3.1% of patients without acute PE, highlighting its possible specific character. Additionally, the combinations between atrial fibrillation and negative T waves (16.1% vs. 6.2%) or bundle branch block and negative T waves (11.9% vs. 3.1%) were also much more frequent in the acute PE group, suggesting a link between these changes and the severity of cardiopulmonary impairment. The combination of negative T waves with Qr in V1 or right axis deviation also showed a clear difference between the groups, supporting the idea that multiple ECG changes increase the pre-test probability of acute PE. These findings support the use of composite ECG scores and could guide the development of predictive algorithms that consider the coexistence of multiple ECG changes rather than evaluating them in isolation.

Table 4. Statistical significance of ECG combinations

ECG Combination	χ^2	p-value
S1Q3T3 + negative T waves in V1–V4	4.33	0.0374
Atrial fibrillation + negative T in V1–V4	1.37	0.2411
Bundle branch block + negative T in V1–V4	1.37	0.2419
Qr in V1 + negative T in V1–V4	0.91	0.3411
S1Q3T3 + right axis deviation	0.87	0.3523

Note: ECG = Electrocardiogram; χ^2 = chi-square statistic; p-value = probability value.

The only combination with a statistically significant difference (Table 4) between patients with and without PE was: "S1Q3T3 + negative T waves in V1-V4", reinforcing its value as a high-specificity composite ECG indicator. Among the combinations analyzed, only the association "S1Q3T3 + negative T waves in V1-V4" showed a statistically significant

difference between patients with and without pulmonary embolism (p = 0.0374), supporting its potential as a composite ECG marker with increased predictive value.

Table 5. Performance of the ECG score

Indicator	Value (%)
Sensitivity	89.9%
Specificity	93.8%
Positive Predictive Value (PPV)	98.7%
Negative Predictive Value (NPV)	63.8%
Overall Accuracy	90.5%
Positive Likelihood Ratio (LR^+)	14.38
Negative Likelihood Ratio (LR^-)	0.11
F1 Score	94.2%

***Note**: The ECG score was considered positive at a threshold ≥5. ECG = Electrocardiogram; PE = pulmonary embolism.*

The analysis of the performance of the composite ECG score (Table 5), applied to a cohort of 200 patients (168 with confirmed acute PE and 32 without PE), showed a sensitivity of 89.9% and a specificity of 93.8%, indicating an excellent ability to correctly identify patients with acute PE, as well as to exclude unconfirmed cases. The high PPV of 98.7% confirms that an ECG score ≥5 is strongly associated with the presence of acute PE and may justify initiating treatment even in the absence of immediate imaging. The overall accuracy of 90.5% reveals the robustness of this score in clinical contexts. The positive likelihood ratio (LR^+) of 14.38 indicates a significant increase in post-test probability of acute PE, while the negative LR^- of 0.11 supports the utility of the score in ruling out the diagnosis when values fall below the threshold. Lastly, the F1 score of 94.2%, which combines sensitivity and PPV, reflects an optimal balance between detecting true cases and avoiding overdiagnosis (Fig. 1).

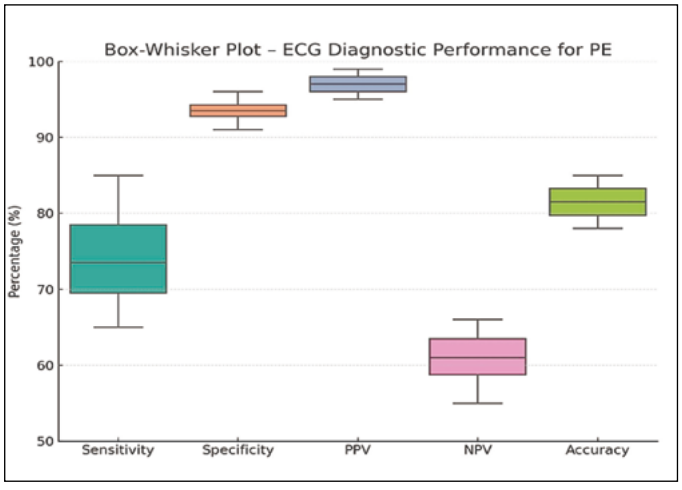

Fig. 1 Box-Whisker Plot showing the variability in diagnostic performance metrics of ECG criteria for PE.

***Note**: The Y-axis represents percentage values (%), and each box indicates the distribution of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. Boxes indicate interquartile ranges; whiskers represent variability; central lines indicate medians.*

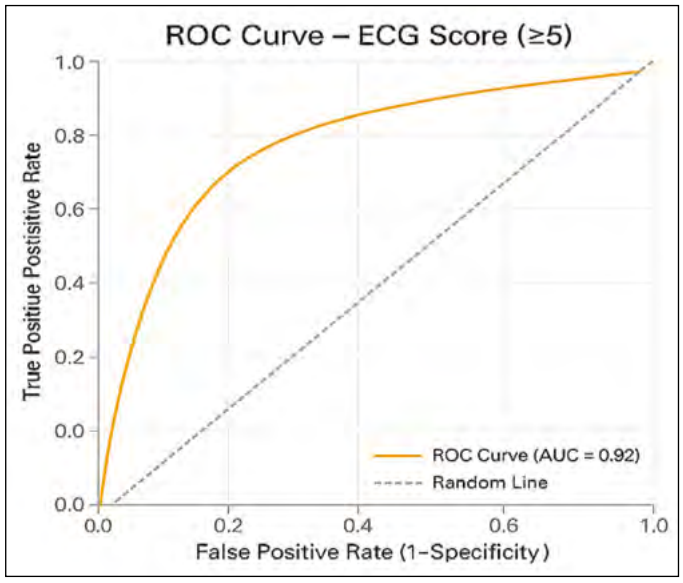

Fig. 2 ROC curve for the ECG composite score (threshold ≥5), illustrating its diagnostic performance in discriminating between patients with and without pulmonary embolism.

***Note**: The Y-axis represents the true positive rate (sensitivity), and the X-axis represents the false positive rate (1 – specificity).*

The plot underlines that specificity and PPV are higher and more stable, while sensitivity and NPV show lower values and greater dispersion. A Receiver Operating Characteristic (ROC) curve was generated to evaluate the diagnostic performance of the composite ECG score for acute pulmonary embolism (Fig. 2).

The analysis yielded an AUC of 0.92, indicating excellent discriminative ability of the ECG score (cutoff ≥5) in distinguishing patients with and without acute PE. The ROC curve demonstrated a steep rise toward the upper-left corner, reflecting high sensitivity with a relatively low false-positive rate.

Discussion

The rapid diagnosis of pulmonary embolism (PE) remains a major challenge in clinical practice, particularly in settings where imaging is not immediately available. Our study helps to fill an important gap in the current literature by demonstrating that the use of a composite ECG score ≥5 provides significant diagnostic value and practical utility in the initial triage of patients with suspected acute PE.

Our findings confirm that certain combinations of electrocardiographic changes, such as S1Q3T3 combined with negative T waves in V1-V4, have greater predictive value compared to isolated signs. While these changes are not exclusive to PE, their simultaneous presence suggests acute right ventricular overload, supporting the pathophysiological hypothesis that PE produces sudden strain on the right heart chambers, which is reflected in distinct ECG changes [6].

The ROC value of 0.92 for the ECG score demonstrates excellent discriminative capacity, surpassing the perfor-

mance previously reported for individual ECG scores or isolated models (e.g., the Daniel score) [2]. A sensitivity of 89.9% and a specificity of 93.8% support the clinical applicability of this model in settings with limited access to imaging or in cases of hemodynamic instability.

Moreover, the positive predictive value of 98.7% suggests that a score ≥5 may justify early initiation of treatment, including anticoagulation, even before tomographic confirmation – potentially reducing the risk of major complications or death.

By integrating multiple ECG parameters with pathophysiological relevance, the proposed score offers a more "global" assessment of right ventricular dysfunction, overcoming the limitations of isolated ECG signs. This multidimensional approach has the potential to be used as a pretest algorithm, enhancing rapid clinical decision-making, particularly in overcrowded emergency departments [2-4].

Nevertheless, it is important to emphasize that ECG should not be seen as a substitute for CT angiography, but rather as a supportive tool that can contribute to risk stratification – especially when combined with other tools such as the Wells score, D-dimer levels, or echocardiography [1].

Conclusions

The ECG composite score ≥5 demonstrates excellent diagnostic utility for acute pulmonary embolism and may serve as a rapid, noninvasive triage tool in emergency settings. Its high discriminatory capacity, supported by sensitivity, specificity, and likelihood ratios, validates its integration into early clinical decision-making. Compared to isolated ECG signs or traditional scores, the composite ECG approach offers an objective advantage in identifying right ventricular strain. Future validation in larger, multicenter cohorts is warranted to confirm its applicability in diverse clinical environments.

Competing interests

None declared.

Authors' contributions

DR – conceived the study design and performed the statistical analysis, CT – participated in the diagnosis and treatment of patients with pulmonary embolism, SM – reviewed the work critically, NC – participated in the study design and critically reviewed the work. All the authors approved the final version of the manuscript.

Ethics approval

The study protocol was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova (minutes No. 2/25, March 30, 2023).

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RESEARCH ARTICLE

Optimizing surgical management of thyroid nodules: a prospective study on an individualized approach

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ABSTRACT

Introduction. Thyroid nodules are a common condition in medical practice, and the correct selection of patients for surgical treatment is essential in their management. Thyroidectomy is indicated with predilection for nodules suspected of being malignant, with progressive growth or with compressive symptoms.

Materials and methods. The study included 89 patients diagnosed with unilateral or bilateral thyroid nodules who met the indications for surgical treatment. All patients underwent preoperative evaluation, including clinical examination, standard laboratory tests, thyroid ultrasonography, hormonal profiling, and fine-needle aspiration in cases with a high suspicion of malignancy.

Results. Out of the total number of patients, 2 (2.25%) patients had contraindications to surgery due to cardiac comorbidities associated with high anesthetic-surgical risk. Total thyroidectomy was performed in 41 patients (46.09%), hemithyroidectomy in 44 patients (49.41%), and combined surgeries in 2 cases (2.25%). Postoperative outcomes were favorable in all patients, with no complications registered during the follow-up period. Hormone replacement therapy was administered based on postoperative thyroid hormone levels. Patients who did not undergo surgery were followed up regularly.

Conclusions. The extent of thyroidectomy should be tailored to each patient based on the estimated risk of malignancy and the clinical-imaging features of nodules. Total thyroidectomy is recommended in cases with suspected malignancy or bilateral disease, whereas lobectomy may be sufficient for unilateral benign nodules or low malignancy risk.

Keywords: thyroid nodules, thyroidectomy, surgical management.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Thyroid nodules represent a prevalent clinical entity in the adult population, posing a complex multidisciplinary challenge due to the current lack of a universally accepted management among healthcare professionals.

The research hypothesis

An individualized surgical approach to thyroid nodules, guided by clinical, imaging, cytological, and histological criteria, reduces the rate of overdiagnosis and improves postoperative outcomes.

The novelty added by manuscript to the already published scientific literature

Our study examines the optimal clinical management of patients with thyroid nodules, with particular emphasis on relevant considerations related to their surgical approach.

Introduction

Thyroid nodules are defined as abnormal, localized proliferations within the thyroid gland, and encompass a large spectrum of lesions, including colloid or cystic nodules, lymphocytic thyroiditis nodules, nodular or multinodular goiters, autonomously functioning nodules and tumors such as thyroid adenoma or carcinoma [1-3].

The incidence of thyroid nodules has increased over recent decades, largely due to the widespread use of imaging modalities such as neck ultrasonography, CT, and MRI, which frequently identify incidental nodules. Epidemiological studies suggest a strong correlation with aging, female sex, iodine deficiency, and exposure to ionizing radiation [4].

Current guidelines issued by professional societies provide frameworks for the evaluation of thyroid nodules followed by surveillance or treatment recommendations, which are usually based on cytologic results, while histopathological diagnosis remains the „gold standard" [5]. From this perspective, the management of thyroid nodules remains debatable regarding surgical indications, the extent of thyroidectomy, and the tactics in indeterminate or low-risk lesions.

Surgeons have a decisive role in the multidisciplinary management of patients with thyroid nodules, particularly when surgical treatment should be considered according to clinical, radiologic, or cytologic findings [6, 7].

The aim of our study is to optimize the diagnostic and surgical treatment strategies in patients with thyroid nodules through individualized approaches based on malignancy risk and clinico-imaging features.

Material and methods

This prospective study was conducted in the Department of Surgery No. 5 of the *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova, between 2022 and the first semester of 2025. After applying inclusion criteria, 89 patients diagnosed clinically and/or radiologically with thyroid nodules were enrolled into the study. Prior to enrollment, all participants signed written informed consent forms in accordance with the approval of the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy Minutes No. 84, from June 07, 2017.

Diagnostic workup included initially medical history collection and physical examination. Thyroid hormonal status was assessed through TSH, FT4 and FT3 levels. In addition to standard investigations, the serum values of autoimmune markers - anti-TPO (anti-thyroperoxidase), anti-TG (anti-thyroglobulin) antibodies and the tumor marker - calcitonin, were determined for appraisal of the underlying etiology of thyroid nodules.

Neck ultrasonography was performed in all patients as a first-line imaging tool for the visualization of the thyroid gland and regional lymph nodes. Thyroid nodules were depicted by location, size, shape, composition, echogenicity, margins, presence of calcifications, and associated lymphadenopathy and were reported in categories according to the Thyroid Imaging Reporting and Data System (TI-RADS), as follows: TI-RADS 1 – normal thyroid gland; TI-RADS 2 – benign nodules; TI-RADS 3 – probably benign; TI-RADS 4 – suspicious for malignancy; TI-RADS 5 – probably malignant. Doppler mode and sonoelastography have enhanced the diagnostic value of ultrasonography by providing additional information on the vascular patterns and the elasticity of thyroid nodules.

Fine-needle aspiration biopsy (FNAB) was indicated in echographically suspicious for malignancy solitary or dominant thyroid nodules and cytological findings were interpreted and reported in accordance with the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC): I – non-diagnostic or unsatisfactory; II – benign; III – atypia of undetermined significance or follicular lesion of undetermined significance; IV – follicular neoplasm or suspicious for a follicular neoplasm; V – suspicious for malignancy; VI – malignant.

Thyroid MRI (Magnetic Resonance Imaging) was used as an advanced imaging modality to evaluate possible extrathyroidal extension of disease and to determine the presence or extent of metastatic involvement in cervical lymph nodes, especially in cases where ultrasound findings were inconclusive.

Intraoperative frozen section (IFS) was performed in selected cases, particularly those with suspicious preoperative features or indeterminate cytological findings, in order to confirm the malignancy and had an important role in real-time surgical decision-making, especially in determining the volume of surgery – limitation to hemithyroidectomy or extension to total thyroidectomy.

Histopathological findings were crucial in confirming the final diagnosis and determining appropriate treatment strategies. The patients were actively monitored over a 24-month period to assess postoperative evolution and detect any signs of recurrence or complications.

The obtained data were processed and analyzed using IBM SPSS Statistics software, version 26.0 (IBM Corp., Armonk, NY) and Microsoft Excel. Absolute and relative values were calculated, expressed in frequencies (n) and percentages, while continuous variables were summarized using means and standard deviations. A 95% confidence interval (CI) was calculated to estimate the precision of observed effects, and a p-value < 0.05 was considered statistically significant in comparable variables.

Results

The study included 89 patients with thyroid nodules who met the inclusion criteria. The age of the patients ranged from 20 to 77 years, with a significant predominance of women - 78 (87.64%) cases (Table1).

The most commonly reported symptoms among patients included discomfort or pain during swallowing (n = 89; 100%), perceived as a "globus sensation", unexplained fatigue (n = 72; 80.89 %), and a mass (lump) in the anterior cervical region (n = 45; 50.56%) (Table 1). Other less frequent complaints were emotional instability and irritability (n = 34; 38.2%), dyspnea (n = 14; 15.73%), and dysphonia (n = 3; 3.37%).

While most nodules exhibited a slow, symptomatic evolution, and patients were monitored by the family physician or endocrinologist, 12 patients (13.48%) experienced rapid nodule growth within a period ranging from 5 days to 6 months, which prompted earlier surgical referral. At the time of admission, most of the patients presented with unilateral lesions of the thyroid gland as solitary nodules (n = 38; 42.7%), or multinodularity of one lobe (n = 5; 5.61%). Bilateral lesions were consisted of multinodular goiter (n = 32; 35.97%) or toxic diffuse-nodular goiter (n = 8; 8.99%). It is important to note that nodule recurrence was documented in 4 cases (4.49%). Two cases involved contralateral lobe recurrence, one occurred following partial resection of the left thyroid lobe, and one case was identified after total thyroidectomy, according to the medical record excerpt. Combined lesions were present in 2 patients (2.25%). The demographic and clinical data of the patients are summarized in Table 1.

Table 1. Demographic and clinical characteristics of patients

Variable	Value (n, %, 95% CI)
Mean age	48.4 ± 14.2 years
Gender	
Women	78 (87.64 %) (95% CI: 80.8 - 94.5)
Men	11 (12.36 %) (95% CI: 5.5 - 19.2)
Gender ratio	7:1 (p < 0.0001)
Complaints	
Discomfort or pain during swallowing	89 (100%) (95% CI: 100 - 100)
Fatigue	72 (80.89%) (95% CI: 72.7 - 89.1)
Lump in the anterior cervical region	45 (50.56%) (95% CI: 40.2 - 60.9)
Duration of the disease	5 days – 20 years
Preventive diagnosis	
Solitary nodule	38 (42.7%) (95% CI: 32.45 - 52.95)
Multinodular goiter	37 (41.58%) (95% CI: 31.34-51.80)
Toxic diffuse-nodular goiter	8 (8.99%) (95% CI: 3.05 - 14.93)
Recurrence	4 (4.49%) (95% CI: 0.18 - 8.80)
Cyst of the right lobe and isthmus nodule	1 (1.12%) (95% CI: 0 - 3.32)
Left nodular goiter and left parathyroid adenoma	1 (1.12%) (95% CI: 0 - 3.32)
Comorbidities	
Diabetes mellitus, arterial hypertension, etc.	5 (5.61%) (95% CI: 0.84 – 10.4)

***Note**: The results were analyzed descriptively, presenting absolute numbers - n, percentages - %, and 95% confidence intervals – 95% CI. A chi-squared test was used to evaluate the relationship between the gender variables. The resulting p-value indicates a highly significant difference between genders.*

Based on serum thyroid hormone levels, all patients were in a euthyroid hormonal status, which in 8 (8.99%) cases of toxic diffuse-nodular goiter was maintained through the administration of antithyroid medication. Although TSH levels were within the normal range in most cases, in 2 patients (2.25%) they were below the reference range, while FT3 and FT4 remained within normal limits (Table 2).

Elevated levels of either anti-thyroglobulin (Anti-TG) or anti-thyroid peroxidase (Anti-TPO) antibodies (not both simultaneously) were observed in 5 cases (5.61%), and revealed an autoimmune thyroiditis etiology of the nodules.

In 6 cases, serum calcitonin values exceeded 2.0 pg/ml and were associated with follicular adenoma (n = 2), and papillary carcinoma (n = 4). While elevated calcitonin is typically considered a marker for medullary thyroid carcinoma, its increase in these cases may suggest atypical secretion or cross-reactivity.

The ultrasound evaluation revealed that the size of thyroid nodules in greatest diameter in solitary lesions varied between 1.0 cm and 6.0 cm, and from 0.8 cm to 3.5 cm in multinodular lesions.

The majority of thyroid nodules were ultrasonographically classified as TI-RADS 4, followed by TI-RADS 3, while a smaller proportion were categorized as TI-RADS 5, which reflects a predominance of nodules with suspicious features. Doppler ultrasound revealed mixed vascular patterns in the evaluated nodules, while elastography demonstrated predominantly elastic tissue. Sonographic examination revealed laterocervical lymphadenopathy in two cases (Table 2).

FNAB was performed on solitary nodules or dominant nodules within unilateral multinodular goiters measuring 1.0 cm and larger. The distribution of cytological results among the TBSRTC categories was non-significant according to the frequencies (Table 2). Papillary carcinoma was histopathologically confirmed in one case from each category III and IV. In two cases reported as TBSRTC V, malignancy was not determined. Both cases classified as TBSRTC VI were diagnosed with papillary carcinoma. It is noteworthy that 10 patients declined FNAB due to concerns about potential bleeding or other complications.

MRI was used in three cases: two cases of suspicious cervical lymphadenopathy identified via ultrasonography, and in one case for a detailed assessment of a parathyroid lesion.

Table 2. Relevant investigations results

Variable	Value (n, %, 95% CI)
TSH	*1.8 ± 1.42 uIU/ml*
TSH ↓	2 (2.25%) (95% CI: 0 - 5.33)
Hormonal status	
Euthyroidism	81 (91.01%) (95% CI: 85.10 - 96.90)
Euthyroidism (maintained with antithyroid drugs)	8 (8.99%) (95% CI: 3.10 - 14.80)
Thyroid ultrasound	*89 (100 %) (95% CI: 100 - 100)*
TI-RADS 3	30 (33.7%) (95% CI: 23.9 - 43.5)
TI-RADS 4	52 (58.43%) (95% CI: 48.2 - 68.6)
TI-RADS 5	7 (7.87%) (95% CI: 2.2 - 13.5)
FNAB	*13 (14.61%) (95% CI: 8.5 - 23.9)*
TBSRTC II	3 (23.08%) (95% CI: 6.2 - 54.0)
TBSRTC III	2 (15.38%) (95% CI: 2.1 - 45.5)
TBSRTC IV	3 (23.08%) (95% CI: 6.2 - 54.0)
TBSRTC V	3 (23.08%) (95% CI: 6.2 - 54.0)
TBSRTC VI	2 (15.38%) (95% CI: 2.1 - 45.5)
Anterior cervical region MRI	*3 (3.37%) (95% CI: 0 - 7.06)*
Regional lymphadenopathy	2 (2.25%) (95% CI: 0 - 4.89)
IFS	*5 (5.61%) (95% CI: 0.83 - 10.41)*
Benign	4 (4.49 %) (95% CI: 0.20 - 8.78)
Malignant	1 (1.12%) (95% CI: 0 - 3.28)

Note*: *Descriptive analysis of categorical variables included absolute frequencies (n - number), relative frequencies (%- percentage), and corresponding 95% confidence intervals – 95% CI; TSH - thyroid stimulating hormone; TI-RADS - Thyroid Imaging Reporting and Data System; TBSRTC - Bethesda System for Reporting Thyroid Cytopathology; FNAB - fine-needle aspiration biopsy; MRI - Magnetic Resonance Imaging; IFS - Intraoperative frozen section.

Surgical indications were established based on the presence of nodules ≥ 1.0 cm with compressive symptoms, suspicious imaging features of nodules, rapid growth, cytological findings suggestive of malignancy, or the presence of multiple nodules ≥ 0.8 cm and with an association with elevated serum calcitonin values serving as a supplementary indication.

During preoperative assessment, 2 patients (2.25%) were identified with contraindications to surgery due to cardiac comorbidities associated with high anesthetic-surgical risk.

The extent of thyroidectomy was decided according to several factors, including the presence of unilateral or bilateral thyroid lesions, regional metastases, established guideline recommendations, patient preference, the age of the patient and IFS results.

Hemithyroidectomy was performed in patients with unilateral nodular lesions, indeterminate cytology, microcarcinomas, or encapsulated carcinoma, benign IFS results, as well as in 2 cases of bilateral multinodular goiter when the contralateral lesions measured between 3–5 mm. Total thyroidectomies were performed in patients with bilateral multiple lesions, confirmed or highly suspected or unforeseeable malignancy, malignant IFS result, and toxic diffuse-nodular goiter.

The most frequently performed surgery was hemithyroidectomy, carried out in 44 patients (49.41%, 95% CI: 39.11 - 59.77), followed by total thyroidectomy in 41 cases (46.09%, 95% CI: 35.75 - 56.43); right hemithyroidectomy with isthmectomy and left hemithyroidectomy combined with left superior parathyroidectomy were each performed in 1 patient (1.12%, 95% CI: 0 - 3.32) respectively (Figure 1).

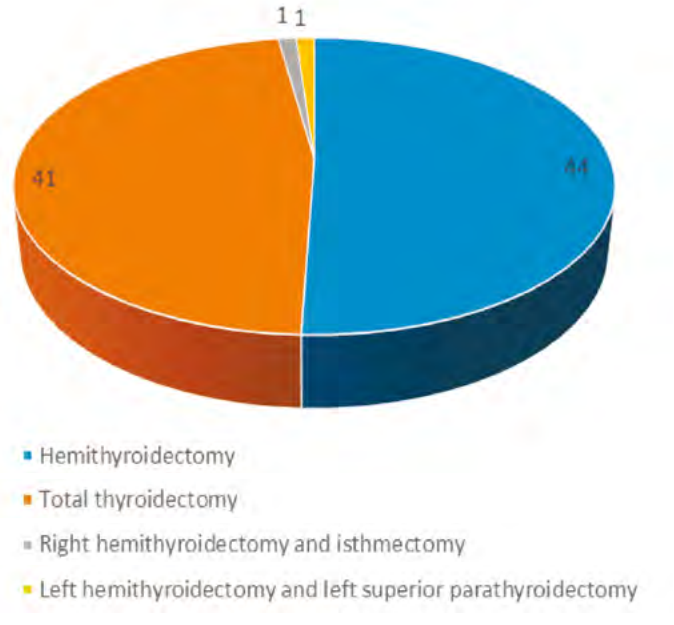

Figure 1. Types of performed surgeries

Note: *The pie chart illustrates the distribution of the performed surgeries. Each slice represents the absolute number of surgical procedures, out of a total of 87 procedures.*

Completion thyroidectomy was performed in one patient, three months after initial hemithyroidectomy, due to histopathological confirmation of papillary thyroid carcinoma. The operation was indicated by the oncologist in order to initiate postoperative radioiodine therapy.

In two cases with preoperative suspicion of cervical lymphadenopathy, lymphadenectomy was not performed. In the first case, the tumor was locally advanced, with pathological lymph nodes firmly adherent to the carotid artery, rendering dissection contraindicated due to high surgical risk. In the second case, intraoperatively during revision, the lymph nodes were not identified.

Histopathological examination of the thyroid nodules confirmed a variety of benign and malignant entities, as detailed in table 3, with goiter being the most common finding, followed in frequency by follicular adenoma.

Table 3. Distribution of patients according to histopathological result

Histopathological result	n	%	95% CI
Goiter	52	59.77	49.15 - 70.00
Follicular adenoma	16	18.40	10.50 - 26.30
Papillary carcinoma	14	16.08	8.50 - 23.70
Colloidal cyst	2	2.30	0 - 5.50
Papillary adenoma	1	1.15	0 - 3.40
Oncocytic adenoma	1	1.15	0 - 3.40
Thyroiditic nodule (de Quervain thyroiditis)	1	1.15	0 - 3.40

Note: *The histological results as categorical variables were analyzed descriptively, with reporting of n - absolute numbers, % - percentages, and 95% CI - 95% confidence intervals.*

In our study, it can be observed that the malignancy rate of thyroid nodules was 18.39%, with all malignant nodules being histologically confirmed as papillary thyroid carcinoma, probably reflecting the fact that this type represents the most prevalent form of thyroid malignancy.

The postoperative period was favorable in all the patients. Throughout the 24-month follow-up, no relapse of thyroid pathology and no complications were recorded. The mean duration of the hospital stay was 7.82 days. Thyroid hormone replacement therapy was adjusted individually by endocrinologists based on serum hormone levels. Patients who did not undergo surgery were followed up regularly.

Discussion

A rational and systematic assessment of thyroid nodules is fundamental to achieving successful outcomes, both in surgical and conservative management. Most guidelines and recent publications recommend measuring only the thyroid-stimulating hormone (TSH) [1, 2, 8, 9] as the first step in evaluating a thyroid nodule. We consider that the determination of the complete hormonal profile, of calcitonin, and of autoimmune antibodies is an essential component in the evaluation of thyroid nodules, as it provides valuable information for the etiological background, diagnosis, and risk stratification. Calcitonin has retained its important role as a single biomarker in the screening and early detection of medullary thyroid carcinoma, contribut-

ing significantly to preoperative diagnosis and appropriate surgical planning [10].

As highlighted by Grani et al. (2024), neck ultrasonography remains a cornerstone in the preoperative assessment of thyroid nodules [11]. Similarly, our study demonstrated that, within the appropriate clinical context and supplemented by Doppler mode and elastography, ultrasonography can often obviate the need for biopsy and serve as a reliable tool to guide surgical decision-making.

The role of ultrasonography is also crucial in the evaluation of both unilateral and bilateral multinodular goiters, whose management is poorly described in the literature. Although each nodule carries a risk of malignancy, we do not suggest performing multiple simultaneous biopsies, in accordance with guideline recommendations [1, 2, 8, 12, 13]. To exclude malignancy in unilateral thyroid disease and to assess intraoperatively the extent of thyroidectomy, we prefer to use IFS. This allowed us to adjust the volume of surgery based on intraoperative findings, thereby optimizing patient management. In cases of bilateral multinodular thyroid disease where nodules exceed 0.8 cm, we recommend total thyroidectomy to effectively prevent compressive symptoms such as dyspnea, dysphagia, and voice changes. This approach also reduces the risk of recurrent surgery and allows for comprehensive management of potentially malignant or symptomatic nodules.

In this context, thyroid nodules larger than 2 cm, which anatomically occupy approximately half of a thyroid lobe, can cause anterior neck deformities resulting in cosmetic concerns. When nodules extend posteriorly, they may induce compressive symptoms. Despite the absence of malignancy risk, such nodules warrant consideration for a surgical approach.

To prevent the recurrence of thyroid nodules, which varies between 8.9% and 40% (J. Rudnicki et al.), we advocate for the use of hemithyroidectomy and total thyroidectomy as standard surgical approaches [14]. Partial lobectomies or subtotal thyroidectomies, although historically performed, are associated with a higher risk of disease recurrence due to the potential persistence of residual nodular tissue.

Conclusions

Thyroid nodules remain a significant clinical concern due to their potential for malignancy and their capacity to produce compressive symptoms on adjacent cervical structures. They necessitate careful clinical, laboratory, and imaging evaluation and ongoing surveillance. The timing and indications for surgery must result from a multidisciplinary collaboration involving endocrinologists, family physicians, radiologists, and surgeons, to develop an evidence-based and personalized management plan for patients with suspicious nodules, which is fundamental for ensuring accurate diagnosis, appropriate treatment, and prevention of disease recurrence or progression.

Competing interests

None declared.

Authors' contributions

AB and CC had a crucial role in the conception and design of the work, acquisition and analysis of data. Both authors interpreted the data, drafted and critically reviewed this article. All authors have approved final version of this paper for publication.

Patient consent

Obtained.

Ethics approval

The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (Minutes no. 84, dated 07.06.2017).

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RESEARCH ARTICLE

Descriptive analysis of pediatric laryngeal papillomatosis in Republic of Moldova: epidemiological and clinical characteristics

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ABSTRACT

Introduction. Recurrent laryngeal papillomatosis is a rare but significant pediatric condition caused by human papillomavirus types 6 and 11. Characterized by the repeated growth of benign exophytic lesions in the respiratory tract, recurrent laryngeal papillomatosis poses a considerable burden due to its recurrent nature, frequent need for surgical intervention, and potential impact on respiratory function. This study aims to describe the epidemiological profile of pediatric recurrent laryngeal papillomatosis in the Republic of Moldova, identify high-risk groups, and explore the socioeconomic implications of the disease.

Material and methods. A retrospective, descriptive observational study was conducted using national data from 198 pediatric patients diagnosed with recurrent laryngeal papillomatosis and treated at the *Emilian Coțaga* Pediatric Otorhinolaryngology Clinic between 1981 and 2013. Demographic, clinical, and epidemiological variables were analyzed, including age at onset, sex, incidence, prevalence, and geographic distribution. Statistical analysis included descriptive methods, correlation coefficients, and linear regression models. Data were processed using Microsoft Excel and Python, with significance set at $p < 0.05$, for the 95% confidence interval.

Results. The average annual incidence was approximately 6 new cases, with a peak of 15 in certain years. The estimated prevalence reached 4.2 per 100 thousand children. Most patients were male, and the disease commonly manifested at ages 1 and 4. Correlation analysis revealed strong associations between age, sex, and disease onset, particularly in males. Linear regression confirmed the predictive value of demographic variables, though the model's robustness is limited by sample size. No progression into adulthood was observed, and seasonal or cyclical patterns were not evident.

Conclusions. Pediatric recurrent laryngeal papillomatosis in the Republic of Moldova shows a male predominance and early age of onset, with a consistent but relatively low incidence rate. Demographic indicators may aid in identifying at-risk populations, while early diagnosis and multidisciplinary management are essential to prevent complications. Expanding human papillomavirus vaccination coverage remains a critical public health priority. The findings support the need for prospective, multicenter research integrating vaccination status and immunological biomarkers to enhance disease prevention and control strategies.

Keywords: recurrent laryngeal papillomatosis, epidemiology, children.

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

Recurrent respiratory papillomatosis in children has been studied internationally, but data on its epidemiology, incidence, and demographics in the Republic of Moldova are scarce. The roles of gender, age at onset, and regional differences in case detection and referral remain poorly understood. This study aims to fill these gaps by offering a detailed epidemiological analysis focused on the Moldovan pediatric population and identifying factors that may influence disease presentation.

The research hypothesis

Within the pediatric population of the Republic of Moldova, epidemiological factors and referral patterns significantly influence the incidence and clinical presentation of laryngeal papillomatosis.

The novelty added by manuscript to the already published scientific literature

This manuscript provides the first comprehensive epidemiological analysis of pediatric laryngeal papillomatosis specific to the Republic of Moldova. It identifies unique demographic patterns and potential regional factors influencing disease onset and clinical presentation within this population. Additionally, it highlights gaps in local healthcare management and offers data that can guide targeted interventions and future research.

Introduction

Recurrent laryngeal papillomatosis is a benign condition affecting the mucosa of the respiratory tract, characterized by the repeated formation of exophytic lesions caused by infection with the human papillomavirus. Although often referred to as glottic or laryngeal papillomatosis, the disease can affect any part of the respiratory tract [1-3]. Despite being relatively rare, recurrent laryngeal papillomatosis's tendency to recur and its impact on respiratory function pose a significant challenge for both patients and healthcare systems [4-6]. Males are more frequently affected than females [6, 7]. Clinical manifestations may resemble other common respiratory conditions, such as asthma or bronchitis, which can delay diagnosis and the initiation of appropriate treatment [7, 8]. In severe cases, airway obstruction may require urgent interventions, including tracheotomy, to ensure adequate breathing [9, 10].

The incidence of recurrent laryngeal papillomatosis varies significantly worldwide, influenced by factors such as human papillomavirus infection rates, access to medical care, and case reporting practices. In the pediatric population, the estimated incidence ranges from 0.2 to 2.1 cases per 100 thousand children annually, with reported prevalence between 0.8-4.3 per 100,000. The juvenile form is characterized by the recurrent appearance of exophytic lesions in the larynx, and disease severity can vary widely, often requiring frequent surgical interventions and adjuvant therapies [10-13].

A clear understanding of the epidemiological aspects of recurrent laryngeal papillomatosis is essential for developing effective strategies for the prevention, early detection, and management of this rare but impactful condition affecting children's health. In this context, the present chapter analyzes the demographic distribution of recurrent laryngeal papillomatosis in the Republic of Moldova, based on data collected over recent decades, highlighting trends in incidence and prevalence by age, sex, and time period. Special attention is given to identifying high-risk groups and the local particularities of disease progression [14, 15].

At the same time, the study evaluates the social and economic implications of the disease, taking into account the costs associated with repeated hospitalizations, surgical treatments, and the psychological impact on affected children and their families. The analysis is based exclusively on national statistical data, providing an updated and context-specific overview for the Republic of Moldova. This information may support the development of public health policies, the adjustment of human papillomavirus vaccination strategies, and the improvement of clinical management. By identifying region-specific needs, the study aims to support informed decision-making in the field of pediatric otorhinolaryngology.

Given the lack of consolidated epidemiological data on recurrent laryngeal papillomatosis in the pediatric population of the Republic of Moldova, this study aims to make a significant contribution to understanding the national distribution and progression of the disease. The statistical analysis of cases recorded over an extended period provides a solid foundation for evaluating the impact of this condition on both the healthcare system and patients. The findings may serve as a starting point for developing tailored preventive measures, optimizing therapeutic intervention strategies, and supporting vaccination and medical education initiatives among the general population and healthcare professionals.

Material and methods

The study was designed as an observational, descriptive, cross-sectional study during the years 1981-2013, aiming to assess the clinical-epidemiological characteristics and morbidity associated with recurrent laryngeal papillomatosis in the pediatric population of the Republic of Moldova. Ethical approval for the research was granted by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, under decision no. 9, dated September 20, 2019.

The study analyzed data from 198 patients diagnosed with recurrent laryngeal papillomatosis who were admitted to the *Emilian Coțaga* Pediatric Otorhinolaryngology Clinic during the period 1981-2013.

The study included children up to 18 years old with a confirmed diagnosis of recurrent laryngeal papillomatosis. The diagnosis was established based on clinical and histopathological criteria. Patients with incomplete data or unconfirmed diagnoses were excluded.

The evaluation included demographic data (age, sex, and residence), medical and social factors, clinical details (age at disease onset, lesion sites), and epidemiological metrics (incidence and prevalence). The progression of the disease was followed for each patient over a period varying between 1 and 15 years.

The study was organized into clearly defined stages: setting objectives, selecting the sample, collecting data through

medical records and questionnaires, processing and statistically analyzing the information, synthesizing the results, and formulating conclusions with practical applicability. Comparative analyses of demographic, geographic, and temporal data were performed to reveal key trends and disparities.

Data processing was performed using Microsoft Excel and Python (Jupyter Notebook) software. Both descriptive and inferential statistical methods were applied. Quantitative indicators were expressed using the arithmetic mean (M), median (Me), and mode (Mo).

The Shapiro-Wilk test was applied to assess the normality of the data distribution. Relationships between variables were examined using linear regression, a model that allows the evaluation of dependence between a scalar response and multiple independent variables.

Values of $p < 0.05$ were considered statistically significant, with results reported alongside 95% confidence intervals.

The results were organized into tables, graphs (histograms), and charts (including structural, column, and bar charts). Typological and variation analyses were performed based on the grouping of key indicators.

Results

Figure 1 presents a descriptive analysis of the variables included in the study, providing a detailed overview of the statistical distribution of each evaluated indicator. This enables not only the individual interpretation of the analyzed parameters but also a comprehensive understanding of the relationships among them, highlighting the relevance and consistency of the collected data.

The dataset is complete, comprising 198 observations for each analyzed variable, with no missing values. The average annual number of newly diagnosed cases is 6, with a peak of 15 cases reported in two separate years. The average number of annual medical visits is approximately 30, the majority of which involve male patients. The internal distribution of variables shows a relatively balanced statistical measure distribution between sexes. Disease progression extending into adulthood was not reported in any cases. The estimated prevalence of recurrent laryngeal papillomatosis among children is approximately 4.2 per 100,000.

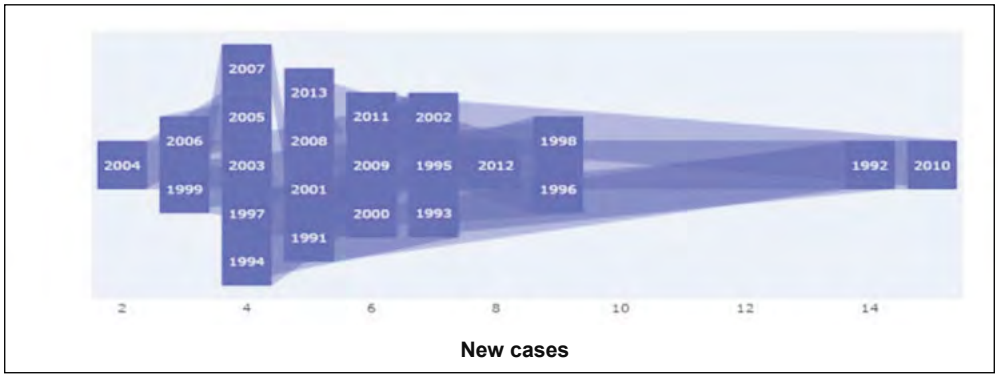

Fig. 1 Frequency of new cases between 1991-2013

Note: *Each block represents a calendar year, plotted against the number of newly diagnosed cases. The absence of pronounced fluctuations supports the hypothesis of a stable incidence over time, with no apparent cyclical or seasonal patterns.*

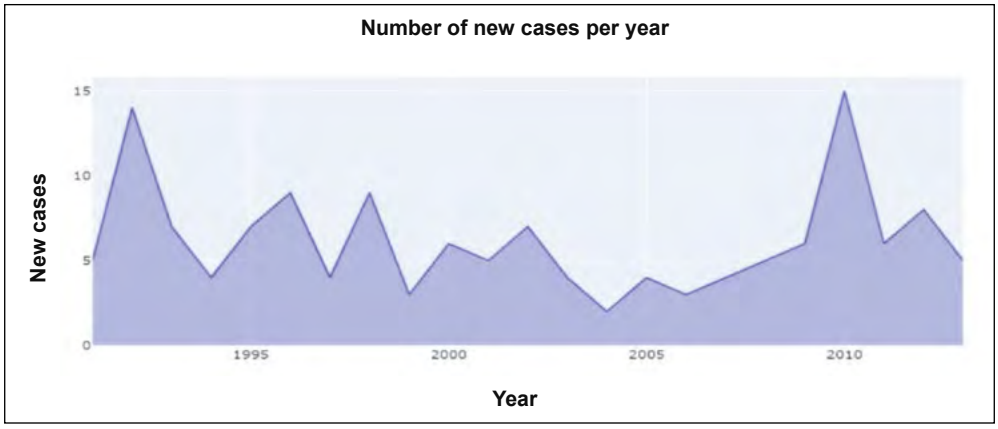

Fig. 2 The distribution of cases per year.

Note: *The graph shows the temporal distribution of new cases over the analyzed period, highlighting interannual variations without a consistent upward or downward trend. Isolated fluctuations may be attributed to reporting factors, access to care, or local epidemiological particularities.*

A retrospective analysis of cases of laryngeal papillomatosis in children in the Republic of Moldova during the period 1991-2013 reveals a fluctuating evolution, characterized by alternating episodes of increased incidence and periods of significant case decline (Fig. 1).

The highest incidence was recorded in 1991, with a total of approximately 15 cases, representing an early peak in the studied timeframe. After this point, the number of cases progressively decreased until 1994-1995, when a slight increase was observed. Another notable rise occurred around 1999, followed by a relatively steady decline in the early 2000s.

A minimum point on the curve was recorded between 2004 and 2006, when the number of cases remained below 5 per year. This relatively calm period was followed by a sudden resurgence starting in 2007, culminating in a second major epidemiological peak in 2010, when incidence again reached approximately 15 cases. Afterward,

a slight decrease was noted, although the number of cases remained above the previous decade's average until 2013.

This uneven evolution suggests the possible influence of cyclical or contextual contributing factors, such as a lack of national human papillomavirus vaccination program, the absence of active human papillomavirus screening in pregnant women, limited access to pediatric Otolaryngology care, and potentially underreporting of cases during certain periods.

The annual distribution of medical consultations for recurrent laryngeal papillomatosis throughout the study period is illustrated in the figure (Fig. 2). Trend analysis highlights that the year 2004 recorded the lowest number of cases, while 2010 saw a peak with a total of 15 reported cases, indicating considerable variation in incidence over time.

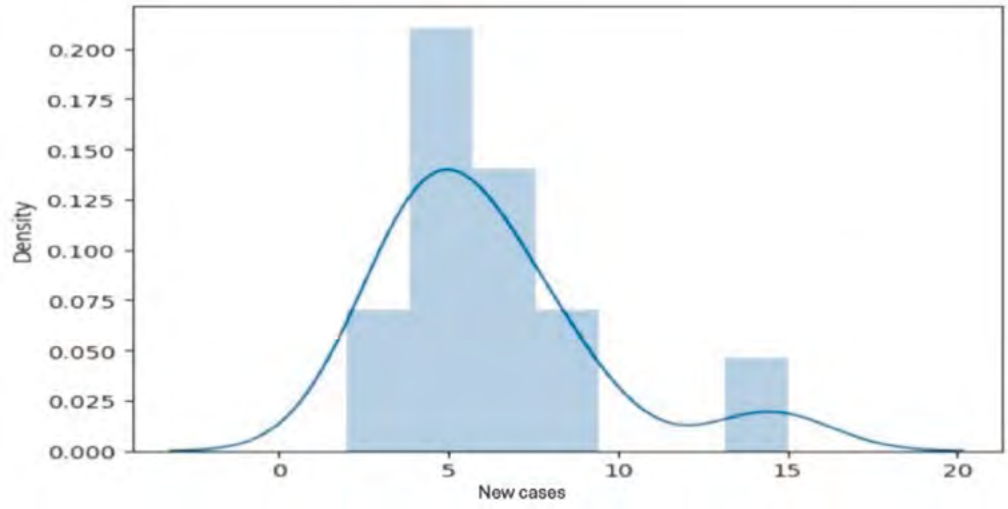

Fig. 3 Annual trend of reported recurrent laryngeal papillomatosis cases (1991–2013).

Note: *The distribution of newly reported cases follows a unimodal, right-skewed pattern, with most years clustering around 5 to 7 cases and a small number of outlier years exceeding 15 cases. This suggests a stable baseline incidence, occasionally interrupted by sporadic peaks.*

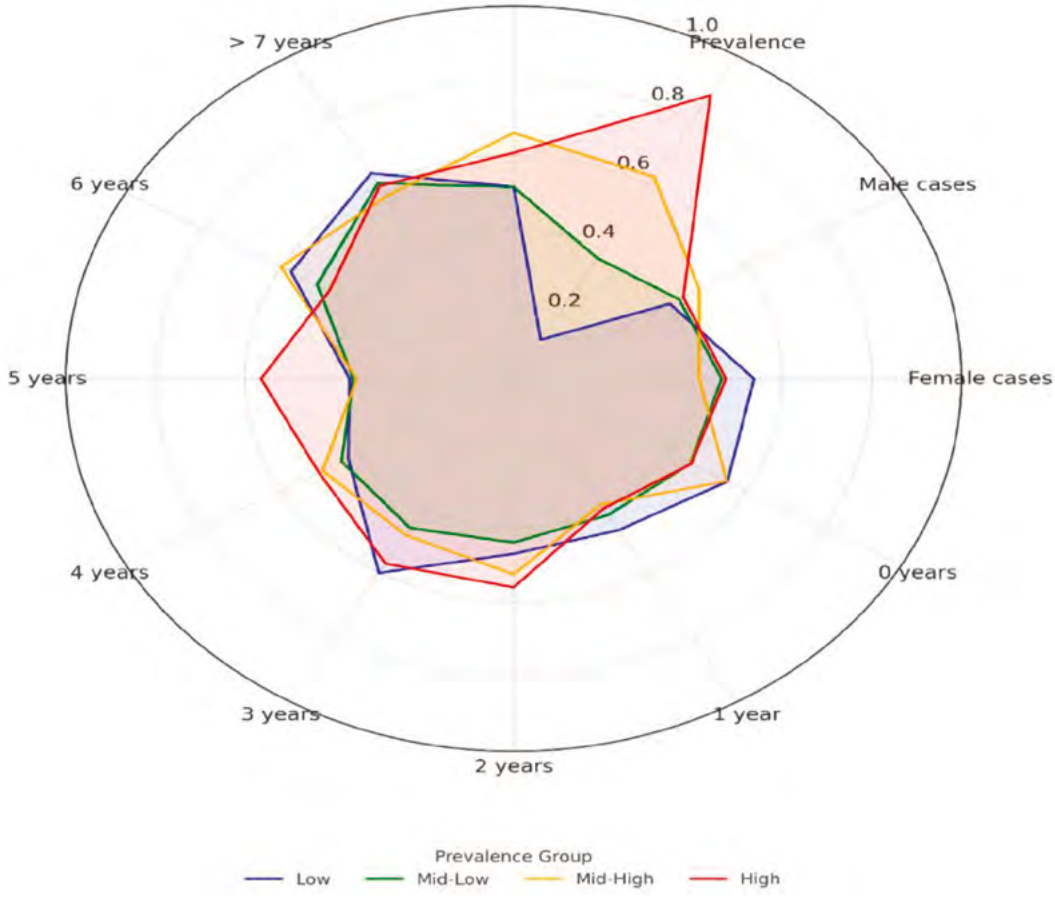

Fig. 4 Correlation between sex, age groups, and the number of new recurrent laryngeal papillomatosis cases

Note: *Radar chart illustrating the normalized median values of selected clinical and demographic variables stratified by four prevalence groups of recurrent laryngeal papillomatosis. Prevalence groups were defined by quartiles of the total prevalence distribution: Low (blue), Mid-Low (green), Mid-High (orange), and High (red). Variables include the number of new cases, overall prevalence, sex distribution (male and female), and age-specific representation from 0 to >7 years. The chart highlights distinct patterns across prevalence strata, with higher values in specific age groups and among males in high-prevalence clusters.*

The annual progression of recurrent laryngeal papillomatosis cases during the study period is illustrated in the figure (Fig. 3). On average, the number of consultations per year remained below 9 cases. However, specific years – such as 1992 and 2010 – demonstrated a notable increase in frequency, reaching approximately 50% above the average. These isolated spikes, which lack a consistent temporal pattern, may indicate the influence of contextual or environmental factors on disease dynamics. Further investigation into these peaks could yield important insights into epidemiological behavior and inform the development of targeted preventive strategies.

In our study, a strong correlation (r = 0.86) was found between "Boys' Cases" and "New Cases," indicating that most new referrals originated from male patients (Fig. 4). Additional correlations between the "1 year" and "4 years" age groups suggest a higher frequency of disease onset at these ages, particularly among boys. Meanwhile, a moderate correlation between "Girls' Cases" and "New Cases" (r = 0.76) reflects a relatively balanced incidence across sexes, with slight age-dependent variations.

Additional correlation analysis reveals a notable association (r = 0.63) between overall prevalence and the "4 years" age group, confirming a higher frequency of cases at this age among boys. Overall, the data suggest a concentration of cases around the ages of 1 and 4 for males, while in females, referrals tend to be more frequent between 7 and 15 years. These observations may be important for understanding the age and sex distribution of laryngeal papillomatosis onset and can help guide early interventions.

In our study, a strong correlation was observed between the number of new cases and the reported incidence per 100,000 population. However, this association may be considered misleading, as the incidence rate is directly calculated from the number of new cases. As a result, these two variables are not statistically independent, and the inclusion of incidence in a separate inferential analysis would not provide additional value.

The Shapiro-Wilk normality test applied to the distribution of new cases in our dataset demonstrated a balanced distribution between sexes, with no significant differences in the frequency of referrals. Nonetheless, a sex-based difference was noted regarding the age at presentation, suggesting a potential influence of gender on the timing of disease onset. This aspect merits further investigation in future research.

In our study, the evaluation of errors obtained through the linear regression method supports the validity of the previous interpretations. The estimated difference between the distribution of referrals for boys and girls compared to the total number of new cases is approximately ±2 cases. Furthermore, the predictive model based on these variables can estimate the number of new cases with a maximum deviation of ±7 cases from the actual data. However, developing a robust and reliable predictive model for forecasting future cases requires a larger sample size and more extensive data collection.

The analysis of statistical coefficients derived from linear regression in our study reveals that variables related to sex distribution (boys and girls) hold the most significant relevance for analyzing and predicting new cases in the upcoming period. While other variables contribute to a deeper understanding of the studied sample, they do not provide sufficient information for extrapolation to a broader theoretical population. Nonetheless, these variables remain valuable for the specific description and interpretation of the patient group included in our study.

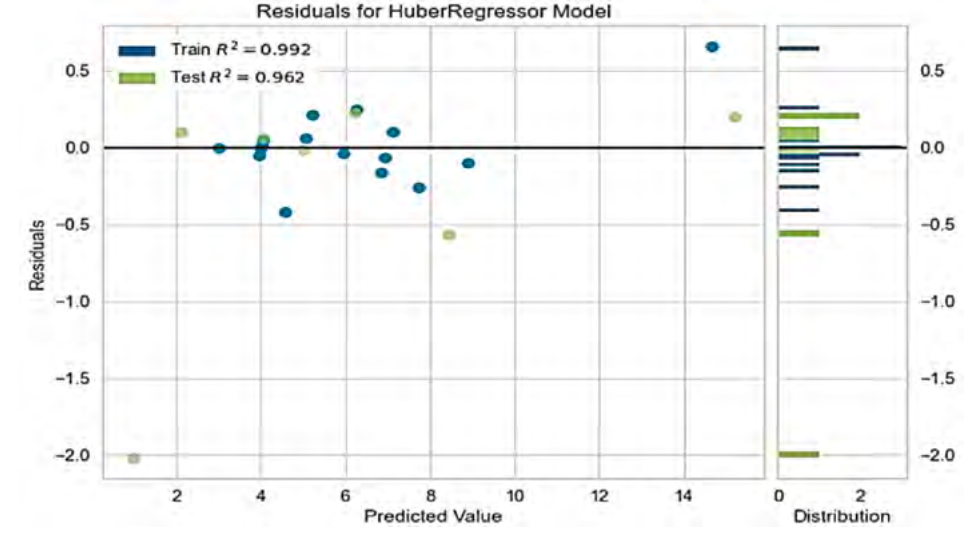

Fig. 5 Exploratory graphical assessment of residual distribution and variance stability in the predictive model

Note: *The figure displays residual values plotted against predicted values for both training (blue) and test (green) datasets. The residuals are mostly centered around zero, with minimal dispersion, indicating good model fit and stability. The distribution histogram (right) confirms the symmetrical spread of residuals, with isolated deviations below –2 in the test set.*

The error analysis resulting from the linear regression test applied to the predictive model demonstrates an approximately 90% homoscedastic distribution of errors, indicating that the variance of the residuals remains constant across the range of predicted values (Fig. 5). This consistency in error variance is essential for the validity of linear regression assumptions and ensures that coefficient estimates are efficient and unbiased. Consequently, the model proves

to be robust, minimizing the risk of systematic errors in its predictions. However, for improved accuracy and generalizability, further analyses are recommended to identify any potential outliers or heteroscedasticity within specific data subsets.

Discussion

Recurrent laryngeal papillomatosis is a condition with significant clinical and social implications, especially among children, where disease recurrence and airway involvement can lead to severe complications. The present study, focused on the epidemiological and clinical analysis of pediatric patients in the Republic of Moldova, provides valuable insight into how this disease manifests and progresses within a specific regional context. The results align with recent global observations while also offering new data useful for planning clinical and public health interventions.

One of the most important findings of our study is the confirmation of a higher prevalence of recurrent respiratory papillomatosis (RRP)among boys compared to girls, a trend also reported in the international literature [15, 16]. The biological explanations for this sex difference remain under investigation; however, recent data suggest that immune response and susceptibility to human papillomavirus types 6 and 11 infections may be differently modulated based on sex [17, 18]. These variations may influence both the frequency of infection and the severity of clinical manifestations. Additionally, socio-cultural factors and differential access to healthcare services may play an important role in case detection and reporting, as highlighted by studies emphasizing inequalities within healthcare systems [19].

The average age at disease onset, around 1 and 4 years, aligns with the range reported in numerous multicenter studies [20, 21], suggesting a universal pattern of recurrent laryngeal papillomatosis progression in the pediatric population. These ages coincide with critical periods in immune system development and may reflect a window of increased vulnerability to initial human papillomavirus infection.

The average annual incidence identified in our study – approximately 6 new cases per year – is comparable to values published for regions with similar epidemiological profiles [22, 23]. Annual variability, with significant peaks in certain years (e.g., 1992 and 2010), may be attributed to various factors such as changes in public health policies, variability in case reporting, or actual fluctuations in viral transmission.

An estimated prevalence of 4.2 per 100,000 children is an important indicator highlighting the need for sustained preventive interventions. Although human papillomavirus vaccination remains insufficiently implemented in many regions, it has demonstrated increased efficacy in reducing recurrent laryngeal papillomatosis incidence [24, 25]. Implementing and expanding vaccination programs represent a strategic priority, especially given that recurrent laryngeal papillomatosis is directly associated with infection by human papillomavirus types 6 and 11, which are included in broad-spectrum human papillomavirus vaccines.

The application of linear regression in our study allowed the identification of statistically significant variables for predicting new case occurrences, particularly those related to sex distribution. This confirms the critical role of demographic factors in disease progression. However, the limited sample size and the absence of detailed data on other factors – such as vaccination status or the presence of comorbidities – limit the capacity to develop complex and robust predictive models [26].

The importance of controlling homoscedasticity and validating models through error analysis is well emphasized in recent methodological studies, which recommend close attention to error variance consistency to ensure accurate estimates and valid interpretations [27]. Our models largely meet these criteria but also highlight the need for further analyses, potentially using machine learning methods or nonlinear models that can better capture data complexity.

Early diagnosis of recurrent laryngeal papillomatosis is crucial for preventing severe complications, which may include airway obstruction, acute respiratory failure, and the need for invasive interventions such as tracheotomy [28]. In this context, educating primary healthcare providers, especially pediatricians and family doctors, about the symptoms and risk factors of recurrent laryngeal papillomatosis is essential to reduce diagnostic delays.

Current clinical management of recurrent laryngeal papillomatosis relies on repetitive surgical interventions; however, the high rate of recurrences necessitates the use of adjuvant therapies. New antiviral and immunomodulatory treatments have shown promising results in reducing recurrence frequency and disease severity [28]. Nonetheless, access to these treatments may be limited in some regions, including the Republic of Moldova, underscoring the need for effective interdisciplinary collaboration and appropriate resource allocation.

Although our study provides important data, the retrospective design entails inherent limitations, such as potential data incompleteness and the inability to control certain variables. Additionally, the lack of detailed information on vaccination history and other environmental factors poses a barrier to comprehensive assessment of causes and risks associated with recurrent laryngeal papillomatosis.

To better understand the involved immunological mechanisms and the predictive potential of biomarkers, prospective and multicenter studies are indispensable [29]. Such studies could integrate molecular, immunological, and epidemiological assessments, providing a solid basis for developing personalized treatment and prevention strategies.

Moreover, expanding human papillomavirus vaccination programs and monitoring their impact on RRP incidence should be a priority in public health policy in the Republic of Moldova and other countries with similar epidemiological profiles [30].

Conclusions

Recurrent laryngeal papillomatosis in children remains a rare but impactful condition, with early onset and clear demographic predispositions that underline the importance of timely diagnosis and multidisciplinary care. Despite

current efforts, significant gaps persist in understanding its pathogenesis and recurrence patterns, warranting further prospective and integrative research. Expanding Human Papillomavirus vaccination coverage stands out as a necessary public health intervention, with the potential to reduce both clinical burden and long-term socioeconomic impact.

Competing interests

None declared.

Authors' contributions

Both authors conceived and designed the study, collected and analyzed the data, and drafted the manuscript. Both authors critically reviewed and approved the final version of the manuscript.

Informed consent for publication

Obtained.

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Ethics approval

Ethical approval was obtained from the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, under decision no. 9, dated September 20, 2019.

Provenance and peer review

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RESEARCH ARTICLE

Prevalence and severity of depression, anxiety, and cognitive impairment in acute ischemic stroke patients: a cross-sectional study

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ABSTRACT

Introduction. Post-stroke depression (PSD) and anxiety are common neuropsychiatric sequelae of stroke, occurring in roughly one-third of survivors. Cognitive impairment is also frequently observed, affecting up to half of stroke patients. These conditions adversely impact rehabilitation and quality of life. This study aimed to determine the prevalence and severity of depression, anxiety, and cognitive deficits in patients with acute ischemic stroke.

Material and methods. We conducted an observational study involving 99 patients with acute ischemic stroke, assessed within approximately two weeks of symptom onset, who were admitted to a tertiary care unit. Depression and anxiety were assessed using the Patient Health Questionnaire-9 (PHQ-9) and Generalized Anxiety Disorder-7 (GAD-7) scales, supplemented by the clinician-rated Hamilton Depression (HAM-D) and Anxiety (HAM-A) scales. Cognitive status was evaluated with the Mini-Mental State Examination (MMSE). Descriptive statistics (proportions, means ± SD) were used to summarize the prevalence and severity of each condition.

Results. The cohort had a mean age of 64.8 ± 8.1 years and was 63.5% male. Vascular risk factors were prevalent, with 88% of patients having hypertension and 33% having diabetes. Based on patient-reported measures, 16% of patients exhibited moderate depressive symptoms (PHQ-9 ≥10), while 42% reported moderate to severe anxiety (GAD-7 ≥10). Clinician-administered assessments identified 35% of patients with moderate to severe depression (HAM-D ≥17) and 35% with clinically significant anxiety (HAM-A ≥18). The vast majority of patients (~92%) reported at least mild depressive symptoms, although only 0–1% met the criteria for severe depression.

Conclusions. Depression, anxiety, and cognitive deficits are highly prevalent in the acute phase of ischemic stroke, with approximately one in three patients experiencing clinically significant depression or anxiety and one in four exhibiting cognitive impairment. These findings underscore the importance of early neuropsychological assessment and intervention as part of acute stroke care to improve rehabilitation outcomes.

Keywords: stroke, depression, anxiety, cognitive impairment, prevalence, rehabilitation.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

The precise prevalence, co-occurrence, and severity patterns of post-stroke depression, anxiety, and cognitive impairment *in the acute phase* – as well as how these relate to health-related quality of life – are insufficiently characterized in our setting.

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The research hypothesis

Among patients with acute ischemic stroke, depression, anxiety, and cognitive impairment are highly prevalent and frequently co-occurring. Greater severity of these symptoms is independently associated with lower EQ-5D scores and a higher number of unmet needs, as measured by the Camberwell Assessment of Need Short Appraisal Schedule (CANSAS).

The novelty added by manuscript to the already published scientific literature

This research provides the first cross-sectional estimates in our context (N=99) using standardized tools (PHQ-9, HAM-A, MMSE, EQ-5D, CANSAS), quantifies multimorbidity between affective and cognitive symptoms, identifies clinical correlates of reduced quality of life, and supports routine early screening in acute stroke units.

Introduction

Stroke is a leading cause of adult disability worldwide. Beyond motor and speech deficits, stroke survivors frequently develop psychological and cognitive complications that can hinder recovery. Post-stroke depression (PSD) is the most common psychiatric condition after stroke and post-stroke anxiety (PSA) is also prevalent [1]. Recent literature indicates that roughly 30% of stroke survivors experience clinically significant depression, and a similar proportion experience anxiety during the first year. Cognitive impairment after stroke is another major concern: estimates of post-stroke cognitive impairment (PSCI) range from about 40% to as high as 60% of patients, depending on timing and assessment criteria. These post-stroke neuropsychiatric problems are not benign: PSD, in particular, is associated with worse functional outcomes and higher mortality, underscoring the need for monitoring and treatment [2]. Notably, anxiety can be independent of depression and affect rehabilitation engagement, which supports screening separately for both conditions rather than assuming they always co-occur [3]. Likewise, untreated anxiety and cognitive deficits can impede recovery by reducing engagement in therapy and self-care.

Despite their impact, depression and anxiety often go under-recognized in acute stroke settings, and cognitive deficits, especially in milder forms, may be overlooked if formal neuropsychological testing is not performed. The acute phase of stroke (days to weeks post-event) is a critical window when early detection of these issues could allow timely intervention. However, much of the existing data on PSD and PSA prevalence comes from chronic or subacute phases of stroke or heterogeneous populations. Fewer studies have focused specifically on the acute post-stroke hospitalization period. Moreover, prior research in this area from Eastern Europe and low-to-middle income countries is limited, underscoring the need for local data to guide clinical practice.

The aim of the study. In this context, we conducted an original research study to evaluate the prevalence and severity of depression, anxiety, and cognitive impairment in patients with acute ischemic stroke. We hypothesized that a substantial proportion of acute stroke patients would exhibit at least mild to moderate depressive or anxious symptomatology and measurable cognitive deficits even in the early post-stroke phase. By quantifying these issues and characterizing their severity, we aim to highlight the importance of routine mental health and cognitive screening in stroke units. This study also seeks to provide baseline data for our center, which can inform the development of integrated neurorehabilitation strategies addressing both the physical and psychological needs of stroke patients.

Materials and methods

Study design and setting. We performed a cross-sectional observational study at the Institute of Emergency Medicine in Chișinău, Republic of Moldova, in a tertiary care unit. The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, issued on 9 July 2024 and recorded in Minutes No. 41/21 May 2024. All procedures complied with the EU GDPR (Reg. 2016/679) and ICH-GCP guidelines; every member of the research team holds a current GCP certificate. Written informed consent was obtained from each participant or, when applicable, from a legally authorized representative.

We used the officially licensed Romanian versions of the EQ-5D (including its Visual Analogue Scale), CANSAS, PHQ-9, GAD-7, HAM-D, HAM-A and MMSE, all of which have been previously validated in Moldovan clinical populations.

Before enrolment began, the assessors (two psychiatrists and one clinical psychologist) completed a 4-hour calibration workshop led by senior supervisors; inter-rater reliability on 10 pilot cases was excellent ($\kappa \geq 0.82$ for the HAM-D total score). Evaluations were performed at the bedside in a quiet ward room, typically lasting 20-30 min, with rest pauses ad-libitum to minimize fatigue. For patients with reading difficulties, items were read aloud verbatim; scoring followed the manuals without modification.

The study is part of a larger project on post-stroke neuropsychiatric outcomes. Patients were recruited between December 2023 and May 2025. Inclusion criteria were: age ≥18 years, acute ischemic stroke confirmed by clinical evaluation

and imaging (CT/MRI), within approximately two weeks of stroke onset. We enrolled consecutive patients admitted for acute ischemic stroke who were medically stable enough to undergo a structured interview and brief cognitive testing. Exclusion criteria included hemorrhagic stroke, transient ischemic attack, severe aphasia, altered level of consciousness, or any condition that precluded informed consent or valid neuropsychological assessment (e.g., pre-stroke dementia or severe psychiatric illness). We ultimately included 99 patients with acute ischemic stroke (referred to as the stroke cohort). Although a control group of patients without stroke was initially planned, it is not included in the present analysis; therefore, no control comparisons are reported.

Assessments. Within 7-14 days post-stroke (median ~10 days), each patient underwent a standardized evaluation by a study physician and psychologist. Demographic and clinical data were recorded, including age, sex, education level, employment status, vascular risk factors (hypertension, diabetes mellitus, etc.), and stroke characteristics. Stroke severity was documented using neurological examination and imaging reports. However, formal stroke severity scores (NIHSS) were not uniformly available for all patients and thus are not reported here. We did note whether the stroke was a first-ever vs. recurrent event.

- ***Depression and anxiety.*** Depression symptoms were assessed using the Patient Health Questionnaire-9 (PHQ-9), a 9-item self-report scale validated in stroke populations for screening depression severity. PHQ-9 scores range from 0 to 27. We used standard cut-offs to categorize depression severity as minimal (0-4), mild (5-9), moderate (10-14), moderately severe (15-19), or severe (20-27). A PHQ-9 score ≥10 was taken as indicative of at least moderate depression, a threshold commonly used to identify clinically significant depression symptoms requiring follow-up [1]. Anxiety symptoms were measured with the 7-item Generalized Anxiety Disorder scale (GAD-7), similarly categorized into minimal (0-4), mild (5-9), moderate (10-14), and severe (15-21) anxiety; GAD-7 ≥10 was considered positive for moderate-to-severe anxiety. In addition to these self-report instruments, trained clinicians administered the Hamilton Depression Rating Scale (17-item HAM-D) and Hamilton Anxiety Rating Scale (14-item HAM-A) for a subset of patients to corroborate self-reported findings with clinical interview-based ratings. HAM-D scores ≥17 and HAM-A scores ≥18 were defined as moderate or greater symptom severity based on conventional cut-offs.
- ***Cognitive function*.** Global cognitive status was evaluated using the Mini-Mental State Examination (MMSE). The MMSE assesses orientation, attention, memory, language, and visuoconstructional abilities, with scores ranging from 0 to 30. A score below 24 was used to define cognitive impairment, a widely accepted threshold indicative of at least mild cognitive deficit. Because the MMSE has known ceiling effects and may miss executive dysfunction, we interpreted our cognitive findings cautiously. Patients with severe comprehension deficits or dysphasia who could not complete the MMSE were excluded from cognitive analyses (these largely overlapped with the exclusion criteria mentioned above).
- ***Other measures.*** Health-related quality of life was documented using the EQ-5D-5L questionnaire (results not the focus of this report). We also recorded any use of psychotropic medications. At the time of evaluation, none of the patients were on antidepressant therapy (baseline "additional treatment (AD)" with antidepressants was recorded; all patients in this acute phase had "No" for current antidepressant use).

Data analysis. Descriptive statistics were used to summarize the cohort's characteristics and assessment results. Continuous variables (e.g., age, scale scores) are presented as mean ± standard deviation (SD) or median [interquartile range] as appropriate, and categorical variables as counts and percentages. The prevalence of depression, anxiety, and cognitive impairment was calculated as the proportion of patients exceeding the defined cut-off scores (PHQ-9 ≥10, GAD-7 ≥10, MMSE <24, etc.). We constructed a frequency distribution of depression and anxiety severity categories. No imputation was done for missing data; only patients who completed a given assessment were included in that specific analysis. All statistical analyses were performed using SPSS 25 and Python (pandas) for data handling. As this was primarily a descriptive study, no hypothesis testing or regression analyses were performed. However, we did explore correlations between scales (e.g., PHQ-9 and HAM-D) to ensure consistency of findings. The threshold for statistical significance (if any tests were applied post-hoc) was set at $p<0.05$.

Results

Participant characteristics. A total of 99 acute ischemic stroke patients were included (mean age 64.8 ± 8.1 years, range 41-89; 63.5% male). Table 1 summarizes the key demographic and clinical features of the cohort. Most patients had low to moderate levels of educational attainment, with 74% having completed only primary or secondary education. Additionally, the majority (approximately 58%) were retired at the time of stroke, reflecting the older age distribution of the cohort. Vascular comorbidities were highly prevalent: 88% of patients had a history of hypertension and 33% had type 2 diabetes mellitus. About one-quarter (25%) had experienced a prior stroke before the index event, indicating recurrent stroke in a substantial subset. In terms of stroke localization, most infarcts were in the middle cerebral artery territory. Detailed neuroimaging data will be reported elsewhere. Neurologically, common deficits included hemiparesis, speech impairment (aphasia in 20% mild/moderate, severe aphasia in 5% leading to exclusion from full neuropsychological assessment), and sensory changes. All patients were within 2 weeks post-stroke (median 10 days). At the time of assessment, they were medically stable, however, many were in early stages of rehabilitation. Table 1 provides an overview of patient characteristics and key outcome measures:

Table 1. Baseline characteristics and neuropsychological outcomes of the stroke patient cohort (N=99).

Variable	Value*
Age, years	64.8 ± 8.1 (range 41–89)
Sex	Male 54 (63.5%); Female 31 (36.5%)
Education level: ≤Secondary	74% (primary 8%, gymnasium 66%)
Employment status: Retired	58% (actively employed 28%; unemployed 8%)
Hypertension (history)	75 (88%)
Diabetes mellitus	28 (33%)
Previous stroke (recurrent)	21 (25%)
PHQ-9 Depression score	7.2 ± 2.6 (mild average)
Moderate-to-severe depression	14 (16%) – PHQ-9 ≥ 10
GAD-7 Anxiety score	9.6 ± 2.9 (mild-to-moderate average)
Moderate-to-severe anxiety	36 (42%) – GAD-7 ≥ 10
MMSE score	25.2 ± 4.1
Cognitive impairment (MMSE <24)	22 (26%)

Note: *PHQ-9: Patient Health Questionnaire-9; GAD-7: Generalized Anxiety Disorder-7 scale; MMSE: Mini-Mental State Exam. Percentages for depression, anxiety, and cognitive impairment are based on N=85 with complete data. Patients with severe aphasia or other barriers were unable to complete PHQ-9/GAD-7/MMSE and are excluded from those calculations. *Continuous variables shown as mean ± SD; categorical variables as n (%). Depression, anxiety, and cognitive impairment prevalence are calculated among the 85 patients who completed those assessments.*
No inferential comparisons are reported in this baseline table; all values are descriptive. In subsequent analyses, continuous variables were compared with the independent-samples t-test (or Mann–Whitney U when assumptions were violated) and categorical variables with the χ^2 test or Fisher's exact test, as appropriate.

Prevalence of depression. Symptoms of depression were widespread in this cohort, though often of mild intensity. The mean PHQ-9 score was 7.2 ± 2.6, which corresponds to the mild depression range on average. A remarkable 92% of patients scored ≥5 on the PHQ-9 (indicating at least mild depressive symptoms).

Figure 1 illustrates the distribution of depression severity categories, with most patients (75%) falling in the mild range. Cognitive screening revealed a mean MMSE of 25.2 ± 4.1; 26% scored <24, indicating cognitive impairment.

Most patients (75%) reported mild depressive symptoms (PHQ-9 score 5-9). Moderate depression (PHQ-9 10-14) was present in 16% of patients, while none scored in the severe range (≥20) in this acute phase. As shown, the largest proportion fell into the "Mild" category (5-9 points) with 64 patients (75%). Fourteen patients (16% of the sample) had PHQ-9 scores between 10 and 14, indicating moderate depression. Importantly, no patients met criteria for *severe* depression (PHQ-9 ≥20), and none fell in the 15-19 "moderately severe" range either, suggesting that very severe depressive symptoms were absent or rare shortly after stroke. When considering clinician-rated depression, the HAM-D scale (administered to 85 patients) yielded a mean of 16.0 ± 5.9. According to HAM-D, 30 patients (35% of those assessed) had scores ≥17, consistent with at least moderate depression, including 13 (15%) with severe depression (HAM-D ≥24). The discrepancy between PHQ-9 and HAM-D results (fewer patients endorsing severe symptoms on self-report) might reflect under-reporting or the challenges of self-assessment in acute illness. Nonetheless, using either instrument, we estimate that roughly 15-35% of acute stroke patients in our cohort experienced clinically significant depressive symptoms.

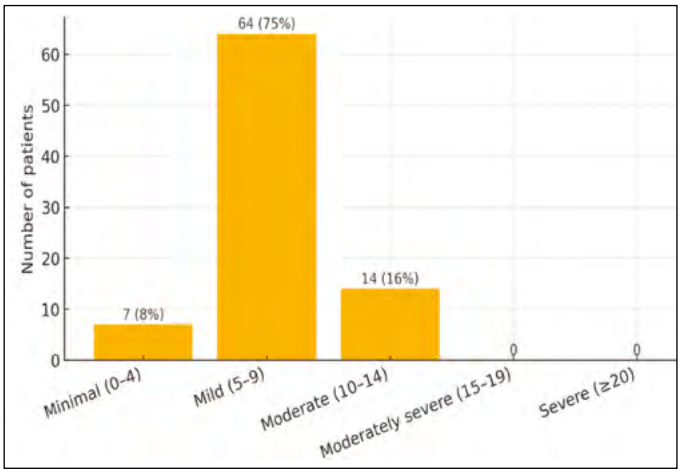

Fig. 1 Distribution of depression severity (PHQ-9) among acute ischemic stroke patients (n=85)

Note: *Bars show the number of patients; values above bars give absolute counts and cohort percentages. Depression was classified as Minimal (0-4), Mild (5-9), Moderate (10-14), Moderately severe (15-19), and Severe (≥20). Most participants (64/85; 75%) reported mild symptoms, 14 patients (16%) fell in the moderate range, and 7 (8%) had minimal symptoms. No patient scored in the moderately severe or severe ranges (≥15) during this acute post-stroke phase, indicating that very marked depressive symptomatology was absent or rare within the first two weeks after stroke.*

Prevalence of anxiety. Anxiety symptoms were even more prominent. The average GAD-7 score was 9.6 ± 2.9, on the upper end of the mild range (5-9) and nearing moderate. A GAD-7 score ≥10 (moderate or severe anxiety) was recorded in 36 patients, representing 42% of those assessed – a substantial portion. Specifically, 31 patients (36%) had moderate anxiety (GAD-7 10-14) and 5 (6%) had severe anxiety levels (GAD-7 ≥15). Thus, about 4 in 10 acute stroke patients suffered moderate-to-severe anxiety symptoms. Self-reported anxiety was corroborated by clinician ratings: the HAM-A scale mean was 17.3 ± 5.8, and 30 patients (35%) had HAM-A ≥18 (moderate or severe anxiety), of whom 9 (11%) were in the severe range (HAM-A ≥25). Many patients described constant worry about their health, fear of disability, or feelings of tension and restlessness in the hospital. Notably, there was considerable overlap between depression and anxiety: among patients with PHQ-9 ≥10, the majority also had GAD-7 ≥10, and vice versa, consistent with a comorbid depressive-anxiety syndrome. We found that 14% of the total sample had combined moderate depression *and* anxiety (both PHQ-9 ≥10 and GAD-7 ≥10). However, it was also evident that some patients had significant anxiety without depression (e.g., "worried well" about recovery), which underscores the need to screen for both conditions separately rather than assuming they always co-occur.

Cognitive impairment. On cognitive testing with the MMSE, the cohort's mean score was 25.2 ± 4.1 (out of 30).

Sixty-three patients (74%) scored in the "normal" range (24-30). Meanwhile, 22 patients – representing 26% of those tested – scored below 24, consistent with cognitive impairment. Most of these fell in the mild impairment range (MMSE 18-23); only 3 patients (3.5%) had MMSE <18, indicating moderate-to-severe cognitive impairment. The cognitive domains commonly missed included short-term recall and complex commands, whereas orientation and basic language were relatively preserved in many cases. It should be noted that patients with severe aphasia (≈5% of the original 99) could not be meaningfully tested with MMSE and were excluded from the MMSE analysis, so the true prevalence of cognitive impairment might be slightly underestimated. Nonetheless, about one-quarter of acute ischemic stroke survivors in our sample showed objective evidence of cognitive deficits even at this early stage. This finding aligns with expectations that vascular cognitive impairment can manifest acutely, particularly with strategic infarcts or high vascular risk burden.

Additional findings. Nearly all patients (95%) reported some degree of physical disability or limitation on the EQ-5D, and the mean EQ-5D visual analog scale (patient-rated health state) was 71.5 ± 14.8 (on a 0-100 scale, where 100 represents perfect health), reflecting a moderate self-perceived health status post-stroke. We observed that patients with higher depression/anxiety scores tended to rate their overall health and recovery confidence lower (qualitatively). No significant differences in depression or anxiety prevalence were found between men and women in this sample, although women had a non-significantly higher mean PHQ-9 (by ~1 point). Patients with cognitive impairment (MMSE <24) were slightly older on average than cognitively intact patients (66.3 vs. 64.3 years), but this difference was not statistically significant in our sample. Those with cognitive impairment also tended to have lower education levels (e.g., all patients with ≤4 years of schooling were cognitively impaired) and a higher frequency of prior strokes, suggesting that these factors may contribute to early post-stroke cognitive deficits.

Summary of key outcomes. In summary, within the acute phase of ischemic stroke, about one-third of patients experienced clinically relevant depression or anxiety symptoms, and one-quarter had measurable cognitive impairment. Mild symptoms were extremely common, with only a minority of patients being entirely free of any depressive or anxious symptoms. These results underscore that psychiatric and cognitive sequelae are the rule rather than the exception, even shortly after stroke onset.

Discussion

In this study of acute ischemic stroke patients, we found a high prevalence of affective symptoms and cognitive deficits even in the immediate post-stroke period. To our knowledge, this is one of the first detailed reports from our region quantifying these issues in an acute stroke setting. Our findings are broadly consistent with the international literature, with some nuances specific to the acute post-stroke timeframe.

Comparison with prior studies. The rate of post-stroke depression (PSD) observed in our cohort (approximately 16% with PHQ-9 ≥10 in-hospital and 35% with moderate depression per HAM-D) aligns with the lower end of the range reported in meta-analyses. A 2023 systematic review by Liu et al. noted an overall depression prevalence of around 27% in stroke survivors, with early assessments (within 3 months) yielding ~24-29% depending on the method [1]. Our self-reported depression rate (16%) is slightly lower, possibly because we captured patients within days of stroke when some were still mobilizing coping resources, or because some patients underreported symptoms due to the hospitalization context. In the first two weeks post-stroke, studies have noted a wide range (12-32%) of depression prevalence [4], so our PHQ-9 finding falls within this interval. By contrast, the clinician-rated depression prevalence (HAM-D 35%) was higher, suggesting that clinical interviewing detected depressive signs (e.g., sad affect, sleep disturbance, apathy) that patients did not fully report on questionnaires. This discrepancy highlights the importance of active clinical screening; relying solely on self-report may underestimate PSD immediately post-stroke, when patients might attribute symptoms to stroke recovery rather than depression per se. Nevertheless, by any measure, a substantial subset of our patients had clinically significant depression very early after stroke. This is notable because early-onset PSD is known to often persist and is linked to worse outcomes if not addressed [1]. Known risk factors for PSD include female sex, a prior history of depression, and stroke severity [5].

The prevalence of post-stroke anxiety (PSA) in our study (~40% with GAD-7 ≥10) was high and in line with, or slightly above, prior estimates. Literature on PSA has been less extensive than that on PSD, but a recent narrative review stated that anxiety affects roughly one-third of stroke survivors in the first year [6]. Our acute-phase data suggest that anxiety might even be more common than depression initially. One possible explanation is that hospitalization and uncertainty about recovery may acutely heighten anxiety. Patients often voiced concerns about disability, future strokes, or loss of autonomy, which can drive anxiety symptoms in the hospital. Nelsone and colleagues (2023) found that 17.6% of patients reported anxiety at 3 months and 15.7% at 12 months after a mild to moderate stroke, and these symptoms were significantly associated with poorer perceived recovery, although the study did not cover the acute hospitalization period [7]. A new 2024 meta-analysis focusing on young stroke patients found 39% had anxiety symptoms, almost exactly matching our 42% in a mixed-age cohort. Thus, our findings reinforce that anxiety is a frequent and often under-recognized problem in acute stroke care. Clinicians should be aware that even patients who do not meet full criteria for depression might be experiencing significant anxiety that could benefit from intervention (e.g., counseling, relaxation techniques, or cautious short-term anxiolytic use) [8].

Regarding cognitive impairment, about one-quarter of our patients had MMSE-defined impairment acutely. This is somewhat lower than long-term studies showing ~40-60% of stroke survivors have cognitive deficits when assessed within the first year [9-10]. The lower figure in our study likely reflects two factors: timing and assessment tool sensitivity. First, some cognitive sequelae of stroke may evolve or become more evident over time (for instance, vascular cognitive impairment can worsen due to post-stroke neurodegeneration or the uncovering of pre-existing deficits). Immediately after stroke, certain patients may not yet manifest the full extent of cognitive dysfunction, or they may partially recover cognitive function that was transiently affected (e.g., due to acute encephalopathy or swelling). Indeed, a proportion of early post-stroke cognitive impairment can improve, as suggested by studies in which some patients show cognitive recovery by 3-6 months [11]. Second, the MMSE has limitations in detecting mild executive dysfunction or attention deficits common in vascular cognitive impairment. Tools like the Montreal Cognitive Assessment (MoCA) are more sensitive in stroke cohorts. Had we used MoCA or a detailed neuropsychological battery, we might have identified a higher prevalence of subtle cognitive deficits (particularly in domains like processing speed or executive function). Therefore, our figure of 26% likely represents the more overt cases of cognitive impairment. It is worth noting that all patients with MMSE impairment had either a large cortical stroke or multiple vascular risk factors, aligning with known risk factors for PSCI such as age, low education, diabetes, hypertension, and prior strokes [11]. These risk factors were common in our sample, suggesting our patients remain at risk for further cognitive decline. Follow-up at 6-12 months will be needed to determine whether more patients develop late cognitive impairment or dementia.

Clinical implications of our findings. Our findings carry several important implications for clinical care and stroke rehabilitation. Firstly, the high rates of depression and anxiety in the acute setting underscore the need for routine psychological screening as part of stroke unit care. Brief instruments like the PHQ-9 and GAD-7 can be feasibly integrated into the nursing/neurology assessment protocol. Early identification of PSD/PSA is crucial because these conditions are treatable, and their treatment can meaningfully improve patient outcomes. For example, evidence suggests that treating post-stroke depression can enhance participation in rehabilitation therapies and is associated with better functional recovery [12]. Untreated depression or anxiety, on the other hand, may lead to reduced motivation, poorer engagement with physiotherapy, longer hospital stays, and diminished functional gains [2, 3, 13]. In our setting, we observed informally that patients with moderate-to-severe mood symptoms were often less active in therapy sessions and more likely to refuse or withdraw from some activities, corroborating these concerns. Early involvement of mental health professionals (such as consultation-liaison psychiatrists or clinical psychologists) could help implement supportive counseling, psychoeducation, or pharmacotherapy when appropriate. Interventions such as therapeutic optimism, involving family support, and managing sleep or pain issues can also alleviate depression/anxiety.

Secondly, recognizing cognitive impairment in the acute phase is important for discharge planning and rehabilitation tailoring. Patients with cognitive deficits may struggle with learning new rehabilitation strategies, adhering to safety precautions, or managing medications after discharge. Simple measures, such as providing written instructions, involving caregivers in education, and occupational therapy cognitive training, can mitigate risks. Moreover, early cognitive rehabilitation strategies might improve outcomes; some studies have shown that targeted cognitive training in post-stroke patients can modestly enhance cognitive function and even influence functional recovery. Our data suggest that even mild cognitive impairment was present in one-quarter of patients, so incorporating cognitive evaluation (e.g., MoCA screening before discharge) is advisable for most stroke survivors. This approach will help identify those who may benefit from neuropsychological follow-up or cognitive rehabilitation programs.

Thirdly, the fact that none of our patients were on antidepressants during the acute phase (and few on anxiolytics) reflects a potential gap in care. There is ongoing debate about prophylactic antidepressant use after stroke. Trials like FLAME (fluoxetine for motor recovery) initially suggested that SSRIs might improve motor outcomes, although later, larger trials did not confirm a clear benefit and raised safety concerns (e.g., falls, seizures). Current guidelines do not recommend routine prophylactic antidepressants for all stroke patients, but they do emphasize treating established depression or anxiety. Our hospital has since instituted a policy to have a psychiatrist evaluate any stroke patient who screens positive for depression or anxiety before discharge, to consider treatment. In the context of our study, had we identified major depression in any patient (e.g., PHQ-9 >20 or HAM-D >23, which we did not in this acute sample), initiation of an SSRI would have been strongly considered. For moderate depression/anxiety, options include psychotherapy (even brief CBT-based interventions or guided self-help), in addition to or instead of medications.

Study limitations. Several limitations must be acknowledged. Most importantly, our study lacked a control group of non-stroke patients. This limits our ability to attribute the observed depression and anxiety solely to the stroke event, as opposed to general hospitalization or prior psychiatric history. Nonetheless, the prevalence we observed is much higher than the general population point prevalence of mood disorders for this age group, suggesting a stroke-related effect. Another limitation is the single-center, relatively small sample size. With 99 patients (85 completing all assessments), our estimates, especially for subgroups (e.g., severe depression = 0%), should be interpreted with caution. The study may have been underpowered to detect certain differences (for instance, sex differences or correlations with stroke location). There is also potential selection bias: patients with very severe strokes (e.g., large infarcts

causing coma or global aphasia) could not be assessed and were excluded, which might underestimate the true burden of neuropsychiatric complications in all stroke patients. Conversely, patients with minor strokes might have been discharged early and missed; however, given our hospital's practice, most acute strokes were admitted for at least a few days, so we believe our sample is representative of moderate-to-severe stroke hospitalizations.

Additionally, the assessment tools have inherent limitations. The PHQ-9 and GAD-7, while validated and convenient, are screening tools and not equivalent to a clinical diagnosis. We did not perform structured psychiatric interviews (e.g., SCID) to formally diagnose major depressive disorder or anxiety disorders according to DSM-5 criteria, which means we are reporting "symptom prevalence" rather than diagnosed disorder prevalence. Some patients might have transient adjustment reactions or subclinical symptoms that would not qualify as disorders upon detailed evaluation. However, from a clinical standpoint, even subclinical symptoms can be important to address in stroke recovery. Our reliance on the MMSE for cognition is another limitation – a more detailed cognitive battery would provide richer information on domains such as executive function and could reveal higher impairment rates. We plan to incorporate the MoCA in future assessments.

Finally, this study only captures a cross-sectional snapshot in the acute phase. We do not have longitudinal data in this report to determine how depression, anxiety, or cognition evolve in these patients (though follow-ups at 3 and 12 months are underway in our project). Therefore, we cannot infer the persistence or prognosis of these conditions from our data. Some patients who were not depressed at discharge may develop depression later (post-stroke mood disorders can have delayed onset in a minority of cases), and vice versa. Similarly, cognitive impairment could worsen (due to post-stroke neurodegeneration) or improve (recovery, neuroplasticity) over time. Our acute-phase findings thus underscore an initial high burden, but longitudinal research is needed to inform when and how to intervene most effectively.

Conclusions

This study demonstrates that depression, anxiety, and cognitive impairment are common and consequential in patients hospitalized with acute ischemic stroke. Even within two weeks of stroke onset, about one-third of patients showed clinically meaningful symptoms of depression or anxiety, and one-quarter had cognitive deficits on screening. These figures reinforce that post-stroke neuropsychiatric complications begin early and should be proactively monitored. We found that most patients had at least mild depressive or anxious symptoms, underlining the need for routine psychological support as part of comprehensive stroke care.

Using a brief (20-30 min), structured battery of validated self-report and clinician-rated scales at the bedside in a real-world Moldovan hospital, we showed that such screening is both feasible and valuable, while also providing much-needed data from an Eastern European population often underrepresented in stroke research. The consistency of our results with international literature suggests that they are generalizable to similar clinical settings.

Our findings add to the growing recognition that stroke recovery is not only about physical rehabilitation but also about addressing mental health and cognitive function. Early identification and management of PSD and PSA might improve participation in therapy, functional recovery, and overall quality of life for stroke survivors. We advocate that stroke units implement standard screening for depression, anxiety, and cognition, and establish referral pathways for psychological and cognitive interventions. Future research should explore the efficacy of early interventions (pharmacological or psychotherapeutic) in ameliorating these conditions and whether such treatments translate into better stroke outcomes.

Competing interests

None declared.

Authors' contribution

MB and RC conceived the study, performed the statistical analysis, and drafted the manuscript. RC, MB, and MaB conducted the data collection. DJ assisted with data management and operational coordination. IN and JC supervised the overall project and coordinated the scientific implementation. JC contributed to the methodological framework and interpretation of findings. LB, AE, AB, and CA assisted with the literature review and critically revised the manuscript. All authors reviewed the work critically and approved the final version of the manuscript.

Informed consent for publication

Obtained.

Ethics approval

The Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy granted ethical approval to the study protocol (Favourable Opinion No. 3, issued on 9 July 2024), as documented in Minutes No. 41 of 21 May 2024, and written informed consent was obtained from each participant or, when applicable, from a legally authorised representative.

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RESEARCH ARTICLE

The relationship between zinc levels and immunological biomarkers in oral fluid in children affected by dental caries

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ABSTRACT

Introduction. Dental caries is one of the most widespread diseases in the human population, and the causes of increased susceptibility to dental caries remain of continuing interest to researchers. Active immunological components of oral fluid significantly influence the evolution of dental caries. The aim of this research is to study the relationship between zinc levels, the antimicrobial peptide LL-37, and transforming growth factor beta-1 in oral fluid, and the incidence of dental caries in children.

Material and methods. In this observational cohort study, 398 children aged between 3 and 15 years were clinically examined. The research group included 132 children with dental caries, and the control group consisted of 266 caries-free children. The Plaque Index (PI) and caries experience indices were evaluated. Caries risk was assessed using the *Cariogram* software. The levels of zinc, the antimicrobial peptide LL-37, and transforming growth factor beta-1 in oral fluid were determined using standard EliTeh kits, in accordance with the manufacturer's recommendations. The study was conducted in compliance with ethical standards and with written informed consent obtained from the children's parents. Data were processed automatically using the open-source software RStudio, version 2024.09.1+394.

Results. In children from the research group, a significant decrease in the levels of zinc, the antimicrobial peptide LL-37, and transforming growth factor beta-1 was detected in oral fluid, showing an inverse relationship with caries morbidity indicators.

Conclusions. Significantly decreased levels of zinc, the antimicrobial peptide LL-37 and transforming growth factor beta-1 in oral fluid of caries-susceptible children may indicate an increased risk of caries. Low levels of zinc, the antimicrobial peptide LL-37, and transforming growth factor beta-1 in oral fluid may also serve as indicators of rapid progression of dental caries – a fact that should be taken into account when planning individualized preventive measures.

Keywords: zinc, transforming growth factor beta-1 (TGF- β1), antimicrobial peptide LL-37, dental caries, oral fluid.

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Key messages

What is not known yet about the issue addressed in the submitted manuscript

Although a large number of salivary or oral fluid biomarkers have been proposed for the early diagnosis and prediction of dental caries, not all biomarkers have the same degree of accuracy. Controversial data are often presented in the scientific literature regarding the accuracy of predicting dental caries using immunological biomarkers from oral fluid (LL-37 and TGF- β1). Thus, the extent to which estimating Zn levels and immunological biomarkers in oral fluid (LL-37 and TGF- β1) contributes to increasing the predictive

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value and clinical applicability of combined biomarkers use remains underexplored.

The research hypothesis

Zinc levels and immunological biomarkers (LL-37, TGF-β1) in children affected by dental caries are lower compared to those in caries-free subjects.

The novelty added by the manuscript to the already published scientific literature

This manuscript makes an original and valuable contribution to the literature by comprehensively evaluating the relationship between zinc levels and immunological biomarkers in oral fluid (LL-37 and TGF-β1) in children with dental caries compared to those without dental caries.

Introduction

Dental caries (DC) is a major oral health problem worldwide and in the Republic of Moldova [1, 2]. The degree of DC prevalence is one of the key indicators of the population's health status and the effectiveness of medical and dental care in the country [3]. Reported since ancient times, and alongside the progress of civilization, the prevalence of DC in the population has increased significantly [3, 4]. Dental caries is considered the disease with the longest evolution period in human life, occurring with high prevalence and incidence in all regions of the globe, giving it an endemic-epidemic character [1, 2]. Despite scientific advancements and modern dental equipment, the prevalence and incidence of DC among the growing population of our country remain high, with no apparent trends toward reducing morbidity indicators [5].

DC and its complications influence the development of the dento-maxillary apparatus through the effect of both simple and complicated caries, often resulting in early tooth loss [6]. Treatment of dental caries in young children is frequently neglected for various reasons, and the impact of untreated caries is manifested not only through pain, sleep disturbances, and odontogenic infections but can also adversely affect the child's growth and development [7, 8].

It is known that oral fluid (OF) plays an important role in maintaining oral health due to its immunological and non-immunological components, such as antimicrobial proteins and enzymes [9]. In recent years, research has focused on identifying salivary biomarkers associated with the onset and progression of DC, including zinc (Zn), antimicrobial peptides (especially LL-37), and immunoregulatory cytokines such as transforming growth factor beta-1 (TGF-β1) [10]. Zn has been shown to inhibit enamel demineralization by approximately 49% through its ability to reduce acid production in the dental biofilm by inhibiting glucose transferase [11]. Experimental studies have also demonstrated that adequate Zn intake can stimulate the expression of these protective factors, thereby contributing to the prevention of DC [12, 13]. LL-37 is a cathelicidin produced by epithelial cells and neutrophils, acting as a broad-spectrum antimicrobial agent and modulator of inflammation. Studies in children of various ages by Almoudi et al. (2021) reported a negative correlation between LL-37 levels and the severity of DC [14, 15], as well as a positive relationship with salivary pH and salivary flow [15]. Conversely, no association has been observed between TGF-β1 content in OF and the extent of DC-related damage [16].

Thus, a large number of studies have demonstrated that OF biomarkers can be used to diagnose several oral diseases, including DC. However, not all biomarkers have the same degree of accuracy, and currently only some of them have been proven reliable. The use of combined biomarkers, on the other hand, could have higher accuracy compared to the use of isolated biomarkers [14-16]. Therefore, it is relevant to study the dynamics of OF biomarkers to develop individualized, accurate quantitative parameters for personalized caries risk assessment and DC prediction.

The aim of the research is to study the relationship between the levels of zinc (Zn), the antimicrobial peptide LL-37, and transforming growth factor beta-1 (TGF- β1) in oral fluid and the incidence of dental caries in children.

Material and methods

To achieve the purpose of the work, a prospective observational cohort study was conducted. This study included 398 conventionally healthy children aged between 3 and 15 years, who were divided into two groups. The examined children were from urban areas (196; 49.2%) and rural areas (202; 50.8%).

The research group (L_1, caries group) consisted of 132 children with carious lesions (33.2%, 95% CI 29-38), while 266 caries-free children (66.8%, 95% CI 62-71) formed the control group (L_0, caries-free group). All subjects were native to and residents of the Republic of Moldova. The distribution of children into age groups followed the target groups recommended by the World Health Organization (WHO) [17]: 3, 6, 12, and 15 years old. The 3-year-old group included 98 children (24.6%, 95% CI 20-29), the 6-year-old group 90 children (22.6%, 95% CI 19-27), the 12-year-old group 102 children (25.6%, 95% CI 21-30), and the 15-year-old-group 108 children (27.1%, 95% CI 23-32). Subjects were evenly distributed by gender: 199 girls (50.0%, 95% CI 45-55) and 199 boys (50.0%, 95% CI 45-55). Of all children examined, 196 (49.2%, 95% CI 44-54) came from ur-

ban areas, and 202 (50.8%, 95% CI 46-56) from rural areas. Therefore, the children in both groups lived under similar socio-economic conditions, and the distribution of subjects in groups L_1 and L_0 was proportional according to age, gender, and living environment.

Table 1. General characteristics of the studied cohort

Variable	N = 398	95% CI
Group		
caries research group (L_1)	132 (33.2%)	29%, 38%
caries-free control group (L_0)	266 (66.8%)	62%, 71%
Age		
3	98 (24.6%)	20%, 29%
6	90 (22.6%)	19%, 27%
12	102 (25.6%)	21%, 30%
15	108 (27.1%)	23%, 32%
Gender		
female	199 (50.0%)	45%, 55%
male	199 (50.0%)	45%, 55%
Area		
rural	202 (50.8%)	46%, 56%
urban	196 (49.2%)	44%, 54%

Note: *N - total number of patients and relative frequencies; 95% CI – 95% Confidence Interval*

A clinical examination of the children was performed, and OF was collected to assess the levels of Zn, TGF-β1, and LL-37. Data collection was conducted using the WHO Oral Health Questionnaire for Children (2013) to record oral status. The degree of DC damage was assessed by calculating caries experience indices: the DC prevalence index (PI) and indices reflecting DC severity, including dmft/DMFT and dmfs/DMFS [17]. The DMFT/dmft index represents the sum of carious permanent or primary teeth extracted due to caries and restored. The DMFS/dmfs index represents the sum of carious surfaces extracted due to caries and restored in permanent or primary teeth. The mean values of the DMFT/dmft and DMFS/dmfs indices were estimated by dividing these indices by the total number of subjects in the research and control groups. To achieve the complex and personalized prediction of DC, *Cariogram* software was used [18].

Biochemical studies of OF included the determination of Zn, transforming growth factor beta-1 (TGF- β1), and antimicrobial peptide LL-37 levels in all children included in the study. OF was collected unstimulated, in the morning, on an empty stomach, in sterile plastic tubes that were subsequently transported to the Scientific Laboratory of Biochemistry of the *Nicolae Testemițanu* State University of Medicine and Pharmacy. OF analysis was performed without dilution, with all stages of the study carried out according to the manufacturers' instructions.

Determination of Zn levels in OF was performed using ELISA Total-Microtiter Plates kits (Epitope Diagnostics). The analysis and detection of cathelicidin LL-37 peptide were performed using ELISA kits (enzyme-linked immunosorbent assay). Assessment of the TGF-β1 level in OF was performed using ELISA kits (Human TGF beta1 ELISA set, BD Biosciences).

The study was conducted in compliance with the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, number 17 dated 02.06.2015.

The data were processed automatically using the open-source software RStudio, version 2024.09.1+394 (https://www.rstudio.com). The use of these modern tools, widely recognized in academic and research environments, enabled rigorous, efficient, and fully reproducible analysis of the dataset. Their selection played a crucial role in ensuring the transparency of the analytical process, the validity of the results, and the potential for independent replication. The source code used for data preprocessing and analysis is available and can be provided upon request, offering the opportunity for verification of results or integration of the methodology in future related research.

To describe the numerical variables, fundamental descriptive statistics were computed: minimum and maximum values, arithmetic mean with standard deviation, median (Me), and interquartile range (IQR). These measures of central tendency and dispersion provided a detailed and precise depiction of the distribution of each variable, facilitating the identification of outliers, potential skewness, and deviations from normality. For comparing two independent groups with respect to the distribution of quantitative variables, the non-parametric Mann-Whitney-Wilcoxon test was applied, given its robustness and insensitivity to non-Gaussian distributions or extreme values.

Graphical representations of numerical variables were generated using box plots, complemented by jitter plots and violin plots, offering an intuitive visualization of central tendencies, variability, and group differences.

For categorical variables, both absolute and relative frequencies were calculated, accompanied by 95% confidence intervals for proportion estimates. This approach enabled a robust statistical characterization of category-level distributions. To test hypotheses related to categorical variables, Pearson's Chi-squared test was used. In all cases, regardless of sample size or frequency distribution, the Monte Carlo simulation method was employed, generating 100,000 random samples to ensure a robust estimation of the p-value. This approach was chosen to maintain the validity of statistical inference even in situations with small cell counts or imbalanced group sizes.

Additionally, linear regression analysis was performed to further explore associations between variables and to quantify the magnitude of the observed effects.

All statistical analyses were conducted using a conventional significance level (α) of 0.05. Results were interpreted accordingly: p-values below this threshold were considered statistically significant. The practical and clinical relevance of the findings was further evaluated in the context of the present study, taking into account the observed effect sizes and their potential implications for public health decisions or clinical interventions.

Results

The evaluation of the results regarding the degree of damage caused by DC at the beginning of the study showed that in children from the research group (L_1), the values of the caries experience indicators were 4.00 (Me, IQR = 3.25) for the dmft/DMFT index and 8.00 (Me, IQR = 7.00) for the dmfs/DMFS index (Wilcoxon rank sum test = 35,112, $p_{adjusted}$ < 0.001) (Table 2).

Table 2. Values of caries experience indices in children

Variables	Caries group (L_1) N = 132	95% CI	Caries-free group (L_0) N = 266	95% CI	Wilcoxon rank sum test	p-value	q-value
DMFT/ dmft	4.74 (2.65) 4.00 (3.25) 1.00 13.00	4.3, 5.2	0.00 (0.00) 0.00 (0.00) 0.00 0.00	0, 0	35,112	<0.001	<0.001
DMFS/ dmfs	8.06 (4.91) 8.00 (7.00) 1.00 24.00	7.2, 8.9	0.00 (0.00) 0.00 (0.00) 0.00 0.00	0, 0	35,112	<0.001	<0.001

Note: *DMFT: D-Decay, M-Missing, F-Filled, T-Tooth; dmft: d-decay, m-missing, f-filled, t-tooth; DMFS: D-Decay, M-Missing, F-Filled, S-Surfaces; dmft: d-decay, m-missing, f-filled, s-surfaces; N-number of patients; CI-Confidence Interval (for L_0, only one point zero); Statistical test: Wilcoxon rank sum test; p-value - probability of obtaining results as extreme as the observed ones, or more extreme, assuming that the null hypothesis is true; q-value – p value Hochberg correction for multiple testing.*

The complex and personalized prediction of DC using the *Cariogram* software highlighted a moderate caries risk in children with carious lesions, the chances of avoiding new carious cavities being 53.00% (Me, IQR = 19.50). In caries-free children, the chances of avoiding new carious cavities were 82.00% (Me, IQR = 18.00), the caries risk being low. A significant difference was detected between the groups of children L_1 and L_0, (Wilcoxon rank sum test = 3.171, $p_{adjusted}$ <0.001) (Figure 1).

The results obtained from the clinical examination and DC prediction, confirmed by the study of the salivary biomarker complex (Zn, TGF-β1, and LL-37), are presented in Table 3.

In the OF of children with carious lesions (L_1), significantly reduced levels of TGF-β1 were detected: 0.31 (Me, IQR = 0.27) pg/mL, compared to caries-free children, 0.44 (Me, IQR = 0.32) pg/mL (Wilcoxon rank sum test = 11,048 $p_{adjusted}$ = 0.001). The assessment of LL-37 in the OF of children affected by DC revealed low values of this antimicrobial peptide – 6.45 (Me, IQR = 1.48) ng/mL, compared to caries-free subjects, 8.85 (Me, IQR = 2.88) ng/mL, the difference being statistically significant (Wilcoxon rank sum test = 7,047, $p_{adjusted}$ = 0.001).

Figure 2 shows the results of the assessment of Zn levels in the OF of children. In children affected by DC, a statistically significant lower level of Zn in OF was detected: L_1 = 20.83 (Me, IQR = 9.73) pg/mL, compared to subjects in the control group, L_0 = 38.07 (Me, IQR = 21.08) pg/mL (Wilcoxon rank sum test = 5,574, $p_{adjusted}$ = 0.001).

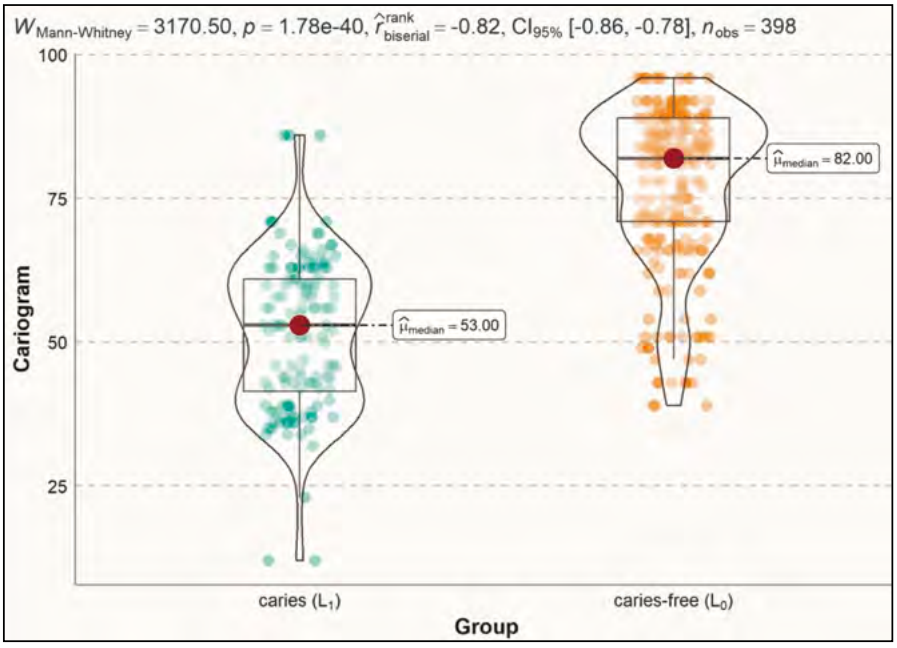

Fig. 1 Chances of avoiding new carious cavities estimated using the *Cariogram* software in children with dental caries and caries-free children.

Note: *$W_{Mann-Whitney}$ = 3170.50 - Wilcoxon rank sum test value; CI - Confidence Interval, n_{obs} – number of research and control groups; n - number of patients; caries (L_1) - research group; caries-free (L_0) - control group.*

Table 3. Values of immune system biomarkers in oral fluid in children

Variables	Caries group (L_1) N = 132	95% CI	Caries-free group (L_0) N = 266	95% CI	Wilcoxon rank sum test	p-value	q-value
Zinc	22.43 (7.35) 20.83 (9.73) 11.09 54.21	21, 24	39.40 (16.51) 38.07 (21.08) 14.79 97.76	37, 41	5,574	<0.001	<0.001
LL-37, ng/mL	6.66 (2.86) 6.45 (1.48) 1.35 27.92	6.2, 7.1	8.59 (2.31) 8.85 (2.88) 1.25 14.08	8.3, 8.9	7,047	<0.001	<0.001
TGF-β1, pg/mL	0.35 (0.21) 0.31 (0.27) 0.04 0.95	0.32, 0.39	0.50 (0.26) 0.44 (0.32) 0.02 1.51	0.47, 0.53	11,048	<0.001	<0.001

Note: *LL-37-antimicrobial peptide; TGF-β1- transforming growth factor beta-1; N-number of patients; CI- Confidence Interval; Statistical test: Wilcoxon rank sum test; p-value - probability of obtaining results as extreme as the observed ones, or more extreme, assuming that the null hypothesis is true; q-value – p value Hochberg correction for multiple testing.*

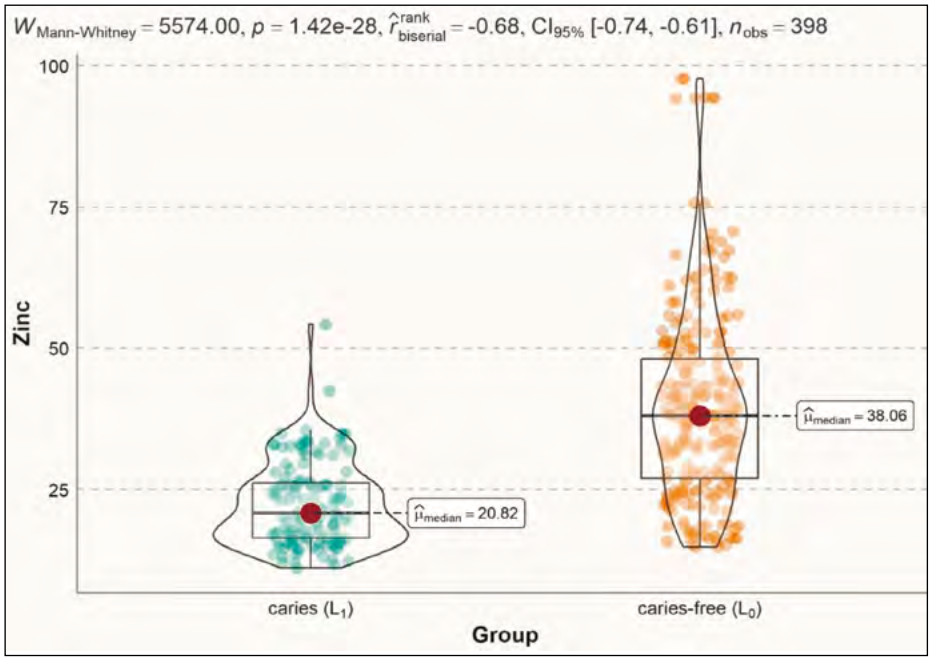

Fig. 2 Zinc levels in oral fluid in children.

Note: $W_{Mann\text{-}Whitney}$ = 5,574 - Wilcoxon rank sum test value; CI-Confidence Interval, n_{obs} – number of research and control groups; n - number of patients; caries (L_1) - research group; caries-free (L_0) - control group.

Discussion

OF represents a very well-organized, specific biological environment with unique and universal properties. It is a dynamic medium that reflects all changes occurring in the body, including those associated with carious disease. Due to its characteristics, OF is considered a valuable tool in the diagnosis of DC and offers multiple advantages as a diagnostic method: it allows better acceptance and cooperation from anxious patients [11], as well as rapid, simple, and non-invasive identification of biomolecules with the potential to improve early diagnosis, prognosis, monitoring of disease evolution, and assessment of the effectiveness of applied treatments [11, 19].

In the present study, significantly reduced levels of Zn, LL-37, and TGF-β1 were detected in the OF of children with carious lesions. Our results are consistent with those of previous research by Ajeel N.A. et al. (2024) [20]. The authors argue that the multifunctional cytokine TGF-β1 is one of the main regulators of the immune response, possesses strong anti-inflammatory properties, and inhibits the production of other pro-inflammatory cytokines [16]. It is assumed that the physiological function of TGF-β1 in odontoblasts is related to tooth mineralization [12]. It has also been suggested that TGF-β1 participates in the antimicrobial protection of the tooth, although this mechanism is not yet fully elucidated.

Several studies have obtained similar results, showing lower levels of LL-37 in the saliva of subjects with high caries activity compared to caries-free children [13, 16]. LL-37 has previously been shown to prevent biofilm formation on tooth surfaces, inhibit the growth and colonization of *Streptococcus mutans* [21], reduce the thickness of existing biofilms and the adhesion of microorganisms to the tooth

surface, thereby decreasing the production of inflammatory markers [13, 21, 22], and to enhance the antimicrobial capacity of anti-inflammatory cells such as neutrophils [14, 15, 21].

The analysis of the results of the present study revealed significantly lower levels of Zn in the OF of children with carious lesions. We believe that Zn deficiency led to a decrease in the levels of the immunoregulatory proteins TGF-β1 and LL-37 in the OF, which resulted in a rapid and severe evolution of the carious process in children of different ages, as confirmed by the low chances of avoiding new carious cavities estimated using the *Cariogram* software. At the same time, significantly higher values of Zn in the OF were observed in children free from DC. These results suggest that Zn may exert a protective role, possibly through its direct antibacterial action and by stimulating the production of the immunoregulatory molecules TGF-β1 and LL-37, and confirm the results of studies conducted by Talukder, P. et. al, 2011 and Morio K.A. et al., 2023 [12, 13]. Our results are also consistent with the study conducted by Sharma A. and Subramaniam P., 2021, which demonstrated the ability of Zn to inhibit glucosyltransferase and block dental biofilm formation [23].

Thus, the results obtained in the present study established that the increased levels of Zn, TGF-β1, and LL-37 in OF in caries-free children indicate that these components have an important role in maintaining oral microbial homeostasis and in preventing the occurrence of DC, as confirmed by the increased chances of avoiding new carious cavities estimated using *Cariogram* software (82.00%), the caries risk being low. Therefore, the combined use of Zn, TGF-β1, and LL-37 biomarkers in OF may ensure higher accuracy, with greater sensitivity and specificity for the personalized prognosis of DC in children. The results obtained support the further development of personalized approaches in the early diagnosis and prediction of DC, based on the biochemical profile of OF.

Conclusions

In children affected by dental caries, significantly decreased levels of Zn, LL-37, and TGF-β1 were found in OF, compared to the balanced levels of these biomolecules in the OF of caries-free children. Low levels of Zn, LL-37, and TGF-β1 in OF may be an indicator of rapid progression of DC, a fact that needs to be taken into account when planning individualized preventive measures.

The combined assessment of OF biomarkers represents a non-invasive and innovative method for DC prognosis, with higher sensitivity and specificity. However, for the clinical use of OF biomarkers, it is necessary to develop standardized protocols and conduct large studies in which the influence of various confounding variables is controlled.

Competing interest

None declared.

Contribution of authors

SP conceived the study, participated in its design, performed the statistical analysis, and drafted the manuscript. OT consulted on the study design, processing of oral fluid samples, assessment of Zn levels and immunological biomarkers, interpreted the results, and contributed to the drafting of the manuscript. AS participated in the study design, interpreted the results, and guided the drafting of the manuscript. All authors critically reviewed the paper and approved the final version of the manuscript.

Ethics approval

The study was conducted as part of a doctoral research project approved by the Committee of Research Ethics of *Nicolae Testemițanu* State University of Medicine and Pharmacy (Minutes No.17, dated 02.06.2015).

Patient consent

Obtained.

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RESEARCH ARTICLE

A simple method for preparing herbal reference standards based on salting-out solvent extraction

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ABSTRACT

Introduction. The expansion of the range of herbal medicinal products requires the availability of a large number of different reference standards for their analysis. A possible solution is the use of herbal reference standards (HRS), which, in turn, requires the development of simple production methods that meet all the requirements for reference standards.

Material and methods. Using ten plant species containing polyphenolic compounds, a general scheme for preparing HRS was developed and tested, which includes extraction of plant material with low concentrations of a polar organic solvent (usually 20% isopropanol), subsequent salting-out of the organic phase with ammonium sulfate, and drying the organic extract on the surface of anhydrous lactose. The composition of the obtained HRS and intermediates was determined by high-performance liquid chromatography using primary chemical reference standards.

Results. For all studied plant species, satisfactory values of the yield of target components, good solubility of the prepared HRS, and similarity of the chromatographic profiles of HRS and the corresponding plant material were obtained.

Conclusions. A simple and inexpensive method for preparing HRS, based on salting-out solvent extraction of target components, is proposed.

Keywords: herbal reference standards, salting-out solvent extraction, polyphenolic compounds, phytochemical analysis, pharmaceutical analysis.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

The possibility of obtaining herbal reference standards (HRS) in the form of dry extracts using the same solvent for treating the plant material followed by salting-out extraction of target compounds.

The research hypothesis

Preparing the primary plant extract with an organic solvent that can be salted out in a subsequent technological stage should reduce time and solvent consumption, thereby simplifying the entire HRS preparation process.

The novelty added by manuscript to the already published scientific literature

Using the same water-organic mixture for preparing the primary plant extract and for subsequent salting-out extraction showed a good yield of medium-polar compounds, such as polyphenols, resulting in a very simple and inexpensive method for producing HRS.

Introduction

The constant expansion of the range of medicinal and dietary products of plant origin, as well as the increasing requirements for their quality, has led to the wide use of instrumental (especially chromatographic) methods of analysis. The multicomponent composition of plant materials poses a serious problem associated with the need to use a significant number of reference chemical substances for calibration and selectivity testing, many of which are expensive or difficult to access.

The European Pharmacopoeia and other international pharmacopoeias propose the use of herbal reference standards (HRS) as a solution to this problem [1-4]. HRS are herbal materials or extracts, usually dry, intended, in most cases, for use as primary standards for qualitative analysis (identification of chromatographic peaks and/or system suitability testing). Pharmacopoeias do not recommend, but do not exclude, the use of HRS in quantitative analysis. In this case, HRS are considered secondary standards and should be standardized against primary chemical standards. In the absence of appropriate pharmacopeial reference standards, non-pharmacopoeial reference standards that meet the requirements of Ph. Eur., chapter 5.12 "Reference standards", are developed and implemented. At the stage of extensive studies carried out during the development of new pharmaceutical products, appropriately characterized primary and working in-house reference materials are used [5].

Factors limiting the use of HRS for quantitative analysis are the hygroscopicity of dry extracts, which imposes high requirements on packaging and storage conditions, solubility problems associated with matrix components, and the uncertainty of chromatogram processing results caused by peak interference and matrix effects [6]. A solution to the problem of HRS hygroscopicity and solubility may be the removal of both the most polar and least polar groups of matrix components at the stage of primary extract purification [7].

The salting-out liquid extraction (also known as homogeneous liquid extraction) method is based on the separation of a mixture of solvents miscible under normal conditions (usually water and a polar organic solvent) by the addition of strong electrolytes. It has been used by various authors for the extraction of metal ions from aqueous solutions [8], determination of preservatives and sweeteners in juices [9], α-dicarbonyl compounds in beer [10], iodate in food-grade salt [11], in biomedical analysis [12, 13], and many other cases. It was previously used to obtain HRS from such plant species as *Melissa officinalis* L., *Hypericum perforatum* L., *Crataegus monogyna* Jacq., *Urtica dioica* L., and *Sambucus nigra* L., showing good efficiency in removing highly polar ballast components [7]. In this case, salting-out extraction with isopropanol in the presence of ammonium sulfate was used after the distillation of ethanol from the primary water-alcoholic extract, which increased the labor intensity and energy consumption of the entire technological process and required the use of two different organic solvents.

In this article, the possibility of salting-out liquid extraction of target components directly from the primary plant extract, without prior removal of the organic solvent, and the efficiency of extraction of various groups of polyphenolic compounds using this approach are discussed.

The aim of the study is to develop the most efficient and cost-effective method for preparing HRS, based on salting-out liquid extraction.

Material and methods

Plant material. Wild bergamot (*Monarda fistulosa* L.), Melissa (*Melissa officinalis* L.), Tansy (*Tanacetum vulgare* L.), and St. John's wort (*Hypericum perforatum* L.) were harvested from the plantation of the Scientific-Practical Centre in the Domain of Medicinal Plants of *Nicolae Testemițanu* State University of Medicine and Pharmacy; Nettle (*Urtica dioica* L.), Hawthorn (*Crataegus monogyna* Jacq.), and Elder flower (*Sambucus nigra* L.) – from the spontaneous flora (municipality Chișinău, township Codru). Collected aerial parts or appropriate organs of the studied plants were air-dried and fragmented to a particle size of 0.65-1.4 mm. Green tea leaves (*Camellia sinensis* (L.) Kuntze) according to the technical specification TU U 19421419.001-99 ("Monomakh" JSC, Ukraine), fresh orange (*Citrus* × *sinensis* (L.) Osbeck) according to the international standard CODEX STAN 245-2004 (Spain), and grapefruit (*Citrus paradisi* Macfad.) according to the international standard CODEX STAN 219-1999 (South Africa) were purchased from a local grocery store.

Apparatus. The plant material was extracted in a cylindrical flow extractor with a 50 mL internal volume, using a peristaltic pump for solvent delivery. Analysis of plant material, extracts, and the final preparations was performed using "Agilent 1260 Infinity" liquid chromatograph with a diode-array detector.

Chemicals. Primary reference substances, solvents, and reagents of analytical grade used in the study were purchased from Sigma-Aldrich (USA), Merck, Fluka, and Stanchem (Germany).

Methodology. The following general scheme was elaborated and used for preparing HRS:

10-20 g of dried and fragmented plant material (depending on its bulk density and swelling coefficient) was loaded into a flow extractor. A 20% isopropanol or another selected solvent was fed into the bottom of the extractor at room or controlled temperature and at a flow rate of 15-25 mL/h using a peristaltic pump, until 2.5–3 parts (*V/M*) of the primary extract were obtained. After taking an analytical sample, ammonium sulfate was dissolved in the extract to saturation (approximately 0.45-0.5 grams per milliliter of primary extract at room temperature). The mixture was centrifuged for 5 min at 3000 min^{-1}, the upper liquid layer was separated, its volume was measured, and a sample was taken for analysis. The organic extract was then applied in 2 stages to a 2-fold (*M/V*) amount of anhydrous lactose with intermediate drying in an open porcelain cup at 70°C and continuous stirring. Final drying was performed for 16-20 h at 40°C. The target product was then lightly ground and sieved (0.65 mm mesh size).

In the case of HRS preparation from fruit juices, 40 mL of freshly squeezed juice was mixed with 10 mL of isopropanol, heated to 80°C to coagulate protein substances, cooled to +4°C, and centrifuged. The centrifugate was saturated with ammonium sulfate, the phases were separated by centrifugation, and the upper layer was applied in 3 stages to 1.5-fold (*M/V*) amount of anhydrous lactose and dried as described above.

The HRS obtained by the described method were dissolved for analysis in 20% ethanol (about 100 mg per 10 mL), visually assessing the completeness of dissolution. The chromatographic profiles of HRS and the original plant material were compared using the "fingerprint method". The quantitative content of the identified components was also determined using primary reference substances.

Results

For all plant species included in the study, good similarity of the chromatographic profiles of HRS and the corresponding raw materials was achieved, which allows the obtained HRS to be considered suitable for peak identification and testing of chromatographic system selectivity. All prepared HRS were completely soluble in 20% ethanol, except Wild bergamot HRS, which needed to be dissolved in 1 mL of dimethyl sulfoxide before dilution with the main solvent, due to the presence of flavone glycosides poorly soluble in water-alcohol mixtures.

The composition of the extractants used, the extraction temperature, as well as the content of the identified components and their yield from the initial plant material, are given in Table 1.

Table 1. Basic conditions for the extraction of plant material and polyphenols composition of the obtained HRS and intermediate products

Plant material	Extractant and temperature	Quantified compounds	Concentration (yield from plant material)		
			Primary extract	Salted-out extract	HRS
Wild bergamot herb	20% IPA, 20-22°C	Rosmarinic acid	1.98 mg/mL (57.7%)	9.69 mg/mL (56.4%)	4.19 mg/g (52.5%)
		Monardic acid A as salvianolic acid A	3.77 mg/mL (71.6%)	12.3 mg/mL (46.7%)	5.29 mg/g (43.2%)
		Flavone glycosides as cynaroside	7.34 mg/mL (77.3%)	15.7 mg/mL (33.1%)	6.99 mg/g (31.7%)
		Flavanone glycosides as naringin	1.56 mg/mL (67.4%)	6.29 mg/mL (54.4%)	2.75 mg/g (51.1%)
Melissa leaf	20% IPA, 20-22°C	Rosmarinic acid	7.32 mg/mL (74.7%)	33.1 mg/mL (71.3%)	13.6 mg/g (64.9%)
Tansy flower	20% IPA, 20-22°C	Hydroxycinnamic acids as chlorogenic acid	9.00 mg/mL (52.4%)	42.0 mg/mL (51.4%)	17.7 mg/g (46.9%)
Nettle leaf	20% IPA, 20-22°C, then + H_3PO_4 to pH 3.0	Chlorogenic acid	2.49 mg/mL (92.7%)	12.6 mg/mL (86.3%)	5.25 mg/g (77.1%)
		Cafeoilmalic acid as chlorogenic acid	4.61 mg/mL (95.3%)	23.8 mg/mL (92.6%)	10.4 mg/g (86.7%)
Hawthorn leaf and flower	20% IPA, 20-22°C	Hydroxycinnamic acids as chlorogenic acid	3.42 mg/mL (73.3%)	11.8 mg/mL (42.1%)	5.05 mg/g (38.9%)
		Flavonol glycosides as hyperoside	0.93 mg/mL (62.8%)	4.07 mg/mL (45.6%)	1.74 mg/g (41.0%)
		Flavone glycosides as vitexin-2-O-rhamnoside	3.73 mg/mL (69.5%)	16.1 mg/mL (50.0%)	6.92 mg/g (45.1%)
Elder flower	15% ACN + 10% IPA, 50°C	Hydroxycinnamic acids as chlorogenic acid	3.71 mg/mL (65.3%)	11.3 mg/mL (50.5%)	5.27 mg/g (49.7%)
		Flavonol glycosides as rutoside	3.82 mg/mL (68.5%)	11.9 mg/mL (54.0%)	5.42 mg/g (52.2%)
St. John's wort	15% ACN + 10% IPA, 45°C	Hydroxycinnamic acids as chlorogenic acid	1.15 mg/mL (55.6%)	3.87 mg/mL (44.2%)	1.53 mg/g (37.5%)
		Flavonol glycosides as rutoside	0.54 mg/mL (49.8%)	2.20 mg/mL (41.0%)	0.84 mg/g (34.9%)
		Hypericin + pseudohypericin	0.28 mg/mL (23.8%)	1.12 mg/mL (22.7%)	0.44 mg/g (19.2%)
Green tea leaf	20% IPA, 20-22°C	Caffeine	5.05 mg/mL (78.9%)	22.3 mg/mL (76.6%)	8.92 mg/g (67.6%)
		Total catechins as (-)-epicatechin	36.2 mg/mL (74.4%)	154 mg/mL (69.6%)	60.0 mg/g (59.6%)
Orange juice	IPA, 25% from juice volume	Hesperidin	–	1.20 mg/mL (53.8%)	0.80 mg/g (52.6%)
Grapefruit juice	IPA, 25% from juice volume	Naringin	–	5.09 mg/mL (77.0%)	3.10 mg/g (73.6%)

Note: HRS – herbal reference standard; IPA – isopropanol; ACN – acetonitrile.

A special study on the efficiency of salting-out agents was carried out using Wild bergamot extracts, containing various groups of polyphenolic compounds, as an example (Table 2).

Table 2. The efficiency of salting-out extraction from 20% isopropanol using saturating concentrations of various salts (for some polyphenolic compounds of Wild bergamot)

Salting-out agent	Analyte concentration (yield from the primary extract)			
	Rosmarinic acid	Monardic acid A	Flavone glycosides	Flavanone glycosides
Ammonium sulfate	8.63 mg/mL (100%)	10.7 mg/mL (73%)	15.5 mg/mL (51%)	5.84 mg/mL (95%)
Magnesium sulfate	6.23 mg/mL (96%)	9.24 mg/mL (85%)	19.8 mg/mL (78%)	4.63 mg/mL (100%)
Sodium sulfate	5.77 mg/mL (100%)	8.43 mg/mL (87%)	16.1 mg/mL (80%)	3.79 mg/mL (92%)
Sodium thiosulfate	7.62 mg/mL (77%)	6.45 mg/mL (39%)	10.8 mg/mL (31%)	5.63 mg/mL (80%)
Sodium citrate	6.02 mg/mL (44%)	5.25 mg/mL (23%)	12.9 mg/mL (27%)	4.86 mg/mL (49%)
Sodium nitrate	3.87 mg/mL (42%)	2.06 mg/mL (13%)	4.75 mg/mL (15%)	3.00 mg/mL (45%)
Sodium nitrite	1.09 mg/mL (7.9%)	0.25 mg/mL (1.1%)	2.41 mg/mL (5.0%)	2.86 mg/mL (29%)
Sodium chloride	3.38 mg/mL (29%)	1.39 mg/mL (7.1%)	3.67 mg/mL (9.1%)	2.90 mg/mL (35%)
Sodium acetate	n/s	n/s	n/s	n/s
Ammonium acetate	n/s	n/s	n/s	n/s
Ammonium chloride	n/s	n/s	n/s	n/s
Potassium chloride	n/s	n/s	n/s	n/s
Potassium bromide	n/s	n/s	n/s	n/s
Potassium iodide	n/s	n/s	n/s	n/s
Potassium dihydrogen phosphate	n/s	n/s	n/s	n/s
Potassium oxalate	n/s	n/s	n/s	n/s

Note: n/s – no separation of liquid phases.

Discussion

Isopropanol was used as the organic component of the extraction mixture for most plant materials because it is sufficiently polar and can be easily salted out from aqueous solutions. Previous studies [7] showed good extraction of a wide range of polyphenolic compounds into the organic phase using the salting-out method. At concentrations of about 20%, it provides a sufficiently high yield of most polyphenolic compounds during the extraction of plant material while minimizing the extraction of undesirable low-polar components, such as lipids and resins. Removal of the most polar ballast (carbohydrates, hydroxy acids, inorganic salts) is ensured at the subsequent stage of salting out the organic phase. The availability and relatively low toxicity of isopropanol are also attractive properties.

However, in some cases, we observed insufficient extraction of all or individual target components from the plant material. For Elder flower at room temperature, an abnormally low (15-20%) yield of both chemical groups of polyphenols was obtained due to the high activity of polyphenol oxidase. In St. John's wort, we observed a significant conversion of quercetin glycosides into the aglycone due to hydrolase activity. In both cases, the problem was solved by adding acetonitrile (a protein-denaturing agent) to the extractant and increasing the extraction temperature. Complete replacement of isopropanol with acetonitrile led to a low yield of the most polar components; therefore, a small amount of isopropanol was retained in the extractant.

Salting out of the organic solvent and target components in all cases was performed with ammonium sulfate, as used by other authors [9, 11, 12]. The high water solubility of this salt allows minimizing the time required for the salting-out procedure and obtaining extracts with a high yield of target components and minimal water content. This circumstance facilitates the subsequent drying of the organic extracts. The advantage of this salting-out agent is demonstrated in Table 2, which shows that the highest yield of all components is provided by salts of polybasic acids, especially those of sulfuric acid. At the same time, ammonium sulfate gave the maximal concentration of most analytes due to the lowest water extraction.

When the primary water-organic extract is saturated with ammonium sulfate and centrifuged, the mixture usually separates into three liquid phases. The upper layer is an organic extract containing the target components; the lower layer is a concentrated aqueous solution of ammonium sulfate; and between them lies a resinous layer of small volume, representing a concentrated aqueous solution of highly polar organic compounds, such as mono- and oligosaccharides, with solid particles of polysaccharides, proteins, and other substances that are poorly soluble in organic solvents and concentrated electrolyte solutions. A solid sediment, consisting of excess ammonium sulfate and poorly soluble inorganic salts, such as calcium sulfate, may be present at the bottom of the centrifuge tube.

In most cases, primary plant extracts are naturally slightly acidic. An exception in this study was Nettle extracts, with pH values of 7.3-7.5, which resulted in a low yield of hydroxycinnamic acids, especially caffeoylmalic acid, upon salting out. In this case, o-phosphoric acid was used to adjust the pH.

In general, the obtained results confirm the suitability of the proposed method for the production of HRS, at least from polyphenol-containing plants. The method is time-saving (all operations, except the final drying, were performed within one working day) and material-saving,

both in relation to plant raw materials and to solvents and reagents. This allows its wide use in research and educational practice. The obtained HRS were found to be the most convenient as in-laboratory working standards for the study of non-pharmacopeial plant species, their screening, phenotyping, technological, and other studies associated with large volumes of analytical work, allowing for significant savings of expensive chemical reference standards.

The use of fresh plant juices eliminates the stage of obtaining the primary extract and simplifies the entire technological process. However, the target substances must pass into the squeezed juice in sufficient amounts. Most likely, HRS prepared from fresh juices will be suitable for the analysis of the juices themselves, particularly in the food industry. Thus, the HRS samples obtained in this study from grapefruit and orange juices may be useful for identifying cases of adulteration of grapefruit juice by the presence of hesperidin.

Conclusions

Extraction of plant material with a 20% isopropanol solution, followed by salting out the organic solvent with ammonium sulfate, in most cases showed a sufficiently high and uniform yield of various groups of polyphenolic compounds. Subsequent drying of the organic extracts on the surface of anhydrous lactose allowed the obtaining of non-hygroscopic, easily soluble dry extracts suitable for qualitative (as primary standards) or quantitative (as secondary standards) analysis of the corresponding plant materials and preparations. As a result, a simple and inexpensive method for preparing herbal reference standards using salting-out liquid extraction was proposed.

Competing interests

None declared.

Authors' contributions

IC designed the study, conducted the laboratory work and performed its technological part, interpreted the data, and drafted the first manuscript. AC collected and processed the plant material, performed the analytical part of the laboratory work, and revised the manuscript. The final version of the manuscript was approved by all authors.

Ethics approval

Not needed for this study

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RESEARCH ARTICLES

Toxico-hygienic and economic aspects of acute non-occupational chemical poisonings in the Republic of Moldova during the period 2019-2023

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ABSTRACT

Introduction. Acute non-occupational chemical poisoning is a current public health problem in the Republic of Moldova, affecting both children and adults. The global increase in the use of chemical substances and mixtures raises the risk of chemical poisoning, which ranks fourth among the causes of injury in children, after road traffic injuries, burns, and drowning. It also generates a significant economic burden, and its quantification contributes to making informed decisions regarding the efficient allocation of resources.

Material and methods. A descriptive, retrospective, cross-sectional study was conducted based on statistical data from the statistical form *f.18-săn* and other national reports for the period 2019-2023. Cases of chemical poisoning were analyzed, excluding other types of poisoning. The evaluated indicators included incidence, mortality, distribution by age group and etiology, as well as the direct costs of their treatment.

Results. The analysis of statistical data revealed a total of 9,579 intoxications, with an overall incidence of 69.5 cases per 100,000 inhabitants. The most common causes were medications, which accounted for 41.8% (n = 4,002), followed by alcohol – 18.8% (n = 1,798), toxic gases – 14.5% (1,385 cases), and pesticides – 4.4% (n = 417). During the 2019-2023 period, children had a higher incidence, with 130.58 cases per 100,000 children, while adults recorded 52.21 cases. The peak incidence of chemical poisonings was recorded in 2019, at 64.8 cases per 100,000 population (24%, 95% CI: 23.12–24.83), while the lowest was in 2020, at 56.5 cases per 100,000 population (15.6%, 95% CI: 14.88–16.34). Over the five-year period, treatment costs for chemical poisoning totaled 58 million lei, highlighting the burden on the health system.

Conclusions. Acute non-occupational chemical poisonings continue to be a significant public health problem in the Republic of Moldova, generating not only an impact on the morbidity and mortality of the population but also a considerable economic burden on the health system, highlighted by treatment expenses that amounted to over 57 million lei during the 2019-2023 period. Preventive, educational, and informational measures for the population, including awareness campaigns, are essential for reducing the number of acute poisonings of chemical etiology.

Keywords: poisonings, medicines, alcohol, gas, pesticides, prevention, expenses.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

In the field of ANCP, several gaps exist, such as the impact of poisonings and their long-term health consequences, as well as the

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incomplete identification of causal agents in many cases. Additionally, the costs of ANCP treatment, especially indirect ones, are not evaluated in the Republic of Moldova. Furthermore, the lack of clinical protocols for adults limits the effectiveness of the medical response to these poisonings.

The research hypothesis

Identifying the particularities of ANCP and implementing information campaigns will contribute to preventing and reducing their incidence and mortality, as well as to lessening the economic impact on health.

The novelty added by the manuscript to the already published scientific literature

This is the first comprehensive retrospective study in Moldova that estimates the incidence, mortality, distribution by etiology and gender, and the economic impact of ANCP (2019–2023) in children and adults. It includes a detailed assessment of direct medical costs and identifies temporal trends through regression analysis. The results served as the basis for developing practical guidelines and prevention materials for ANCP.

Introduction

Recently, at the global level, there has been an increase in the use of chemical substances and mixtures in various areas of human activity, such as industry, agriculture, and households [1].

This contributes to an increased risk of accidental or intentional exposure to chemical agents, with a direct impact on public health. According to data from the specialized literature, approximately 3,000 chemical substances are involved in the production of acute chemical poisonings, out of a total of over 100 million known natural or synthetic compounds (over 350,000 artificially synthesized) at the global level [2-5].

Acute non-occupational chemical poisonings represent a current public health problem, with a major impact on morbidity and mortality worldwide [6-7]. They constitute one of the main causes of patient presentation to emergency departments and admission to intensive care units, affecting particularly vulnerable populations, such as children and adolescents [8-10].

According to the World Report on the Prevention of Injury in Children, poisonings of chemical origin rank fourth in incidence in 16 countries, after road traffic injuries, burns, and drowning [11-12], with an incidence of 1.8 cases per 100,000 children [13].

Studies conducted in several countries (Turkey, France, etc.) focusing on cases in emergency departments have highlighted that the main chemical substances causing poisonings are medicines, alcohol, gases, pesticides, and household chemicals. The use of these substances has increased considerably, bringing not only economic benefits but also costs related to the treatment of poisonings and their consequences [1]. Thus, the annual costs associated with acute poisonings of chemical origin have reached 1.5-2.1 billion euros in France and Italy, and the university hospital in Gent, Belgium, in 2017 spent 1.5 million euros, i.e., 3.6% of all emergency admissions, with an average cost of 1,287 euros per patient [1, 14, 15]. In Illinois, USA, the costs for treating people with pesticide poisoning reached 7.9 billion dollars annually [16, 17], while in Chile and Colombia they were 1.4 million dollars and 892,336 dollars, respectively, for treating cases of acute paraquat poisoning [18].

Another problem in this area is the use of chemicals in suicide attempts, a phenomenon reported both in developed countries, accounting for 10–36.2%, and in developing countries, with a share between 65 and 79.2%, where pesticides are one of the main causes of voluntary deaths [19-22].

According to data from the World Health Organization, suicide by chemical poisoning represents a major public health problem [23] and is the second leading cause of death among young people aged 15 to 29, after road accidents [24, 25].

The Republic of Moldova is no exception to the global trend regarding acute poisonings of chemical origin, and the analysis of cases reported during the period 2019-2023 is essential for identifying the chemical substances involved in such accidents–medicines, alcohol, pesticides, household chemicals, etc. – and for classifying poisonings according to the age of the patients. The impact of chemical poisonings, including economic costs, continues to represent a challenge for the public health system in the country.

Thus, the identification of causal factors, consequences, and vulnerable groups, including the costs generated by this condition, is essential for the development of measures to prevent acute poisonings of chemical etiology.

Material and methods

A descriptive, retrospective, cross-sectional study was conducted, aimed at estimating the epidemiological and toxico-hygienic aspects of acute non-professional poison-

ings of chemical etiology in the Republic of Moldova during the period 2019-2023. The research was based on a multilateral process that included the collection, analysis, and synthesis of statistical data on cases of acute poisonings with medicines, alcohol, gases, pesticides, nitrates, and household chemicals registered in the Republic of Moldova. The information was extracted from existing statistical forms, such as Statistical form f.18-săn, Urgent notification forms regarding ANCP f. no. 058-3/e, the Register of persons with acute exogenous non-professional poisonings of chemical etiology, f. no. 360-1/e, and National Reports of the National Agency for Public Health (State Supervision of Public Health in the Republic of Moldova), and was used to assess the incidence and mortality of such poisonings, both among children up to 18 years old and the adult population (≥ 19 years old). The analysis included the description of the annual evolution of incidence and mortality from ANCP, the distribution by age categories, etiological structure, and causal factors. Data processing was carried out using Microsoft Excel 2021, calculating statistical indicators such as averages, confidence intervals (95% CI), determination coefficients (R^2), and establishing the corresponding linear regression formulas (Y).

The research protocol was approved and received a favorable opinion from the Research Ethics Committee of the "Nicolae Testemițanu" State University of Medicine and Pharmacy, according to meeting minutes no. 68 dated May 21, 2018.

The study focused on direct treatment costs, collecting and analyzing data on expenditures related to patients hospitalized with ANCP in the Republic of Moldova during the 2019-2023 period. It also analyzed the applicability and challenges of economic evaluation, especially cost-benefit analysis, as a support tool in public health decision-making and in the development of prevention strategies. The retrospective, descriptive, and economic study was conducted within two reference medical institutions: the Mother and Child Institute, which predominantly serves the pediatric population, and the *Holy Trinity* Municipal Clinical Hospital, intended for the treatment of adult patients. Primary information was extracted from hospital admission registers, medical records, accounting records, and internal statistical reports of the two medical institutions involved in the study.

The total cost of treatment for a pediatric case was calculated according to the formula:

$$\textbf{Cost}_{\textbf{per case}} = \text{MCI} \times \text{Annual contracting fee}$$

MCI (Medical Complexity Index) is established by the attending physician (it is regulated annually by the National Health Insurance Company and the Ministry of Health, according to the provisions established in the standard contract for the provision of hospital services concluded with public medical and health institutions).

For the adult population, the analysis included data on the length of hospitalization, medications administered, investigations performed (including laboratory tests and imaging examinations), as well as all related cost components. The economic estimate was based on quantifying expenses using the following formula:

$$\mathbf{C_{tpc}} = C_s + C_a + C_m + C_{sm} + C_i$$

where:

C_{tpc} – total cost per case
C_h – cost of hospitalization
C_a – cost of alimentation
C_m – cost of medicines
C_{sm} – cost of sanitary materials
C_i – cost of investigations

The direct costs of acute non-occupational chemical poisonings include all medical expenses incurred for case management, from initial diagnosis to recovery.

Results

The incidence and mortality of acute non-occupational chemical poisoning (ANCP) are determined by demographic factors, etiology, intentionality, and the knowledge level of the population, highlighting the essential role of epidemiological surveillance and toxico-hygienic monitoring in prevention strategies and effective therapeutic management.

The retrospective analysis of statistical data regarding ANCP in the Republic of Moldova during the period 2019-2023 reveals an alarming trend, with a total of 9,579 cases, of which 41.3% occurred among the pediatric population and 58.7% in people over 19 years of age. The increased vulnerability of children is confirmed by an average annual incidence higher than that observed in the adult population, with 130.58 cases recorded per 100,000 children compared to 52.21 cases per 100,000 adults. The peak of ANCP incidence was reached in 2019, with a total of 64.81 cases per 100,000 population or 24% (n = 2,296 cases, 95% CI: 23.12–24.83), and the minimum value was recorded in 2020, with an incidence of 56.51 cases per 100,000 thousand inhabitants or 15.6% (n = 1,494 cases, 95% CI: 14.88–16.34) (Table 1).

Children aged 0-3 years were the most affected, with the highest share of ANCP (43%), an annual average of 339 cases, and 2 deaths. Adolescents aged 15-18 years represented 23.1% of cases, with intentional cases predominating, of which 6 resulted in death.

At the same time, during this period, 156 fatal cases were recorded as a result of ANCP, of which 11 occured in children and 145 cases in adults. A trend of decreasing deaths was observed, due to the implementation of prevention measures, including the annual organization of weeks dedicated to informing, raising awareness, and educating the population on chemical risks and the consequences of ANCP.

Of the total number of cases in the analyzed period (n = 9,579 cases of ANCP), the most frequent causal factor identified was medicines, with an incidence of 146.8 cases per 100,000 population (n = 4,002), equivalent to 42% (95% CI: 40.79–42.77). This highlights the predominant role of medicines in the etiology of the analyzed cases. Alcohol intoxication was in second place, involved in 63.6 cases per 100,000 inhabitants (n = 1,798), or 19% (95% CI: 18–19.56), with a μ coefficient of 0.1877.

Table 1. Incidence of ANCP in the Republic of Moldova during 2019-2023

Year	Total			Children			Adults		
	n (%)	Incidence	95% CI	n (%)	Incidence	95% CI	n (%)	Incidence	95% CI
2019	2,296 (24)	64.81	23.12-24.83	893 (22.6)	126.20	21.32-23.93	1,403 (25)	49.49	23.82-26.08
2020	1,494 (15.6)	56.51	14.88- 16.34	600 (15.2)	100.21	14.1-16.33	894 (15.9)	43.72	14.96-16.87
2021	1,954 (20.4)	74.39	19.6-21.22	854 (21.6)	144.13	20.35-22.92	1,100 (19.5)	54.08	18.53-20.61
2022	1,944 (20.3)	75.79	19.5- 21.11	802 (20.3)	138.51	19.07-21.58	1,142 (20.3)	57.50	19.26-21.37
2023	1,891 (19.7)	75.87	18.96- 20.55	803 (20.3)	143.88	19.09-21.6	1,088 (19.3)	56.25	18.32-20.39
Total	9,579	347.38		3,952	652.92		5,627	261.04	
Average	1,915.8	69.5		790.4	130.6		1,125.4	52.2	

***Note**: ANCP – Acute non-occupational chemical poisonings, CI – Confidence interval, n –total absolute number of cases of acute non-occupational chemical poisoning. *Incidence was calculated based on the annual number of poisonings relative to the total population according to data from the National Bureau of Statistics [26], and expressed per 100,000 inhabitants.*

Gas poisonings accounted for 1,385 cases, or 14.5% (incidence: 50.3 cases per 100,000 inhabitants, 95% CI: 13.77–15.18, μ = 0.1446). Among the causal factors included in the analysis, pesticides followed, with 15 cases per 100,000 population (n = 417), corresponding to 4.4% (95% CI: 3.96%–4.78) (Table 2).

Table 2. Distribution of acute non-occupational poisonings of chemical etiology in the Republic of Moldova during 2019–2023 (by etiology)

Categories	Total	% of total	Incidence (per 100,000)	95% CI	μ
Medicines	4,002	41.78	146.8	40.79–42.77	0.4178
Alcohol	1,798	18.77	63.6	18–19.56	0.1877
Gas	1,385	14.46	50.3	13.77–15.18	0.1446
Pesticides	417	4.35	15	3.96–4.78	0.0435
Nitrates	26	0.27	0.9	0.19–0.4	0.0027
Others	1,951	20.37	70.8	19.57–21.19	0.2037
Total	9,579	100	347.4		

Note: CI – Confidence interval, μ – mean, the average annual incidence of acute non-occupational chemical poisonings

The analysis of the incidence of acute medicinal poisonings (T36–T50) during the 2019–2023 period demonstrated an upward trend, with an increase in the number of cases from 22.86 to 34.03 per 100,000 inhabitants, despite the decrease in population size. The linear regression model, including the coefficient of determination (y = 3.212x – 6462.1; R^2 = 0.8364), indicated a strong correlation between the year and the incidence, highlighting an increase in the frequency of poisoning cases in recent years (Fig. 1).

In the case of acute alcohol poisoning (T51), the analysis of incidence and linear regression (y = -0.547x + 1118.2; R^2 = 0.1242) showed a decrease from 16.46 cases per 100,000 population in 2019 to 10.09 cases in 2021 (Fig. 2).

During 2019-2023, acute non-occupational gas poisonings (T58) recorded a relatively stable trend (y = 0.798x - 1602.7, R^2 = 0.3585), which indicates stagnation of the phenomenon (Fig. 3).

The incidence of acute pesticide poisoning (T60) ranged from 2.5 to 3.82 cases per 100,000 population, with a total incidence of 15.03 cases per 100,000, showing a moderate downward trend. This demonstrates the effectiveness of the regulatory measures regarding the management of phytosanitary products (pesticides), including preventive actions and educational initiatives in this area. However, the continued persistence of reported cases confirms the need to implement additional measures and improve access to information on the safe use and storage of pesticides (Fig. 4).

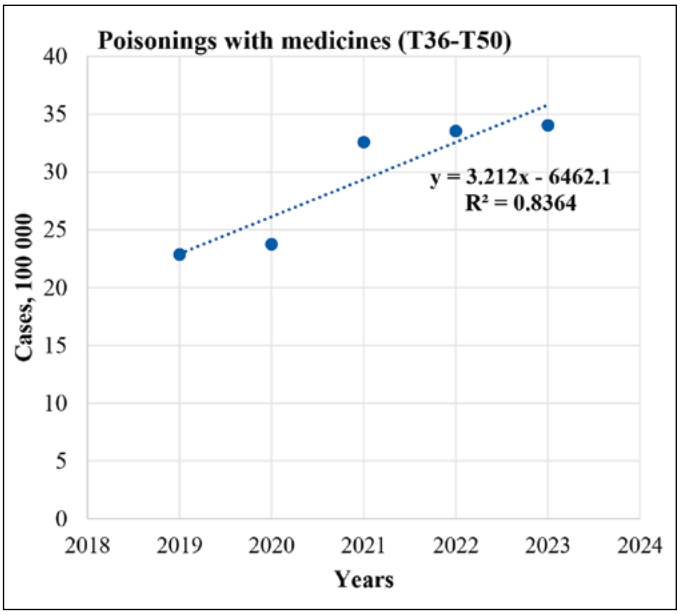

Fig. 1 Evolution of the incidence of acute medicinal poisonings and linear regression, 2019-2023

***Note:** R^2 – coefficient of determination, y – linear regression. * The codes T36-T50 are part of the International Classification of Diseases, 10th Revision (ICD-10) [27], and refer to: T36-T50 — Poisoning by drugs, medicaments, and biological substances.*

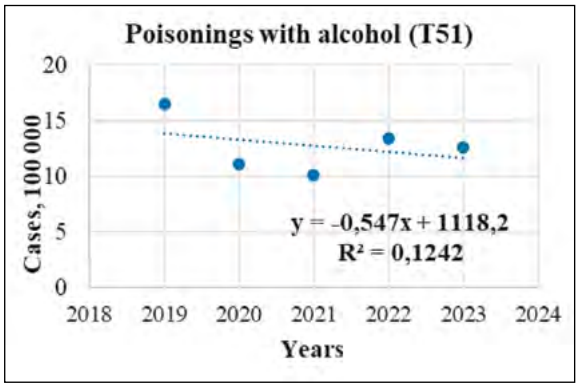

Fig. 2 Evolution of the incidence of acute alcohol poisoning and linear regression, 2019-2023

Note: *R^2 – coefficient of determination, y – linear regression.*

** The code T51 is part of the International Classification of Diseases, 10th Revision (ICD-10) [27], and refers to: T51 — Acute alcohol poisoning.*

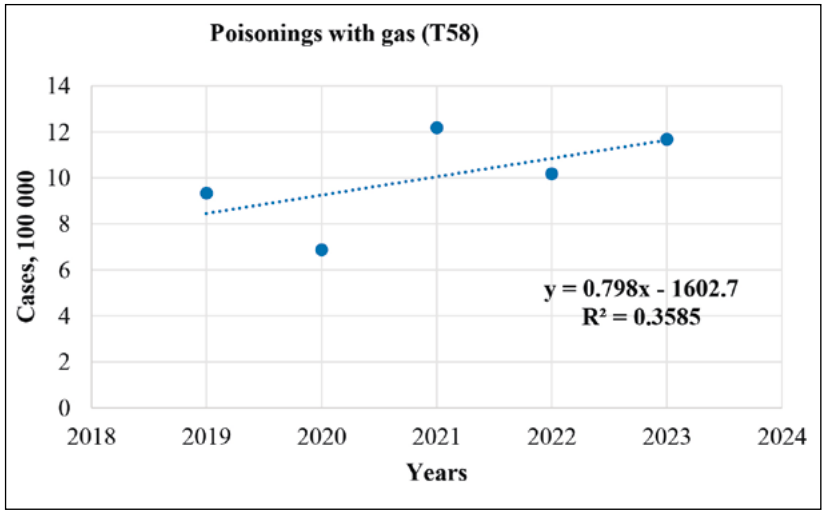

Fig. 3 Evolution of the incidence of acute gas poisonings and linear regression, 2019-2023

Note: *R^2 – coefficient of determination, y – linear regression.*

** The code T58 is part of the International Classification of Diseases, 10th Revision (ICD-10) [27], and refers to: T58 — Acute poisonings by gases.*

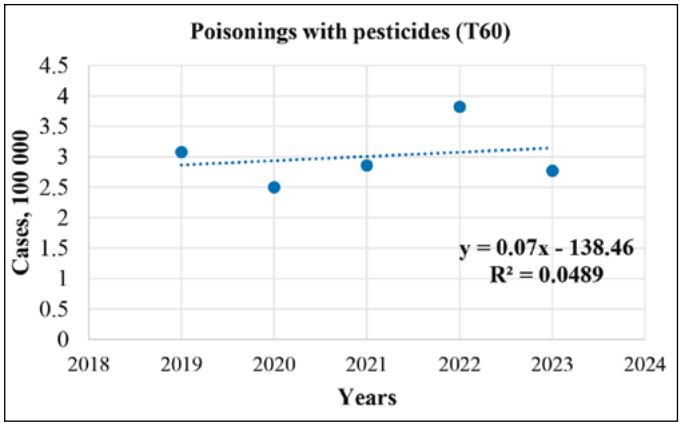

Fig. 4 Evolution of the incidence of acute pesticide poisoning and linear regression, 2019-2023

Note: *R^2 – coefficient of determination, y – linear regression.*

** The code T60 is part of the International Classification of Diseases, 10th Revision (ICD-10) [27], and refers to: T60 — Acute poisonings by pesticides.*

At the same time, the study identified the causes of ANCP, highlighting variations between children and adults. For example, in children, the main causes were natural curiosity (39%) and easy access to medicines, phytosanitary products (pesticides), and household products. An alarming proportion of 39% of ANCP cases in children were intentional or suicidal, often due to family conflicts or personal/emotional problems. In adults, chemical overdose was reported in 61% of cases, followed by suicidal poisoning (47.5%), and a low level of knowledge regarding the real risk of chemicals (22%), which confirms the need for educational and preventive measures in this area.

The economic analysis of the impact of ANCP during the 2019-2023 period highlighted a considerable volume of direct expenses generated by the treatment of patients intoxicated with potentially toxic chemical products, totaling 57,991,955.1 lei. Out of the total number of cases, 3,952 involved children under 18 years of age, with total expenditures amounting to 31,191,649.5 lei, while 5,627 cases involved adults aged 19 years and older, generating expenses of 26,800,305.56 lei.

Medicine poisonings were the most frequent and generated expenses of 22,436,099.1 lei, representing 39% of the total costs. Among children, 1,723 cases were recorded, generating 12,578,197.4 lei or 40.3% of the pediatric expenses. Among adults, 2,279 affected individuals accounted for 9,857,901.69 lei, representing 37% of total adult treatment costs.

Alcohol poisoning was reported in 1,798 cases, totaling 9,907,251.58 lei or 17.1% of the total expenditures. Children were affected in 529 cases, with expenditures of 3,511,060.57 lei, which constituted 11.3% of the expenditures for children. In adults, 1,269 cases were reported, with expenditures of 6,396,191.01 lei, or 23.9% of the total for adults.

In the gas poisoning category, 1,385 cases were registered, with 8,005,908.77 lei spent (13.8% of the total expenses). Of these, 510 cases occured in children, with a cost of 3,619,523.66 lei (11.6%), and 875 adults were affected, with expenses of 4,386,385.11 lei (16.4%).

Pesticide poisoning accounted for 417 cases and total expenses of 2,620,087.64 lei, representing 4.5% of the total expenses (Table 3).

Table 3. Medical expenses for ANCP in children and adults, 2019-2023

Etiology	Total			Children			Adults		
	n	Total expenditures, lei	%	n	Total expenditures, lei	% from the total in children	n	Total expenditures, lei	% from the total in adults
Medicines	4,002	22,436,099.1	38.7	1,723	12,578,197.4	40.3	2,279	9,857,901.69	36.8
Alcohol	1,798	9,907,251.58	17.1	529	3,511,060.57	11.3	1,269	6,396,191.01	23.9
Gas	1,385	8,005,908.77	13.8	510	3,619,523.66	11.6	875	4,386,385.11	16.4
Pesticides	417	2,620,087.64	4.5	144	1,373,480.62	4.4	273	1,246,607.02	4.7
Others	1,977	15,022,608	25.9	1,046	10,109,387.3	32.4	931	4,913,220.73	18.3
Total per country	9,579	57,991,955.1	100	3,952	31,191,649.5	100	5,627	26,800,305.56	100

***Note*:** *ANCP – acute non-occupational chemical poisoning, n –total absolute number of cases of acute non-occupational chemical poisoning.*

During the 2021-2023 period, direct expenses for the treatment of patients hospitalized with ANCP in the Nephrology, Dialysis, and Toxicology Department of the "Holy Trinity" Municipal Clinical Hospital recorded an increase of 19.2%, from 1,232,514 lei in 2021 to 1,469,003 lei in 2023. The average cost per patient evolved from 4,449 lei to 5,421 lei, highlighting a possible increase in the severity of cases and therapeutic interventions. During this period, the largest shares in the expenditure structure were allocated for hospitalization, with 1,442,941 lei spent, and for medicines, with 860,165 lei. The average length of hospitalization increased from 1.7 days in 2022 to 2.0 days in 2023 (Table 4).

Table 4. Expenses incurred for the treatment of adults with ANCP at the «Holy Trinity» Municipal Clinical Hospital during the years 2021-2023

Year	Number of cases	Hospitalization	Alimentation	Medicines	Sanitary materials	Laboratory tests	Other investigations	TOTAL expenditures	Expenditures per person	Average
2021	277	446,188	21,635	256,711	5,894	222,646	27,944	1,232,514	25,238	2.2
2022	236	320,450	17,850	214,556	12,040	202,657	303,098	1,070,651	25,808	1.7
2023	271	676,302.9	34,477	388,898	7,570	187,615	174,140	1,469,003	23,486	2.0
Total	784	1,442,941	73,962	860,165	25,504	612,918	505,182	3,772,168	74,532	

Note: *ANCP – Acute non-occupational chemical poisoning*

In order to prevent and reduce cases of ANCP, NAPH specialists, in collaboration with public medical and health institutions and educational institutions, have developed practical and methodological guides and conducted annual awareness, education, and information campaigns in this field.

Discussions

The results of the study highlighted that ANCP continues to present a major burden to public health in the Republic of Moldova, affecting both the adult population and children. The analysis of statistical data demonstrated an incidence of acute poisonings of chemical origin more than twice as high among children, with average annual values of 130.6 cases per 100,000 population, compared to 52.2 cases in adults, reflecting their particular vulnerability. This is determined by the exploratory behavior of children, especially young ones, as well as by insufficient supervision, inadequate storage and use of potentially toxic chemicals, and easy access to medicines or household products.

Based on the estimation of the etiological factors, medicines were identified as the main cause of ANCP, a finding that corresponds to international trends reported in the specialized literature. This predominance reflects a deficit in the education and information of the population, including children, regarding the correct and safe management of chemical products under household conditions. Moreover, there is a need to develop and implement additional regulatory measures, including clear labeling, the promotion of secure packaging, and the establishment of stricter standards regarding the accessibility of medicines and other chemical products.

During the analyzed period (2019-2023), the persistence of cases of alcohol, gas, and pesticide poisoning was observed, reflecting accidental exposure due to a lack of awareness of the real risks associated with potentially toxic chemical substances or mixtures. This emphasizes the importance of continuing education and information campaigns in the field of ANCP, as well as prevention measures and first-aid training.

At the same time, the costs associated with the treatment of ANCP (approximately 58 million lei) demonstrate that they represent an economic burden for the public health system. To assess the financial impact of ANCP and justify investments in prevention, the cost-benefit analysis (CBA) method was applied. The analysis demonstrated a cost-benefit ratio of 4.8:1 in favor of prevention. This means that for every 1 lei invested in prevention measures, the health sys-

tem saves 4.8 lei in direct costs of poisoning treatment. This result justifies the implementation of integrated preventive measures, including the creation of a toxicological information center. Investing in prevention is a superior economic strategy, not just a public health necessity. By preventing cases, direct medical expenses are substantially reduced.

Overall, the integration of preventive, educational, legislative, and psychosocial support measures will contribute significantly to the reduction or prevention of the incidence of ANCP and their economic impact in the Republic of Moldova.

Conclusions

1. The study demonstrated that children were significantly affected by ANCP, with an incidence twice as high as that in adults, highlighting their increased vulnerability.
2. During the 2019-2023 period in the Republic of Moldova, acute medicine poisonings predominated and generated the highest costs, reflecting an alarming upward trend.
3. The high costs borne by the healthcare system, especially for the treatment of poisoned children, indicate a major economic impact and pressure on available resources.
4. Data on the cost-benefit ratio favoring prevention highlight the need to implement additional preventive measures, strengthen public education, including among children, and reinforce inter-institutional collaboration through continuous and coordinated actions.

Competing interests

None declared.

Authors' contributions

The authors participated in the design of the study and made significant contributions to the drafting of the manuscript. They critically reviewed the work and approved the final version of the manuscript.

Ethics approval

The study was approved by the Research Ethics Committee of the *Nicolae Testemiţanu* State University of Medicine and Pharmacy of the Republic of Moldova, minutes no. 68 of 21.05.2018.

Patient consent

Obtained.

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REVIEW ARTICLE

Current concepts in the management of bone lesions in multiple myeloma

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ABSTRACT

Introduction. Bone lesions remain a serious, unresolved issue in patients with multiple myeloma. The management of myeloma-related bone disease involves a multimodal approach, including chemotherapy, bone antiresorptive agents (bisphosphonates), radiotherapy, pharmacological pain management, minimally invasive percutaneous orthopedic procedures, and invasive surgical interventions.

Material and methods. A bibliographic search was conducted using databases such as *PubMed, Hinari, SpringerLink, the National Center for Biotechnology Information, and Medline*. Articles published between 2000 and 2025 were selected using the following keywords: "bone lesions in multiple myeloma" in combination with terms such as "conservative treatment," "surgical treatment," "orthopedic surgery," and "minimally invasive techniques" to maximize the search yield. Based on the established search criteria, a total of 286 full-text articles were identified. The final bibliography includes 42 relevant sources, deemed representative of the literature published on the topic of this review article.

Results. Bisphosphonates or denosumab should be considered the standard of care for the treatment of bone disease in patients with multiple myeloma. Cement augmentation (polymethylmethacrylate) is effective in managing painful vertebral compression fractures (percutaneous vertebroplasty, balloon-assisted percutaneous kyphoplasty). Radiotherapy is recommended for uncontrolled pain in cases of spinal cord compression or pathological fractures of long bones, especially in patients who show no response or minimal response to systemic treatment for multiple myeloma. Surgery should be used to prevent and repair pathological fractures of long bones, spinal instability, and spinal cord compression caused by bony fragments. Postoperative radiotherapy should be considered, particularly for long bone fractures, to achieve local disease control and prevent implant failure.

Conclusions. The current concept in the management of patients with multiple myeloma and bone lesions is based on developing an individualized approach that takes into account anatomical, biological, radiological, and social factors. The selection of surgical techniques must be tailored to each patient, based on general medical condition, quality of life and life expectancy, prior response to chemotherapy, fracture location, number, size and distribution of bone lesions, extent of bone invasion, neurological status, and patient expectations.

Keywords: multiple myeloma, bone lesions, conservative treatment, surgical treatment, orthopedic surgery, minimally invasive methods.

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

Despite continuous improvements in outcomes for patients with multiple myeloma due to the increasing availability of effective treatments, skeletal-related complications remain a significant issue, imposing a substantial burden on both patients and health-

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care systems. Therefore, preventing these complications is of paramount importance.

The research hypothesis

The analysis and synthesis of contemporary literature reveal a clear association between the appropriate application of surgical indications and improved clinical outcomes in patients with multiple myeloma and bone lesions.

The novelty added by the manuscript to the already published scientific literature

This article provides a synthesis of the most recent international publications concerning the characteristics and effectiveness of current medical and surgical treatment methods for bone complications in patients with multiple myeloma. The study's findings will contribute to improving treatment protocols for managing and optimizing therapeutic approaches in patients with multiple myeloma.

Introduction

Bone disease – one of the major complications of multiple myeloma (MM) – is characterized by severe bone loss and the development of osteolytic lesions, frequently leading to pathological fractures. Bone lesions occur in approximately 80% of patients with newly diagnosed symptomatic MM and in over 90% during the course of the disease, being associated with significant patient morbidity and mortality. The axial skeleton, particularly the spine, and the proximal long bones are most commonly affected, although any bone may be involved [1-13].

The spine is the most frequently affected site due to myeloma-induced osteoporosis, osteolysis, or vertebral compression fractures (VCFs), accounting for about 60% of bone lesions present at diagnosis and 15–30% of newly developed lesions. These patients have an increased risk of skeletal-related events – including pain, VCFs, spinal instability and spinal cord compression, hypercalcemia, and pathological fractures of long bones – that often require radiotherapy and surgical intervention. All of these factors negatively impact quality of life and significantly reduce overall patient survival [1-13].

The treatment of bone lesions remains a serious, unresolved issue in patients with MM. The management of myeloma bone disease involves a multimodal approach that includes chemotherapy, bone antiresorptive agents (bisphosphonates), radiotherapy, pain management (aspirin, nonsteroidal anti-inflammatory drugs), minimally invasive percutaneous orthopedic interventions (percutaneous vertebroplasty – VP, balloon-assisted kyphoplasty – BKP), and invasive surgical procedures. However, optimal management depends on the individual nature of bone involvement – primarily spinal involvement – and requires careful evaluation and appropriate intervention throughout treatment [1, 2, 5, 11-15].

In this context, this article aims to provide a narrative synthesis of the most recent data regarding the characteristics and effectiveness of modern medical and surgical management strategies for patients with multiple myeloma and bone lesions.

Material and methods

To achieve the stated objective, an initial search of scientific publications was conducted using specialized databases, including *PubMed, Hinari (Health Internet Work Access to Research Initiative), SpringerLink, the National Center for Biotechnology Information, and Medline*. The selection criteria for articles focused on contemporary data regarding pharmacological management, minimally invasive percutaneous orthopedic interventions, and invasive surgical treatments for bone disease in patients with multiple myeloma. The following keywords were used: "bone lesions in multiple myeloma," combined in various ways with terms such as "conservative treatment," "surgical treatment," "orthopedic surgery," and "minimally invasive methods" to maximize search efficiency.

For the advanced selection of bibliographic sources, the following filters were applied: full-text availability, English language, and publication years ranging from 2000 to 2024. After a preliminary analysis of titles, original research articles, editorials, narrative reviews, systematic reviews, and meta-analyses were selected – those that included relevant information and contemporary concepts regarding the effectiveness of various medical and surgical management strategies in patients with MM and bone lesions. Additionally, the reference lists of the selected articles were reviewed to identify further relevant publications not captured in the initial database search.

The information from the included publications was collected, categorized, evaluated, and synthesized to highlight the key aspects of current perspectives on medical and surgical treatment methods for bone disease in MM.

To minimize the risk of systematic bias in the study, comprehensive database searches were performed to identify the maximum number of relevant publications. Only studies meeting validity criteria were included, and reliable exclusion criteria were applied to eliminate irrelevant articles.

Results

Following the processing of information identified from databases such as PubMed, Hinari, SpringerLink, the Na-

tional Center for Biotechnology Information, and Medline, and according to the defined search criteria, a total of 286 articles were found addressing the topic of managing patients with MMBD (multiple myeloma bone disease). After a primary title screening, 58 articles were deemed potentially relevant for this synthesis. Upon further in-depth review of these sources, a final selection of 42 publications was made. These 42 articles were included in the final bibliography, being considered representative of the literature published on the topic of this review article.

Publications whose content did not align with the subject, despite being identified by the search algorithm, as well as articles that were not freely accessible via *HINARI* or through the scientific medical library of the "Nicolae Testemițanu" State University of Medicine and Pharmacy, were subsequently excluded from the list.

Current non-operative interventions for the treatment of vertebral compression fractures (VCFs) include oral and parenteral analgesics, corticosteroids, bisphosphonates, spinal orthoses, and radiotherapy [16].

Bisphosphonates (zoledronic acid and pamidronic acid) are specific inhibitors of osteoclastic activity administered intravenously and represent the cornerstone of treatment and prevention of bone disease in newly diagnosed multiple myeloma (MM), with or without associated bone involvement. These agents inhibit osteoclast activity and induce osteoclast apoptosis, thereby reducing bone resorption and, consequently, decreasing and delaying skeletal complications associated with MM. Bisphosphonates are effective in reducing VCFs and pain; however, they do not completely prevent osteolytic lesions, fail to promote new bone formation or repair of existing lesions, and their role in improving survival remains unclear [2, 3, 5, 7, 9-13, 15, 17-20].

The International Myeloma Working Group (IMWG) experts recommend zoledronic acid for all newly diagnosed MM patients, regardless of the presence of bone disease. Once patients achieve a very good partial response or a good response, following monthly administration of zoledronic acid for at least 12 months and up to 24 months, the treating physician may consider reducing the frequency or discontinuing the treatment [3, 18]. Zoledronate is superior to first-generation bisphosphonates (etidronate) in improving outcomes related to vertebral compression fractures (VCFs) [2, 12, 17, 18].

Although bisphosphonates represent the initial treatment of choice, their long-term use is limited due to adverse effects. These include nephrotoxicity, requiring dose adjustment in hospitalized patients with renal insufficiency, flu-like symptoms, gastrointestinal disturbances during administration, atrial fibrillation, and atypical femoral fractures [9, 10, 12, 15, 18-20]. A critical factor to consider when prescribing high-dose bisphosphonates is the risk of osteonecrosis of the jaw, which can occur in approximately 3–4% to 11.0–11.8% of patients [3, 5, 12, 18].

Current treatments aim to prevent MM-induced bone disease through the use of antiresorptive therapy. New drugs are being developed that act specifically on bone pathology. At present, the use of zoledronic acid or denosumab is recommended at the initiation of treatment in newly diagnosed MM patients with bone lesions [3, 7, 9, 10, 12, 18, 19, 21].

Denosumab is a monoclonal IgG2 antibody and is considered an effective alternative to bisphosphonates for managing bone lesions in MM [2, 12, 13, 18, 20]. Denosumab is effective in preventing systemic osteolytic events and, importantly, improves quality of life in MM patients. A key advantage of denosumab is its significantly lower risk of renal toxicity, making it preferable to zoledronate for patients with MM who have renal dysfunction and hypercalcemia [2, 3, 12, 13, 18, 20].

Drug combinations targeting both myeloma cells and the bone marrow microenvironment may be potentially useful in inducing disease response and halting bone resorption. In this context, thalidomide and lenalidomide represent a new treatment paradigm due to their alternative mechanisms of action, which include disruption of the interaction between plasma cells and bone marrow stromal cells, inhibition of cytokine secretion, anti-angiogenic activity, and immunomodulatory effects, resulting in significant improvement in overall survival [11].

Recent advances in understanding the pathogenesis of bone lesions in multiple myeloma offer new therapeutic approaches and potential targets. There is a growing need for new therapeutic targets that not only prevent but also repair bone destruction, which may enhance treatment outcomes and, most importantly, improve the quality of life in MM patients. Bone anabolic agents (parathyroid hormone, anti-Dkk-1, anti-sclerostin, etc.) show strong potential utility in the treatment of bone lesions in MM. Increasing evidence of the benefits of these agents brings promise for improving the management of bone disease in multiple myeloma patients [19].

The primary indication for radiotherapy is the treatment of bone pain. Other indications include the prevention of pathological fractures, spinal cord compression, or alleviation of symptoms associated with tumor mass. In MM, spinal radiotherapy is typically used in patients with uncontrolled pain or in cases of impending vertebral compression fracture (VCF) or spinal cord compression. Studies conducted on small patient cohorts have demonstrated that complete pain relief (76.4–84%), as well as improvements in quality of life and motor function, were achieved in a considerable proportion of treated cases [3, 11-13, 15, 18, 20, 21].

However, radiotherapy may be associated with an increased risk of vertebral compression fractures (VCFs), which could significantly impact both survival and quality of life. Overall, within a 3-year period, approximately 30.7% of patients reported new VCFs or progression of existing fractures. The analgesic effect of radiotherapy is typically delayed by 10–15 days, and its impact on bone reconstruction is partial, requiring several weeks to fully develop. Therefore, in cases of severe pain, neurological complications, or spinal instability, surgical intervention may be considered [3, 12, 13, 18, 21].

Analgesics are commonly used in multiple myeloma management and include simple analgesics (paracetamol), nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and diclofenac, weak opioids (co-codamol, dihydrocodeine), and strong opioids (morphine, oxycodone, fentanyl) [9, 10].

Thus, bisphosphonates and denosumab remain the standard first-line therapy for MM patients to prevent skeletal-related events. New agents targeting various molecular pathways involved in bone metabolism restoration are under development, and some are currently undergoing clinical trials [13, 20].

***Orthopedic surgery*.** The main objectives of orthopedic surgery in bone lesions among patients with multiple myeloma (MM) are to reduce pain, alleviate symptoms, improve mobility, prevent pathological fractures, restore bone and spinal stability, decompress the spinal cord and nerve roots, enhance quality of life, and support subsequent systemic treatment.

Studies have confirmed the effectiveness of surgical intervention in metastatic spinal disease for improving quality of life and clinical outcomes. Surgery is typically reserved for cases where conservative treatment has failed or when structural integrity and function are severely compromised [6, 8-10, 12, 20, 22-25].

When considering surgical intervention in patients with multiple myeloma (MM), several factors must be included in the decision-making process: potential neurological deterioration, disease stage and prognosis, overall patient condition, lesion location and number, as well as the patient's preferences and expectations. To improve quality of life, the primary objective of surgical treatment is to preserve mobility by reducing pain, maintaining neurological function, and ensuring structural stability [20].

As previously mentioned, MM is often successfully treated with non-surgical methods, including chemotherapy and radiotherapy. In cases of MM without neurological compromise or instability, radiotherapy is considered the treatment of choice. However, when pathological fractures of the extremities or spine are unstable, or when neurological deficits result from nerve compression by tumor masses, surgical intervention is required before radiotherapy or systemic therapy [12, 21, 23, 26, 27].

Indications for surgical treatment in MM include: spinal instability; actual or impending pathological fracture caused by MM; progressive neurological impairment due to spinal cord or nerve root compression by MM; intractable pain attributable to MM; solitary bone plasmacytoma; soft tissue plasmacytoma involving the limbs or spine; actual or impending long bone fracture; and needle or open biopsy providing pathological evidence that supports further treatment [8, 25, 27].

Contraindications to surgical treatment of MM include: poor physical condition; severe cardiac, pulmonary, or renal dysfunction; significant coagulopathy that is difficult to correct; and uncontrolled severe infection [8].

The spectrum of surgical treatment options for the extremities ranges from composite osteosynthesis using bone cement and implants, with stable extramedullary plates or intramedullary nails, to endoprosthetic systems. In spinal surgery, minimally invasive procedures (vertebroplasty, balloon kyphoplasty), selective decompressions, spinal fusion (spondylodesis), and vertebral body replacement procedures are commonly employed. The choice of surgical method and timing of treatment must be individualized based on the risk profile and prognosis of the patient with MM. Postoperative local radiotherapy should always be administered after intralesional tumor resection and surgical stabilization to prevent tumor progression [12, 15, 23, 28, 29].

Current surgical treatment options for the proximal femur include osteosynthesis (intramedullary fixation with nails, intramedullary implants, open reduction and internal fixation with plates and screws), as well as reconstructive options (hemiarthroplasty or total hip arthroplasty and endoprosthetic reconstructions) [25, 30, 31].

Vertebral Lesions. Multiple myeloma (MM) commonly affects the spine, particularly the vertebral bodies. The most frequent site of vertebral compression fractures (VCFs) in MM is the thoracic spine, followed by the lumbar and cervical regions. Bone lesions lead to bone pain, pathological fractures, and spinal cord compression, potentially resulting in neurological deficits. Surgical intervention is indicated when >50% of the vertebral body or the posterior vertebral elements (posterior margin, pedicles) are destroyed, and the tumor has invaded the spinal canal. Sudden-onset neurological deterioration and mechanical instability are the main indications for surgical intervention [16, 32].

When VCFs cause significant pain (with vertebral height loss exceeding one-third and kyphotic spinal deformity), minimally invasive procedures – vertebroplasty (VP) or balloon kyphoplasty (BKP) – may be considered to stabilize the spine and relieve pain [8, 33]. Minimally invasive treatment strengthens the vertebrae, significantly reduces pain, provides mechanical stability, and improves functional outcomes and quality of life [8, 11, 14, 18].

In cases of spinal cord compression from lesions within the spinal canal or unstable spinal fractures, open surgery is often necessary for spinal cord decompression and restoration of spinal stability [8, 11, 21].

Recommended surgical interventions include:

- ***Laminectomy***, used solely for targeted decompression and posterior reconstruction, aiming to preserve the integrity of neurological structures while providing adequate mechanical stability of the spine. In the past, decompressive laminectomy via a posterior surgical approach was considered the treatment of choice; however, it is now rarely used, as it may lead to spinal destabilization and consequently worsen pain and neurological deficits.
- ***Extracapsular intralesional excision*** of the neoplastic mass in cases resistant to chemotherapy and radiotherapy.
- ***Vertebral body reconstruction*** according to the biomechanical needs of the spine (using a prosthesis, acrylic cement – polymethylmethacrylate, autologous bone graft, or vertebral body stent).

- ***Vertebroplasty (VP)*** *or* ***balloon kyphoplasty (BKP)*** – minimally invasive percutaneous procedures –reserved for patients who, after pharmacologic and/or radiotherapeutic treatment, are left with a cavity in the vertebral body that poses a high risk of fracture [8-12, 17, 23, 29, 33, 34].

Although traditional non-operative treatment may provide pain relief, it does not stabilize vertebral compression fractures or minimize progressive kyphotic deformity – outcomes that can only be achieved through vertebral augmentation procedures such as VP and BKP [16].

Vertebroplasty (VP) and Balloon Kyphoplasty (BKP) are minimally invasive procedures used as local treatments for vertebral lesions to rapidly reduce pain and prevent deformity. These procedures are well tolerated and associated with early clinical pain relief, significant reduction in the use of analgesic medications, shorter hospital stays, lower complication rates, improved quality of life, and a favorable platform for subsequent treatment. In addition, they are characterized by low approach-related morbidity and faster recovery, which may accelerate the initiation of adjuvant systemic therapies – making them especially suitable for lesions without major instability. VP involves the percutaneous injection of bone cement (polymethylmethacrylate) into the fractured vertebral body to stabilize it and destroy nerve endings to relieve pain. BKP involves the insertion of a balloon to restore vertebral height, realign the sagittal profile, and create a cavity that can be easily filled with high-viscosity bone cement under low pressure [11, 12, 15, 16, 34-38].

Vertebroplasty (VP) is a minimally invasive, simple, safe, and effective procedure for the treatment of painful vertebral compression fractures (VCFs) in patients with multiple myeloma (MM). The technique was first described in 1987 for the treatment of painful vertebral collapses caused by hemangiomas and osteolytic spinal tumors. The main advantages include immediate stabilization of the fractured vertebral body, significant and long-lasting pain relief (both at rest and during activity), a marked increase in vertebral strength and quality of life, thereby reducing the risk of fracture in the treated vertebra or new collapses in adjacent vertebral bodies. Subjective scores showed sustained improvement – approximately 65% of patients required fewer analgesics post-VP, and 70% experienced improved mobility, with discharge occurring within 2-4 hours. The procedure can be repeated at multiple levels, and the pain relief effect is virtually permanent [16, 37, 38].

A study conducted on a cohort of 4,547 patients (3,211 women and 1,336 men) with a mean age of 70.2 years reported a total of 13,437 treated vertebrae. The authors concluded that VP is an effective and safe procedure for managing vertebral fractures, significantly alleviating pain within 48 hours. No major neurological complications were reported, while minor complications were observed in 32.9% of cases [36].

Balloon Kyphoplasty (BKP) is a minimally invasive procedure used to treat painful vertebral compression fractures (VCFs) caused by primary or secondary osteoporosis, osteolytic lesions due to multiple myeloma (MM), bone metastases, or trauma. The goal of BKP is to reduce and stabilize the fracture while restoring vertebral body height, thereby providing immediate and sustained pain relief, improved physical function, and enhanced quality of life, while also preventing subsequent VCFs. After balloon removal, the created intravertebral cavity is filled with cement (polymethylmethacrylate) or bone graft to restore vertebral height [9, 10, 12, 15, 26, 33, 39].

BKP is a well-tolerated, relatively safe, and effective technique in patients with painful neoplastic spinal fractures, including VCFs caused by MM. The procedure results in rapid pain relief and functional improvement, significant restoration of vertebral height, and reduction of segmental kyphosis, helping to prevent further vertebral height loss and reducing the risk of kyphotic deformities. BKP provides long-term benefits in terms of pain and disability. However, potential procedural disadvantages include incomplete fracture reduction or significant loss of reduction after balloon deflation, prior to cement injection [35, 39, 40].

Cement leakage into adjacent neural and vascular spaces is the most commonly reported complication of vertebroplasty (VP) and balloon kyphoplasty (BKP), but it occurs more frequently after VP. Although in most cases cement leakage is clinically insignificant, in extreme situations it can lead to serious complications such as pulmonary or cerebral embolism, neurological deficits, myelopathy, radiculopathy, and, in rare cases, death [16, 38, 40].

VP is associated with a very high incidence of cement extrusion, with reported leakage rates ranging from 30% to 75%, and even up to 85.7% in cases with posterior vertebral wall osteolysis. Moreover, VP does not attempt to restore lost vertebral height or correct the resulting spinal deformity. Kyphotic spinal curvature may compromise spinal biomechanics and increase the risk of subsequent fractures in adjacent vertebrae. Consequently, BKP - developed from VP techniques in the 1990s - was introduced as a more effective treatment option in MM. BKP helps restore lost vertebral height, correct spinal deformities, stabilize the spine, and reduce the incidence of cement leakage. The absence of cement-related complications may be attributed to: (1) the use of high-viscosity cement; (2) the selection of BKP in appropriate cases; and (3) the injection of relatively small volumes of cement (2-8 mL) [16, 35, 37, 39, 40].

Serious complications associated with BKP – such as inflammation, epidural hematoma, rib fractures, cement leakage, pulmonary embolism, and systemic toxicity – are rare. The most frequently reported complication is cement leakage (extrusion), occurring in 7-9% of cases. The vast majority of these events are asymptomatic. Symptomatic cement leakage is reported in only 0.04-1.3% of cases. New VCFs following BKP have been reported in 14.1-17% of patients over a follow-up period ranging from one month to three years [10, 11, 26].

A systematic review of the literature included 23 studies (9 on BKP, 12 on VP, and 2 on both procedures), totaling 923 patients with vertebral fractures due to multiple myeloma

(MM). The authors concluded that both BKP and VP were equally effective in reducing postoperative pain scores and analgesic medication use, with low treatment-related complication rates. However, complications generally occurred more frequently after VP compared to BKP. Both procedures demonstrated similar safety and efficacy profiles [40].

A new procedure called "vertebral body stenting" uses a specially designed stent mounted on a catheter, which can be implanted and expanded within the vertebral body, leading to a significant increase in overall vertebral height. This innovative technique enables complete reduction of VCFs and helps maintain the restored height through the use of the stent. Recently, small-scale studies have shown the effectiveness of a spinal implant – an expandable intravertebral titanium device – that provides long-term pain relief and restoration of hemovascularization in VCFs secondary to MM and other etiologies [16].

Open surgical approaches include direct anterior, posterior, and combined anterior-posterior approaches. The goals of surgical intervention are maximal tumor resection, decompression, spinal reconstruction, and internal fixation. Suitable internal fixation systems include titanium plates, spinal systems with pedicle screws, and lateral mass screw fixation systems. Appropriate reconstructive implants include artificial vertebral bodies, titanium mesh, bone cement, and bone allografts [8].

Spinal surgery plays a fundamental role in cases of vertebral involvement in multiple myeloma (MM) that are partially or entirely unresponsive to pharmacologic and radiotherapy treatments, particularly in cases of disease progression or relapse. Surgical intervention in selected MM patients with spinal involvement and neurological deficits is associated with favorable clinical outcomes, neurological recovery, and an acceptable complication rate. Furthermore, the improved efficacy of medical therapies has led to increased life expectancy, reinforcing the consistent and undisputed utility of spinal surgery – especially regarding functional status [17, 32].

Surgical intervention serves a supportive role in MM management and is performed with the intent to stabilize imminent or existing pathological fractures in cases of refractory pain, neurological complications, or spinal instability. The objectives of surgery include decompression of neural structures, pain control, restoration of spinal stability, and correction of coronal and sagittal spinal alignment. The goal of tumor excision is the partial or complete removal of neoplastic tissue surrounded by healthy tissue. Surgery for vertebral MM lesions is a valuable option for carefully selected patients [17, 41].

According to a U.S. national database, vertebral augmentation (9,643 cases, 65.7%) was the most commonly performed procedure, followed by spinal stabilization with or without decompression (4,176 cases, 28.4%), and decompression alone (868 cases, 5.9%). The study highlighted a growing trend in the use of spinal surgery for inpatient MM management, while the rate of vertebral augmentation procedures is decreasing [14].

However, postoperative complication rates remain high after spinal surgery in patients with multiple myeloma (MM). The overall complication rate has been reported to range between 22.4% and 35.0%, which aligns with complication rates observed after spinal surgery for spinal metastases in the broader literature, ranging from 14% to 34%. Approximately 20% of patients underwent at least one reoperation within 2.5 years following the initial spinal surgery. The most common indications for reoperation were adjacent-level fractures (53%), wound dehiscence and/or infected hardware (19%), and tumor recurrence or spinal cord compression at the operated site (15%) [14, 41].

Thus, balloon kyphoplasty (BKP) and vertebroplasty (VP) are both safe and effective surgical procedures for the treatment of vertebral compression fractures (VCFs). BKP provides comparable long-term pain relief, functional outcomes, and rates of new adjacent VCFs to VP. However, BKP is superior to VP in terms of cement volume injected, short-term pain relief, short- and long-term correction of the kyphotic angle, and lower cement leakage rates. On the other hand, BKP is associated with a longer operative time and higher material costs than VP.

A combination of open surgery and minimally invasive spinal surgery is often used to treat patients with multiple spinal lesions, as it provides the benefits of both approaches - reducing blood loss and other complications [8].

Long Bone Lesions. In MM, long bone fractures are relatively less common than VCFs but typically require hospitalization for early intervention via fixation or replacement of the affected segment. The benefits of surgical treatment for pathological fractures of long bones in the limbs include pain relief, restoration of bone continuity and limb function, and improved quality of life [2, 6, 8, 28].

Pathological fractures of long bones should be operated on as soon as possible, especially in the lower limbs due to weight-bearing demands. Procedures include resection of the affected segment, augmentation with polymethylmethacrylate, internal fixation (using screws, plates, or intramedullary nails in carbon – fiber-reinforced or titanium implants), or replacement with conventional prostheses or megaprostheses [2, 6, 8, 28, 30, 31].

The selection of the fixation system and surgical procedure depends on the patient's general condition, quality of life, and life expectancy; prior response to chemotherapy; fracture location; the number, size, and distribution of lesions; extent of bone invasion; neurological status; and patient expectations [8, 24, 30, 31].

Complications associated with surgical treatment methods for bone lesions in multiple myeloma (MM) have been broadly classified into four main categories:

- Hematoma-related (cauda equina syndrome, lower limb weakness, sudden paraplegia).
- Wound-related (hematoma, wound dehiscence).
- Surgical complications (implant failure or dislodgement, rod breakage, screw loosening, inaccurate screw placement, pathological fracture).
- Medical complications (deep vein thrombosis, acute

renal failure, acute respiratory failure, pulmonary embolism, intraoperative hyperthermia) [17, 27, 31].

To date, there is no consensus on the surgical complication rate in patients with multiple myeloma (MM), with various studies reporting significantly different rates, ranging from 3.9% to 35% [22].

Given that patients with MM and bone lesions are often in advanced stages of the disease with terminal organ dysfunction, one study analyzed the types and incidence of perioperative medical and surgical complications. The overall complication rate following surgery among 58 patients with bone disease in MM was 74.03%, with 45.45% of patients experiencing two or more complications. Surgical complications were reported in only 20.78% of cases (e.g., bleeding or hematomas, adjacent segment fractures, surgical site infections). Among medical complications, the most frequent were moderate to severe anemia requiring transfusion (28.57%), acute renal failure (25.97%), infections (24.68%), and hypercalcemia (10.39%) [4].

Surveillance and Prognosis. Surgical intervention in patients with MM-related bone lesions has been shown to improve overall survival and reduce the rate of postoperative complications [42].

According to a recent study, 58 patients treated for MM-related bone disease were followed for a mean period of 6.13 years, and 37.93% experienced a new fracture during follow-up. The median overall survival after surgical intervention was 32.9 months (ranging from 11.6 to 49.0 months). The estimated overall survival rates at 1, 3, and 5 years postoperatively were 81.17%, 57%, and 34.11%, respectively [4].

According to multivariate logistic regression analysis, patient age, disease duration, International Staging System (ISS), preoperative Karnofsky performance status, hemoglobin <90 g/L, and systemic treatment are independent prognostic factors influencing outcomes in MM patients following surgical treatment of bone lesions [22].

Thus, bisphosphonates or denosumab should be considered the standard of care for the treatment of skeletal disease in patients with multiple myeloma (MM). The choice of a specific agent should take into account several factors such as cost, convenience, patient preference, and toxicity profile. The International Myeloma Working Group (IMWG) recommends zoledronic acid as the preferred treatment option for patients without imaging-confirmed bone disease related to MM, while denosumab is preferred for patients with renal insufficiency.

Cement augmentation (polymethylmethacrylate) is effective in the treatment of painful vertebral compression fractures (VP, BKP). Radiotherapy is recommended for uncontrolled pain in cases of spinal cord compression or pathological fractures of long bones, particularly in patients with absent or minimal response to systemic treatment for multiple myeloma (MM). Surgery should be employed to prevent and repair pathological fractures of long bones, address spinal instability, and decompress the spinal cord in cases involving bony fragments. Postoperative radiotherapy should be considered, especially for long bone fractures, to achieve local disease control and to prevent implant failure. This approach is particularly important in patients who demonstrate minimal or no response to systemic MM treatment.

Conclusions

1. The current therapeutic landscape for bone complications in multiple myeloma supports the individualized use of antiresorptive agents, emphasizing the need for a patient-centered approach based on clinical and practical considerations.
2. Minimally invasive procedures such as balloon kyphoplasty and vertebroplasty remain valuable options for managing vertebral fractures, with specific procedural advantages that can guide surgical decision-making.
3. An integrated therapeutic strategy combining radiotherapy and surgical intervention plays a critical role in managing structural complications, particularly in refractory cases, supporting both symptom relief and disease control.

Competing interests

None declared.

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REVIEW ARTICLE

Stigmatization in medicine: impact on patients, healthcare providers, and ethical standards of care

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ABSTRACT

Introduction. Stigmatization is a social phenomenon that adversely affects not only access to care but also the quality of medical services. In the medical context, stigma occurs when patients – or even healthcare professionals – are treated differently, with prejudice or a lack of empathy, due to certain traits, conditions, or social affiliations.

Material and methods. We conducted a narrative review of stigma in healthcare settings. Searches were performed in PubMed/MEDLINE and Google Scholar, and complemented by consulting official public-health websites (WHO, ECDC, UNAIDS, Romanian MoH/NIPH) for the period 1 Jan 2000 – 27 Jul 2025 (English/Romanian). Search strategies combined terms related to stigma/discrimination, healthcare/quality of care, and vulnerable groups, with backward- and forward-citation tracking. Two reviewers screened against predefined criteria (peer-reviewed studies, reviews, authoritative institutional reports). Opinion pieces, non-healthcare contexts, duplicates, and inaccessible full texts were excluded, and evidence was synthesized qualitatively.

Results. Stigma in healthcare appears as discriminatory behavior that fosters exclusion, leading to delayed diagnoses, treatment abandonment, and loss of trust in the system. Vulnerable groups – such as people living with HIV/AIDS, those with mental disorders, LGBTQ+ individuals, substance users, the homeless, and ethnic minorities – are most affected. HIV-positive patients often face avoidance, while those with psychiatric conditions may be seen as "unpredictable" or dangerous. Such attitudes harm patients' health, deepen inequities, and erode the core ethics of equity and respect. Stigma undermines the patient–provider relationship, discouraging preventive care and adherence to treatment, and can cause complete disengagement. For providers, stigma fosters "dehumanization," unconscious bias, and skewed clinical decisions, leading to substandard care. Healthcare workers experiencing their own health issues may internalize stigma, avoid seeking help, and compromise the care they deliver.

Conclusions. Health-related stigma is widespread and takes multiple forms, profoundly degrading the quality of medical care and hindering patients' access to services. Medical stigma generates serious systemic consequences: patients delay seeking treatment, avoid interacting with the health system, suffer emotional distress and burnout, and face extreme difficulty with social reintegration. These realities underscore the need for strategic interventions in professional education, legislation, and public awareness to combat stigma in healthcare.

Keywords: social stigma, prejudice, vulnerable populations, quality of healthcare, mental health, health personnel.

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Key messages

What is not known yet about the issue addressed in the submitted manuscript

Stigmatization in healthcare remains a subtle yet powerful barrier to equitable access to quality care, and its full impact on patient outcomes and provider behavior is insufficiently understood in Eastern European contexts.

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The research hypothesis:
Stigma within medical systems significantly undermines trust, delays access to care, and compromises treatment adherence.
The novelty added by the manuscript to the already published scientific literature
This article synthesizes current evidence on health-related stigma, focusing on HIV, mental health, and internal stigma among healthcare providers, while highlighting structural gaps and presenting concrete strategies for stigma reduction in healthcare.

Introduction

In an era of remarkable technological and scientific advances in medicine, the relationship between patients and the healthcare system remains paradoxically marked by vulnerability and inequity. One of the least discussed yet highly influential forms of imbalance is stigmatization – a social phenomenon that affects not only access to care but also the quality of medical treatment itself. This subtle but frequent form of discrimination impacts the dignity and well-being of patients and is seldom addressed in depth.

Broadly defined, stigma is a social process by which certain individuals are negatively labeled and excluded based on real or perceived attributes deemed deviant from social norms. Sociologist Erving Goffman described "stigma" as an attribute that deeply discredits an individual in the eyes of society, reducing them "from a whole and usual person to a tainted, discounted one" [1]. In other words, stigma is a "deeply discrediting" trait that diminishes a person's social identity. In healthcare, this process takes on an especially grave connotation: prejudices arise not only from the general society but sometimes from those entrusted with care and life preservation. Stigma can be external (imposed by others) or internal, when an individual internalizes shame and withdraws from necessary care. Stigmatization, as an expression of shame, is often described as having four components: labeling the affected person, generalizing that label to all patients with the same condition, defining them as an "inferior" group, and thus leading to discrimination.

In medicine, stigma involves a distinct power dynamic and vulnerability between patient and healthcare provider. Stigmatization in medicine occurs when patients – or even healthcare professionals – are treated differently, with prejudice or a lack of empathy, because of a particular attribute, illness, or social affiliation. This often-unconscious process creates a climate of exclusion and shame that can have dire consequences: delayed diagnosis, interruption of treatment, patient alienation, and erosion of trust in the health system. Stigma in healthcare can be expressed through biased attitudes, behaviors, or clinical decisions influenced by prejudice against certain categories of patients: those with mental illnesses, substance use disorders, HIV/AIDS, LGBTQ+ patients, people with obesity, or individuals from marginalized ethnic groups. Such stigmatization may occur at the individual level (during doctor–patient interactions) and at the institutional level (through policies or practices that perpetuate inequality) [2]. Indeed, far from being an isolated phenomenon, medical stigma has serious consequences: stigmatized patients tend to avoid the medical system, not follow recommended treatments, or suffer in silence, thereby worsening their health status. Moreover, stigma affects healthcare professionals as well. Due to a culture of silence and pressures of perfectionism, providers can fall victim to stigma themselves – particularly regarding their own mental health – which further perpetuates harm within the system.

This study aims to explore the phenomenon of stigma in medicine from a comprehensive perspective, analyzing the causes of medical stigmatization, the most affected categories of people, the consequences on the doctor–patient relationship, and possible solutions to reduce this harmful behavior. In a patient-centered healthcare system, understanding and combating stigma should become a professional and ethical priority. In a world where health is considered a fundamental right, it is essential to recognize how prejudices can sabotage this right, especially when the humanistic and ethical aspects of medical care are sometimes neglected.

Material and methods

We conducted a narrative review on health-related stigma in healthcare settings. Searches were run in PubMed/MEDLINE and Google Scholar, complemented by targeted consultation of official public-health websites (World Health Organization, European Centre for Disease Prevention and Control, UNAIDS, Romanian Ministry of Health/National Institute of Public Health). The coverage period was 1 January 2000 – 27 July 2025, and publications in English and Romanian were eligible. Search strings combined controlled vocabulary and free-text terms for stigma/discrimination, healthcare/quality of care, and vulnerable groups, with condition/population modifiers (e.g., HIV, mental health, healthcare workers). Reference lists of included papers and their forward citations were screened to extend retrieval.

We included peer-reviewed original studies, reviews, and authoritative institutional reports examining stigma within healthcare settings and reporting empirical findings, conceptual frameworks, or policy-relevant evidence. We excluded editorials and opinion pieces without data, items unrelated to healthcare settings, duplicates, non-English/Romanian publications, and records without accessible full text. Titles, abstracts, and full texts were independent-

ly screened by two reviewers (I.A.-C., L.R.), with disagreements resolved by discussion; data were charted on setting, population, stigma type, outcomes (e.g., access, quality of care, adherence), and implications. Owing to heterogeneity in designs and outcomes, evidence was synthesised qualitatively; no meta-analysis or formal risk-of-bias assessment was undertaken.

Results

Overall manifestations of stigma in healthcare

Stigmatization in the medical field arises when patients – or even medical staff – are subjected to discriminatory, empathy-lacking, or prejudice-based treatment due to an illness, personal trait, or membership in a certain social group. Although often unintentional, such attitudes foster an environment of exclusion and shame, which can have serious effects: delays in establishing diagnoses, treatment abandonment, social isolation, and loss of confidence in health services. In healthcare settings, certain patient populations are consistently exposed to a higher risk of stigma, both from medical personnel and from society at large. Among the most affected groups are people living with HIV/AIDS, patients with mental health disorders, members of the LGBTQ+ community, substance users, the homeless, and ethnic or racial minorities. These groups are often viewed through the lens of negative stereotypes, leading to systemic discrimination, limited access to quality medical services, and deterioration of the therapeutic relationship. For example, HIV-positive patients may be avoided or treated with reluctance by healthcare workers, while patients with psychiatric disorders might be seen as uncontrollable or "dangerous" even in the absence of acute symptoms. Such attitudes not only harm the physical and mental health of patients but can also perpetuate social and health inequities, undermining fundamental principles of medical ethics such as equity, respect, and non-discrimination. A global framework on health-related stigma emphasizes that stigma within health facilities is a significant barrier to care and must be addressed to uphold ethical standards in medicine.

The doctor–patient relationship is an essential pillar of medical practice, built on trust, open communication, and mutual respect. Stigmatization – manifesting as negative attitudes, prejudices, or discriminatory behaviors – profoundly alters this relationship, with major consequences for the quality of medical care. The damage to the therapeutic alliance extends beyond the immediate clinical encounter to public health at large: patients who do not trust the system are less willing to participate in screening programs, preventive services, or vaccination campaigns. For example, in communities with high stigma, transmissible diseases such as HIV or tuberculosis may remain underdiagnosed and undertreated because affected individuals avoid engagement with health services. Research indicates that stigma and prejudice can deter people from utilizing healthcare even when they need it, illustrating how stigma can be as harmful as the diseases it is attached to.

From the patient's perspective, the perception or experience of discrimination by healthcare providers can lead to avoidance of the health system, delayed presentation for medical consultations, or outright refusal of treatment. Numerous individuals living with HIV, mental illness, or conditions associated with social stigma (such as hepatitis C, obesity, or substance dependence) report feeling judged, ignored, or treated superficially in their interactions with medical staff. These experiences erode patients' trust in medical advice and can result in poor adherence to treatment or even complete disengagement from care [3, 4]. When patients anticipate stigma, they may postpone seeking care until conditions worsen or avoid disclosing critical information about their health, leading to suboptimal outcomes.

From the healthcare provider's perspective, stigmatization can lead to the dehumanization of patients, compromising the provider's ability to act with empathy, ethics, and fairness. Implicit biases – often unrecognized by the clinician – can negatively influence clinical decisions: stigmatized patients might receive fewer diagnostic investigations, more cursory examinations, or be excluded from cutting-edge treatments. This creates a vicious cycle in which the stigmatized patient receives inferior care, and the clinician forfeits professional objectivity. For instance, studies have documented that patients who are marginalized (by HIV status, mental illness, etc.) sometimes receive less thorough work-ups or are managed less aggressively due to provider biases. Such practices not only harm individual patients but also contravene evidence-based medicine and fairness. In essence, stigma in the clinic can subvert the standards of care, leading to health disparities among already vulnerable groups [5].

Stigma in HIV care

Stigma surrounding HIV/AIDS remains one of the largest barriers to achieving global HIV control. It impedes testing, disclosure, and treatment adherence, leading to late diagnoses and limited treatment access. HIV-related stigma and discrimination are consistently cited as major obstacles to ending the AIDS epidemic [6]. Many people are reluctant to get tested for HIV or to reveal a positive status, to use prevention methods like PrEP, or to take life-saving HIV treatment specifically because of stigma and fear of negative reactions. According to the first-ever global People Living with HIV Stigma Index report (covering 25 countries and over 31,000 participants), 85% of people living with HIV acknowledged experiencing internalized stigma, and 25% reported actual discrimination by healthcare personnel. In Romania, a national study in 2022 (HIV Outcomes Romania, in collaboration with the *Matei Balș* Institute) involving 1,050 people with HIV found a stigma prevalence of 39.9% compared to the general population. Nearly half (48.4%) of respondents believed that women with HIV are more stigmatized, and 59.5% felt that children living with HIV also experience significant stigma [7]. HIV stigma is often intersectional, overlapping with prejudice related to other marginalized identities. People living with HIV frequently face multiple layers of stigma stemming from factors such as ethnicity, sexual orientation, gender identity, or moral judgments attached to

behaviors or conditions (e.g., drug use, sex work, poverty). In 64 low- and middle-income countries, the prevalence of stigmatizing attitudes toward people with HIV ranges from about 13% (in Rwanda) to 91% (in Samoa), demonstrating vast global variability. Importantly, higher levels of societal stigma correlate with significantly lower rates of HIV testing uptake.

Stigma in healthcare settings directly affects patient care for those with HIV. In a large European survey of 3,272 patients across 54 countries, 26% reported fear of being treated differently by healthcare professionals if their HIV status was known, 23% had been outright denied care or faced treatment delays due to HIV status, and 33% felt they were treated poorly in healthcare facilities because of being HIV-positive. Within medical facilities, 66% of HIV patients reported encountering direct discrimination from staff, and about 30% delayed testing or disclosure of their status due to fear of stigmatization [8]. These findings illustrate how stigma can permeate clinical interactions, resulting in substandard care and patient disengagement. Despite a global commitment to eliminate HIV-related discrimination in healthcare settings – and the availability of validated tools to measure stigma and evidence-based interventions to reduce it – efforts to incorporate stigma-reduction activities broadly into healthcare remain limited. Training of healthcare workers has proven effective in closing knowledge gaps that can perpetuate HIV stigma, but training alone does not address other factors (such as entrenched attitudes and institutional cultures) that produce and reinforce stigma. Innovative approaches, such as applying quality improvement methods to stigma reduction, have been suggested to identify root causes of discrimination at both individual and system levels and to create contextually appropriate, evidence-based responses. One study demonstrated that a quality improvement intervention in healthcare settings can significantly reduce HIV-related stigma and discrimination by targeting structural drivers and workflow changes [9]. Ultimately, stigma against patients with HIV leads to a significant degradation in quality of care, affecting communication, trust, and treatment adherence. When healthcare workers harbor biases against a group of patients, those patients may receive superficial, incomplete, or delayed care, directly compromising health outcomes.

It is imperative to intensify efforts to address HIV-related stigma and discrimination. This includes highlighting its harmful effects to policymakers capable of amending discriminatory laws that perpetuate stigma, as well as implementing community-led monitoring to document and tackle stigma in healthcare and other community settings. High levels of internalized stigma among people with HIV underscore the importance of access to mental health support. Without such support, individuals with HIV are more likely to refuse or discontinue treatment and to experience mental health problems. Mental health services are especially critical for HIV-positive people who are also gay, transgender, sex workers, or people who use drugs, particularly youth in these groups.

Stigma in mental healthcare

Despite mental health conditions affecting nearly one in five people worldwide, an estimated 70-90% of individuals with mental illness report encountering social or professional stigma, and around 60% do not seek help for this reason. Stigma creates a deep barrier that prevents millions from accessing necessary care and impedes recovery. Among the most damaging stereotypes about people with mental illness is the belief that they are unpredictable and dangerous. Public perception of violence risk is often exacerbated by sensationalized media portrayals: both entertainment media and news outlets tend to present exaggerated, distorted representations of psychiatric disorders, reinforcing ideas of unpredictability, danger, and violence. Such portrayals significantly influence public attitudes, leading to widespread misconceptions about mental illness. As a result, a large segment of the population believes that people with mental disorders are not trustworthy and feels uncomfortable with the idea of working or living alongside them. Alarmingly, healthcare professionals are not immune to stigmatizing views – negative attitudes toward mental illness, including conditions like schizophrenia, are also found among medical providers themselves.

Healthcare professionals have considerable influence in shaping health-related attitudes among both the general public and patients. Medical students, in particular, are a critical group to focus on, as they are future health professionals and there is an opportunity to correct stigmatizing attitudes during their education. Studies using instruments such as Link's Social Distance Scale have found that medical students may exhibit moderately negative attitudes, with prevalent social distance and stereotypes toward patients with mental illness. Notably, international medical students showed more unfavorable attitudes compared to Romanian students in one comparative study [10]. Stigma among health providers is not always conscious; often, medical staff operate under culturally or socially ingrained unconscious biases. These biases can affect diagnosis, the clinician–patient interaction, and therapeutic decisions. For instance, a patient with a mental health disorder might not receive the same thorough evaluation or timely treatment for a concurrent medical condition if the provider's biases lead them to attribute symptoms to the psychiatric illness or to assume the patient is less capable of adherence.

In Romania, a systematic review from 2023 highlighted that public stigma toward mental illness is higher than in some other European countries [11]. The researchers reported numerous adverse effects of stigma, including reluctance to seek help or to engage and remain in treatment, as well as an overall increase in comorbidity and mortality among those with mental illness. Stigma also leads to fewer opportunities for education, employment, and social interaction, as well as difficulties in obtaining housing for affected individuals [11]. In light of these concerns, the World Health Organization (WHO) and many international alliances have underscored the importance of reducing both public and structural stigma through anti-stigma interventions and

advocacy for policy change [12]. Currently, mental health services in Romania are available only in certain regions of the country, and collaboration with other health institutions remains limited. This lack of coordination contributes to the discrimination faced by people with mental disorders, impeding their equitable access to care. The overwhelming evidence points to an urgent need to prioritize health professionals as a primary target for anti-stigma interventions.

Findings also reveal two essential aspects: an absence of a clear theoretical framework for addressing community-level stigma and a very small number of publications on this topic in the local context. These gaps can themselves be interpreted as manifestations of structural stigma – they reflect, at least in part, insufficient funding and the undervaluing of mental health compared to other medical fields. The consequences are numerous: existing interventions tend to be isolated, poorly coordinated, and inadequately communicated, resulting in limited long-term impact. Ultimately, all these factors contribute to the perpetuation of stigma and discrimination against people with psychiatric disorders. Structurally, the mental healthcare system in Romania is underfunded and inefficient, which in itself leads to discriminatory outcomes. For example, the absence of psychiatry departments in many general hospitals can be seen as a sign of a stigmatizing structure that segregates mental healthcare, implying it is less of a priority or should be kept separate from "general" medicine. This structural shortcoming exacerbates inequitable care and reinforces stigma.

Stigma among healthcare professionals

Stigmatization within the medical system is not only an external phenomenon affecting patients but also an internal one, impacting healthcare professionals themselves. Medical personnel can be both sources and targets of stigma, especially when they suffer from mental health conditions, infectious diseases (such as HIV or hepatitis), or belong to marginalized social groups. Studies have shown that health professionals who experience depression, anxiety, or burnout often avoid seeking help, fearing that they will be perceived as incompetent or weak by their peers and superiors [13, 14]. This internalized and institutionalized stigma leads to underdiagnosis and lack of treatment for mental health issues among medical staff, directly affecting the quality of care provided to patients. For instance, a significant number of medical students and practitioners report mental health problems but choose not to access support services due to anticipated stigma or career repercussions [14, 15]. In a multi-institutional U.S. survey of medical students, many self-reported psychological distresses yet were reluctant to formally seek help, highlighting the stigma attached to mental health within medical culture [15]. Many clinicians fear that disclosing a need for mental health support could jeopardize their careers – for example, they worry about potential loss of their medical license or discrimination in the workplace if their struggles become known. One survey of female physicians found that most would avoid seeking mental health treatment out of concern that having a documented diagnosis could tarnish their professional record [14]. This deeply rooted "culture of silence" exacerbates risks for both healthcare providers and patients. Providers face a professional and personal paradox: despite being trained to care for others' health, their own health (particularly mental health) is often ignored or minimized. When these professionals suffer from depression, anxiety disorders, stress-related conditions, or burnout, they risk not only personal distress but also becoming targets of stigma from colleagues, the institution, or through self-stigmatization. Self-stigma is one of the most dangerous forms because it deters individuals from seeking the psychological support they need. Moreover, many providers fear that admitting to such issues could carry punitive consequences for their careers – such as being deemed unfit for duty or facing bias in promotion – reinforcing their silence [14].

On the other hand, stigma can also manifest between colleagues. For example, HIV-positive healthcare workers may be excluded from team activities or relegated to demeaning professional roles by their peers. In the absence of clear anti-discrimination policies at the institutional level, this peer-driven stigma persists and contributes to a toxic work culture. During the COVID-19 pandemic, frontline healthcare workers were frequently stigmatized by the public, being seen as "disease carriers." This societal stigma led to social isolation and significant emotional difficulties for many providers. The consequences of unaddressed internal stigma in the health professions are profound. Lack of intervention results in the worsening of providers' mental health symptoms, increases the likelihood of medical errors, and erodes empathy in patient care. Disturbingly, there is also a consistently documented increased risk of suicide among medical professionals – especially physicians, and notably female physicians – which has been linked to the pressures of stigma and not seeking timely care. Instead of being a place of support, the hospital environment can become one of emotional isolation, where vulnerability is hidden behind a veneer of professionalism.

To combat this internalized stigma, it is essential to promote an institutional culture that recognizes mental health as an integral component of professional well-being. Confidential psychological support programs for staff, training in mental health awareness, and internal campaigns to reduce stigma can encourage healthcare workers to seek help without fear of judgment. For example, some hospitals have introduced confidential counseling services and peer support groups for clinicians, resulting in increased utilization of mental health resources by staff. Educating healthcare teams about burnout and depression as common, treatable issues can normalize help-seeking. Additionally, clear anti-stigma and anti-discrimination policies at the workplace – accompanied by enforcement mechanisms – are necessary to change the "hidden curriculum" that a "real" professional must always be resilient and without personal vulnerabilities. Only by acknowledging and treating this internal stigma can the medical system become a safer, more empathetic, and more functional space for both providers and patients.

Discussion

Combating stigma in healthcare requires a coherent, multidimensional approach that targets individual, institutional, and systemic levels. Firstly, continuous education and training of healthcare workers on topics such as cultural diversity, empathetic communication, and recognition of implicit biases are essential to prevent discriminatory behaviors in clinical practice. Sensitizing medical students and professionals to stigma issues can foster empathy and self-awareness, which are crucial for changing attitudes. Secondly, at the institutional level, implementing clear anti-stigma policies – accompanied by mechanisms for monitoring and reporting discriminatory incidents – helps create a safe and inclusive environment for all patients. This may include formal protocols to address patient complaints of discrimination, regular staff training on ethics and patient rights, and leadership commitment to a zero-tolerance stance on stigma. Thirdly, at the structural level, integrating services for marginalized groups (such as people living with HIV, those with mental health disorders, or ethnic minorities) into general healthcare systems can reduce the fragmentation of care and promote equitable access [16]. For example, ensuring that mental health services are available within primary care settings or that HIV care is integrated into general clinics helps normalize these services and reduce "othering" of patients. Public awareness campaigns are also pivotal: partnerships with media, educational institutions, and community organizations can dispel myths and reduce social prejudice by promoting science-based information and messages of empathy. For instance, national anti-stigma media campaigns have been used to humanize people with mental illness or HIV, showing success in improving public attitudes in various countries. Equally important is the involvement of affected communities in designing and implementing stigma-reduction initiatives. Engaging people who have experienced stigma in co-creating programs and policies leads to solutions that are more relevant, credible, and sustainable in the long term [17]. These human rights–centered and socially just approach ensure that interventions address the real needs and concerns of those most impacted by stigma. In summary, a multifaceted strategy – combining education, policy, community engagement, and structural reform – is critical to meaningfully reduce health-related stigma.

To illustrate, several recent programs have demonstrated efficacy in reducing stigma and improving access to care. These examples suggest that effective anti-stigma strategies blend targeted education, participation of vulnerable groups, and rigorous evaluation, yielding significant improvements at both institutional and individual levels:

- *Human Rights Curriculum in Medical Education (Moldova):* Beginning in 2023, *Nicolae Testemițanu* State University of Medicine and Pharmacy in Chișinău, in partnership with UNAIDS and with financial backing from the Embassy of Sweden, introduced "Human Rights in Healthcare" as a compulsory course across all faculties. Rather than being a standalone course, this content is integrated into clinical disciplines such as gynecology, surgery, and infectious diseases. Students are trained in topics including patient confidentiality, communicating with HIV-positive patients, and ethical self-evaluation. Preliminary results are promising: anonymous evaluations indicate a significant increase in medical students' empathy and awareness of patients' rights following the curriculum's implementation [18].
- *ADAPT-ITT Stigma Intervention Adaptation (Tanzania):* In Dar es Salaam, Tanzania, a group of researchers adapted an existing HIV stigma-reduction program using the ADAPT-ITT framework to also address stigma associated with people who use drugs (PWUD). The process was highly participatory, directly involving patients and clinical staff. Key steps included initial formative research, development of a tailored manual, stakeholder workshops, and pilot testing of the adapted intervention. The resulting program aimed to change healthcare providers' attitudes and create a more inclusive, non-judgmental environment, ultimately improving retention of PWUD in HIV treatment. By broadening the focus of an HIV stigma intervention to encompass drug-use stigma, the program recognized the intersecting prejudices that often impede care. Early outcomes suggest improved clinician attitudes and better engagement of PWUD in care [19].
- *Anti-Stigma Training for Healthcare Workers (Bangladesh):* A training program in Bangladesh targeted over 300 healthcare professionals working in adolescent health and reproductive health services. The curriculum included dedicated sessions on HIV-related stigma, LGBTQ+ identities, and common stereotypes regarding sexuality. The training was delivered as a two-day course, with a follow-up reinforcement session six months later. The impact was significant: providers' attitudes toward HIV-positive patients and sexually active youth became markedly more accepting. For example, the proportion of trainees who believed that people with HIV "should feel ashamed" dropped from 35% before training to 16% after training. Likewise, patient satisfaction among young clients rose from 63% pre-intervention to 98% post-intervention, indicating that the changes in provider attitudes translated into improved patient experience [20]. This example underlines how focused education, combined with follow-up support, can substantially reduce stigmatizing beliefs and improve the quality of care.

Notably, stigma can hinder healthcare effectiveness in less direct ways as well. Patients who anticipate judgment or discrimination may withhold important information about their health behaviors or conditions, compromising the accuracy of clinical assessments and public health data

[21]. For instance, someone at risk for HIV might not disclose certain behaviors to a doctor out of shame, leading to missed opportunities for testing or prevention. Such gaps in data can distort public health strategies that rely on honest reporting of risk factors. Stigma also has the potential to erode trust in the healthcare system and in public health authorities, which becomes especially critical in contexts such as pandemics, where community cooperation is essential. During the COVID-19 pandemic, for example, stigmatization of medical personnel and of patients who contracted the virus impeded the implementation of public health measures and increased the public's reluctance to undergo testing or vaccination. Fear of being stigmatized led some to avoid testing for COVID-19 or to hide symptoms, thereby hampering disease control efforts. These instances highlight that reducing stigma is not only a moral or ethical imperative but also a strategic necessity for the effective, equitable, and sustainable functioning of health systems.

Conclusions

Stigmatization in healthcare is a pervasive phenomenon, manifested in multiple forms and profoundly affecting the quality of medical care and patient access to services. Patients belonging to stigmatized groups – such as people living with HIV, individuals with mental health disorders, substance users, or ethnic minorities – often face prejudice and even denial of care. Medical stigma leads many patients to delay seeking treatment or to avoid the health system entirely due to fear of judgment or prior discriminatory experiences. This avoidance behavior contributes to late diagnoses, poor treatment adherence, and worsening health outcomes. In parallel, stigmatization inflicts significant emotional distress on patients (including feelings of shame and low self-worth) and complicates their social reintegration. Stigma in healthcare also violates the principle of equity in access to services and can have system-wide repercussions by undermining trust in medical institutions and public health initiatives.

Addressing health-related stigma is imperative. Strategic interventions in medical education, such as incorporating anti-stigma and empathy training, can prepare healthcare professionals to recognize and counteract their biases. Clear policies and legislation against discrimination in healthcare settings are needed to provide accountability and protect both patients and providers. Furthermore, sustained public awareness and advocacy campaigns are essential to challenge societal prejudices and normalize seeking care for all individuals, regardless of their condition or background. Reducing stigma in healthcare is not only a matter of ethics and human rights but also a critical step toward improving clinical outcomes and strengthening health systems. By fostering an inclusive and compassionate healthcare environment, we can ensure better quality of care, enhance patient trust and engagement, and promote the fundamental principle of health equity.

Competing interest

None declared.

Contribution of authors

IAC conceived the study, conducted the literature review and analysis, and drafted the manuscript. LR supervised the research as academic coordinator, contributed to the study design and data interpretation, and critically revised the manuscript. Both authors reviewed and approved the final version of the manuscript.

Ethics approval

Not needed for this study

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REVIEW ARTICLE

Contemporary approach to pediatric ovarian tumors

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ABSTRACT

Introduction. Pediatric ovarian tumors represent a rare but clinically important category of gynecologic conditions, comprising approximately 1-2% of all childhood malignancies and about 5% of pediatric abdominal masses. While most are benign, a meaningful percentage can be hormonally active, raising diagnostic and therapeutic challenges. Due to nonspecific symptoms such as abdominal pain or distension, early diagnosis is often delayed, potentially compromising fertility preservation and long-term outcomes. A multidisciplinary, age-specific approach is essential to optimize management.

Material and methods. This study is a narrative literature review based on an extensive search across *PubMed, ScienceDirect, SpringerLink,* and *Google Scholar*. The search covered the period from 2008 to 2025 and included terms such as "pediatric ovarian tumors", "germ cell tumors", "sex cord-stromal tumors", "diagnostic imaging", and "fertility preservation". Inclusion criteria encompassed peer-reviewed, full-text articles in English focusing on patients aged 0-19 years. A total of 20 sources, including clinical guidelines and articles, were selected for their thematic relevance and quality of evidence.

Results. Pediatric ovarian tumors show wide clinical and histological variability, with germ cell tumors being the most prevalent malignant subtype. Transabdominal ultrasound is the first-line imaging tool, while Magnetic Resonance Imaging is reserved for complex or inconclusive cases. Tumor markers, such as alpha-fetoprotein, beta-human chorionic gonadotropin, lactate dehydrogenase, and Inhibin B, are essential in differentiating tumor types and guiding management. Surgical decisions prioritize minimally invasive, fertility-sparing approaches when malignancy is unlikely. Long-term follow-up includes hormonal, reproductive, and psychosocial monitoring. Psychological support is particularly important for adolescents. Despite advancements, diagnostic delays, lack of pediatric-specific guidelines, and disparities in care, especially in low-resource settings, remain critical challenges.

Conclusions. Pediatric ovarian tumors require an individualized, multidisciplinary management strategy that integrates early detection, age-appropriate surgical care, fertility preservation, and long-term endocrine and psychological support. This review highlights the need for pediatric-specific protocols and improved access to diagnostics to enhance outcomes and preserve the future reproductive potential of affected children and adolescents.

Keywords: pediatric ovarian tumors, diagnostic imaging, tumor markers, minimally invasive surgery, fertility preservation, multidisciplinary management.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Despite advances in the management of pediatric ovarian tumors, several knowledge gaps persist. There is limited availability of standardized, age-specific diagnostic and therapeutic guidelines tailored to the pediatric population. The interpretation of tumor markers in children lacks universally accepted pediatric reference ranges. The long-term impact of conservative surgical approaches on hormonal function, fertility, and psychosocial outcomes remains insufficiently studied. These gaps are particularly significant

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in low-resource settings, where access to imaging, tumor markers, and pediatric-trained specialists is limited.

The research hypothesis

Adherence to evidence-based, age-specific diagnostic and surgical management guidelines for pediatric ovarian tumors, incorporating imaging, tumor marker profiling, and fertility-preserving techniques, and adapted to local healthcare capacities, can improve reproductive and psychosocial outcomes more effectively than non-specialized, adult-oriented approaches.

The novelty added by the manuscript to the already published scientific literature

This manuscript provides a focused, multidisciplinary overview of pediatric ovarian tumors, emphasizing age-adapted diagnostics, fertility preservation, and long-term endocrine and psychosocial follow-up, topics often insufficiently explored in existing literature. By addressing pediatric-specific needs and healthcare disparities, it offers a more tailored and integrative approach than previous reviews, which are largely based on adult data.

Introduction

Pediatric ovarian tumors represent a rare but clinically important category of gynecologic conditions, comprising approximately 1-2% of all childhood malignancies and about 5% of pediatric abdominal masses, as described by Banli-Cesur et al. (2021), Renaud et al. (2019), and Satoh et al. (2016) [1-3]. Although most ovarian tumors in children and adolescents are benign, the clinical spectrum is wide, ranging from functional cysts and hormonally active lesions to highly malignant germ cell neoplasms, which require careful clinical evaluation and multidisciplinary management [4]. Literature data emphasize that the majority of pediatric ovarian malignancies are of germ cell origin, accounting for 60-80% of cases, especially in girls ≤15 years [1, 2]. Tsikouras et al. (2008) further highlight the predominance of this histologic type in prepubertal patients, requiring age-specific diagnostic and therapeutic protocols [5, 6].

Timely detection of pediatric ovarian tumors is often delayed due to their nonspecific clinical presentation. Braungart et al. (2020) report that abdominal pain, distension, and the presence of an abdominal mass are among the most common symptoms, but these may mimic more frequent pediatric conditions such as appendicitis or urinary tract infections [7]. Children often struggle to articulate their symptoms clearly, contributing to diagnostic delays and potentially compromising fertility preservation opportunities [8, 9]. Ultrasonography, particularly the transabdominal approach, remains the primary imaging modality in pediatric patients due to its accessibility, safety, and diagnostic yield [7, 8]. MRI is increasingly used for complex or equivocal cases, providing tissue characterization and guidance for surgical planning [3, 8]. However, imaging alone is insufficient to confirm tumor histology or evolution. Tumor markers such as AFP, β-hCG, LDH, and Inhibin B are essential auxiliary tools, especially in the evaluation of germ cell and sex cord-stromal tumors [3, 10, 11].

Surgical management in this population must carefully balance oncologic safety with preservation of endocrine and reproductive function. Studies indicate that conservative surgical techniques, including ovarian cystectomy and fertility-sparing procedures, are particularly important in benign or early-stage malignant cases [2, 12]. Laparoscopy, when feasible, is the optimal approach due to its minimally invasive nature and lower risk of postoperative adhesions [8]. Long-term follow-up is essential not only for detecting recurrence, but also for monitoring pubertal progression, fertility potential, and psychosocial adaptation. Survivors of pediatric ovarian tumors may experience anxiety, body image disturbances, or concerns regarding reproductive health, requiring integrated psychological and endocrine support [8, 13].

This review aims to provide a comprehensive synthesis of the current literature on pediatric ovarian tumors, encompassing epidemiological trends, diagnostic strategies, clinical presentation, imaging, tumor markers, surgical and conservative management, fertility preservation, recurrence, and multidisciplinary care.

Material and methods

This study is a narrative literature review designed to synthesize current evidence on pediatric ovarian tumors. A structured literature search was conducted using four electronic databases: *PubMed, ScienceDirect, SpringerLink,* and *Google Scholar,* covering the period from 2008 to 2025. Keywords included: "pediatric ovarian tumors", "germ cell tumors", "sex cord-stromal tumors", "fertility preservation", "diagnostic imaging", and "tumor markers in children". Inclusion criteria encompassed full-text, peer-reviewed articles published in English, focused on the pediatric population (age range 0-19 years), and addressing diagnostic approaches, therapeutic strategies, or multidisciplinary management of ovarian tumors. A total of 20 full-text articles and clinical guidelines were selected based on their relevance, quality of evidence, and thematic contribution. The literature was reviewed and

categorized into the following thematic domains: incidence and epidemiological trends in children and adolescents; clinical manifestations, diagnostic imaging, and tumor marker profiles; surgical indications, with an emphasis on fertility-sparing approaches; recurrence, monitoring strategies, and multidisciplinary follow-up; and strategic directions for improving pediatric gynecological care.

Results

This study provides a comprehensive and updated overview of pediatric ovarian tumors, focusing on their multidisciplinary management approaches. This literature review reveals significant advances in the understanding and management of these tumors, while also highlighting key challenges and knowledge gaps.

Pediatric ovarian tumors remain rare, with an annual incidence estimated at 2.6 per 100,000 girls [2, 4, 9]. Satoh et al. (2016) report a small incidence peak during infancy and adolescence [3]. Hormone-secreting sex cord-stromal tumors are more frequent in pediatric populations compared to adults [14, 15].

The clinical presentation is often nonspecific. As reported by Braungart et al. (2020), abdominal pain is the most common symptom, observed in up to 80% of cases [7]. Pain may be intermittent or acute, sometimes mimicking appendicitis or urinary tract infections [16]. Abdominal distension and a palpable mass are also frequent, particularly with larger tumors (>8 cm) [10, 11]. Mass effect on adjacent organs can cause nausea, vomiting, constipation, increased urinary frequency, or urinary retention [8, 9]. These symptoms often lead to initial referrals to pediatric surgery or gastroenterology, delaying gynecologic evaluation [6, 9]. Functional tumors, such as granulosa cell tumors or Sertoli-Leydig cell tumors, may present with precocious puberty, menstrual irregularities, hirsutism, or virilization (in androgen-secreting tumors). In postmenarchal adolescents, abnormal uterine bleeding may be the first sign of a hormonally active tumor [9, 14, 16, 17]. Up to 20-30% of pediatric ovarian tumors may present as acute abdomen due to torsion of the ovarian pedicle–a surgical emergency–or intracystic hemorrhage or rupture. Ovarian torsion is more likely in benign, mobile cystic lesions, particularly those larger than 5 cm. Asymptomatic ovarian masses are occasionally discovered incidentally [7, 12, 18]. These cases often involve simple cysts or mature teratomas and tipically require close monitoring rather than immediate intervention [19].

In prepubertal and early pubertal girls, transabdominal US is the method of choice due to anatomical and ethical considerations. Transabdominal US can differentiate between simple cysts and solid masses [9]. It may also be used in postmenarchal adolescents with patient consent, especially when further detail is needed [8, 15]. MRI is performed as a second-line tool in cases where US is inconclusive or when more detailed tissue characterization is required prior to surgery [3, 7]. MRI offers several advantages: superior soft tissue contrast, better delineation of tumor components (fat, fluid, hemorrhage), detection of lymphadenopathy or local invasion, and helps distinguish between benign teratomas, hemorrhagic cysts, and malignant tumors. Mature teratomas have a high fat content and mixed signal intensity. Malignant tumors consist of solid components with contrast enhancement, necrosis, or ascites. MRI is especially useful for large or complex masses in planning fertility-sparing surgery, or when the urgency of surgical intervention must be assessed and prioritized [3, 17].

Recent international guidelines advocate for standardized risk assessment, the use of tumor markers (AFP, β-HCG, LDH), and structured follow-up protocols to monitor recurrence and endocrine sequelae [3, 18]. The availability and use of tumor markers have improved early differentiation between benign and malignant tumors, although pediatric reference ranges are not always standardized [6, 17]. Interpretation of tumor markers is more complex in pediatric patients due to age-related reference ranges [6, 17] (Table 1).

Table 1. Main tumor markers and pediatric reference values

Marker	*Pediatric reference interval*	*Use in ovarian masses*	*Suggestive of malignancy*
AFP	0–30 days: 0.6–18,964 1–11 months: 0.6–77.0 1–3 years old (y.o.): 0.6–11.1 4–6 y.o.: 0.6–4.2 7–12 y.o.: 0.6–5.6 13–19 y.o.: 0.6–4.2	↑ in yolk sac tumors and mixed malignant GCT.	Persistently elevated beyond age-appropriate range or rising trend.
β-hCG	At birth (placental transfer): 10–50 IU/L Declines with ½ every 2–3 days. By >3 months: <1.0 IU/L	↑ in choriocarcinoma, some mixed GCT; rarely in dysgerminoma.	Detectable/raised after 3 months of age when not pregnant.
LDH	1–3 y.o.: 160–370 4–6 y.o.:145–345 7–9 y.o.:143–290 10–12 y.o.:120–293 13–15 y.o.:110–283 16–17 y.o.:105–233	↑ in dysgerminoma.	Markedly above age-specific upper limit with solid mass on imaging.
Inhibin B	Low/undetectable prepuberty (<20–100 pg/mL) Peaks mid-puberty (80–90 pg/mL), then falls.	↑ in granulosa cell tumors.	Marked elevation relative to age/puberty stage.
AMH	Broad age-specific variation; examples in girls: approx. 0.08–13.2 ng/mL.	↓ suggests diminished ovarian reserve; not a malignancy marker.	Not a tumor marker; trends used for ovarian function monitoring.
CA-125	Adult-type cutoff commonly used: ≤35 U/mL.	Non-specific; can rise with peritoneal irritation, endometriosis, infection.	Markedly elevated with solid/complex mass and concerning imaging/other markers.

Note: *AFP – alpha-fetoprotein; βhCG – beta-human chorionic gonadotropin; LDH – lactate dehydrogenase; AMH – anti-Müllerian hormone; CA125 – cancer antigen 125.*

Surgery is guided by the suspected risk of malignancy, presence of complications, and imaging features. Emergency indications include ovarian torsion, rupture, or hemorrhagic cysts [7, 8]. When a benign lesion is suspected, minimally invasive procedures, such as laparoscopic cystectomy or tumorectomy, are typically indicated in elective settings [2, 4]. Fertility-preserving resection is feasible and safe in most cases. Conservative management is considered when lesions are <8 cm in diameter, cystic in nature, and associated with normal tumor marker levels. Laparotomy is reserved for large or suspicious masses, or for confirmed malignancies requiring staging [15, 16].

The management of ovarian tumors in children and adolescents presents distinct clinical, diagnostic, and therapeutic challenges. These differences arise from anatomical, physiological, psychosocial, and oncological considerations, which must be carefully addressed to ensure optimal outcomes. There is a greater emphasis on fertility preservation, given the long reproductive lifespan ahead. Thus, ovarian-sparing techniques are prioritized whenever oncologically safe [9, 20]. The need for a contemporary, age-adapted approach to pediatric ovarian tumors is therefore important. This includes improved awareness among pediatricians and gynecologists and the integration of multidisciplinary teams. Laparoscopy is the optimal surgical method in pediatric ovarian tumors when the likelihood of malignancy is low. It ensures faster recovery, better outcomes, and fertility preservation. However, laparotomy remains crucial when dealing with large, complex, or malignant lesions, offering oncologic safety and thorough exploration. The choice of approach must be individualized, guided by imaging, biomarkers, intraoperative findings, and surgical expertise [9, 20].

Fertility-sparing surgery does not compromise oncologic outcomes in early-stage tumors. Braungart et al. (2020) reported high rates of menstrual recovery and pubertal progression post-surgery [7, 8]. AMH levels and follicle count via US are used in long-term fertility assessments. Hormonal dysfunction may occur after bilateral surgery or chemotherapy, necessitating endocrine follow-up [4]. Psychological support is critical, especially in adolescents undergoing treatment with reproductive implications [13, 15]. Preservation of fertility and hormonal function is particularly important in children and adolescents. Ovaries possess regenerative capacity and can often be spared with careful surgical planning [2, 3]. Conservative surgery is indicated in cases with normal or slightly elevated tumor markers, cystic or mixed echogenicity on US, size <8 cm with smooth borders and no ascites, absence of solid components or internal septations, non-hemorrhagic contents, and no evidence of metastasis [19].

Recurrence is uncommon in benign cases but must be monitored closely in malignant or borderline tumors. Risk factors include advanced stage, high-grade histology, incomplete previous resection, or absence of adjuvant therapy. AFP and β-hCG are useful surveillance markers for germ cell tumors, while Inhibin B is monitored in granulosa cell tumors [8]. US and MRI are used to assess residual tissue or recurrence. When recurrence occurs, re-excision, chemotherapy, or combined therapy is applied depending on tumor type [7, 9].

Surgical management of ovarian tumors in pediatric patients must strike a balance between timely intervention, fertility preservation, and oncologic safety. The indication for surgery may arise in emergency or elective settings, depending on the clinical presentation, imaging findings, and suspected pathology. Emergency surgery is required when a child presents with acute symptoms suggestive of complications [7, 8, 12, 20].

Long-term follow-up must also address hormonal health, pubertal staging, and psychosocial reintegration. Structured transition to adult care is essential [6, 8].

The optimal care of children and adolescents with ovarian tumors–whether benign, borderline, or malignant–requires a multidisciplinary team approach. This model ensures individualized, comprehensive, and coordinated care by integrating the expertise of specialists, tailored to the specific clinical, developmental, psychological, and fertility-related needs of pediatric patients. Core members of the multidisciplinary team include: a pediatric oncologist, pediatric surgeon or gynecologic oncologist, radiologist, pathologist, reproductive endocrinologist, pediatric endocrinologist, clinical psychologist or psycho-oncologist, nursing staff, and patient navigation team. The multidisciplinary team approach in clinical practice can improve diagnostic accuracy and reduce unnecessary radical surgery, enhance compliance with evidence-based protocols, lower recurrence rates and improve survival outcomes, protect fertility and pubertal development, and provide a stronger psychosocial support and family-centered care [12, 13].

Discussion

Pediatric ovarian tumors represent a rare but clinically significant group of gynecologic conditions, marked by substantial heterogeneity in histology, symptomatology, and outcomes. Their management requires a tailored, multidisciplinary approach that considers the developmental, reproductive, and psychological contexts unique to children and adolescents. This review consolidates current evidence and practice patterns, identifying both advances and persistent challenges in diagnosis, treatment, and follow-up care.

Germ cell tumors are the most common malignant ovarian neoplasms in pediatric populations, particularly in girls under 15 years, as demonstrated by different authors [1, 2]. Their early recognition is crucial given their high chemosensitivity and favorable prognosis when detected promptly. However, these tumors are often diagnosed late due to their nonspecific presentation, which overlaps with more common pediatric conditions such as appendicitis or urinary tract infections [4, 5]. Sex cord-stromal tumors, although less frequent, can present with early puberty, irregular bleeding, or virilization [14, 15].

The diagnostic approach is strongly guided by imaging assessment and biochemical marker analysis. US remains the first-line diagnostic tool due to its safety and accessi-

bility, with MRI providing superior anatomical detail in complex or equivocal cases [7]. However, neither imaging method can independently confirm malignancy. Tumor markers such as AFP, β-hCG, LDH, Inhibin B, and AMH are essential tests, yet their interpretation in children, especially neonates and prepubertal girls, requires careful consideration of age-specific reference ranges [3, 6, 10]. Expanding access to these markers is a critical step in improving diagnostic accuracy (Table 2).

Table 2. Diagnostic and treatment algorithm – key steps

Phase	*Key steps*
Initial assessment	History (pain, menstrual cycle, acute torsion signs) Tanner stage Pregnancy test if post-menarchal.
First-line imaging	Pelvic ultrasound with Doppler
Second-line imaging	MRI if indeterminate or to refine surgical planning CT reserved for staging/complications.
Tumor markers	Order age-appropriate panel: AFP, β-hCG, LDH; ± inhibin B (granulosa), AMH baseline, ± CA-125.
Risk stratification	Integrate US features + markers + age/puberty: Low-risk (simple cysts) vs. suspicious (solid/complex, elevated markers).
Conservative management	Simple, asymptomatic cysts (generally <5–7 cm): observation ± short-interval US (6–12 weeks).
Torsion protocol	Urgent laparoscopy; detorsion and cystectomy when viable; avoid routine oophorectomy.
Benign-appearing tumors	Minimally invasive ovarian-sparing cystectomy/ tumorectomy whenever feasible.
Suspicious/malignant	Oncologic principles: avoid rupture, perform unilateral oophorectomy if indicated, staging; consider fertility preservation.
Pathology	Frozen section selectively; definitive histology drives adjuvant therapy.
Follow-up	Clinical assessment + ultrasound; markers as applicable; endocrine/fertility monitoring (menses, AMH/ovarian volume).

Note: *Sources: Renaud E.J., 2019; Birbas E., 2023; Bašković M., 2025; Braungart S., 2020; Pio L., 2023; Margioula-Siarkou C, 2023.*

Ovarian-sparing procedures are performed when preoperative imaging and biomarkers suggest benign or low-grade malignancy [2, 7, 9]. Laparoscopic surgery is the optimal choice due to its minimally invasive nature and faster recovery. Nonetheless, laparotomy remains necessary in selected cases, particularly for large or suspicious lesions requiring staging or to avoid capsular rupture [6, 15]. The decision between conservative and radical approaches requires expert surgical judgment and, ideally, intraoperative histological assessment.

Preservation of hormonal function and fertility potential is a cornerstone of pediatric tumor management. Most girls undergoing conservative procedures retain normal pubertal progression and regular menstrual cycles [4, 18]. However, patients undergoing bilateral oophorectomy are at risk of primary ovarian insufficiency, which may require hormone replacement therapy and fertility counseling. Post-treatment evaluation of ovarian reserve using AMH levels and antral follicle counts is becoming standard [13]. Pubertal staging, menstrual tracking, and regular hormonal assays (FSH, LH, estradiol) are critical components of follow-up.

The emotional and psychological impact of a tumor diagnosis during adolescence is profound and warrants careful attention. Studies reported elevated risks for anxiety, depression, altered self-image, and fear of infertility [13, 15]. Family dynamics are also deeply impacted, with parents frequently experiencing guilt and decisional distress, particularly when fertility-sparing options are uncertain. Incorporating psychologists and psycho-oncologists into care pathways improves emotional resilience and treatment adherence.

Although recurrence is uncommon in benign tumors, it remains a concern in malignant and borderline cases, especially in the context of incomplete resection or delayed adjuvant therapy. Markers such as AFP, β-hCG, and Inhibin B are valuable tools for post-treatment monitoring, particularly in germ cell and granulosa cell tumors [9]. Imaging, most often US and MRI, is used to detect recurrence, assess residual lesions, or identify secondary postoperative changes.

Long-term follow-up should extend beyond oncologic surveillance to include endocrine, reproductive, and psychological dimensions [6]. Transitioning care from pediatric to adult gynecology or reproductive endocrinology represents a vulnerable period. In low- and middle-income countries, girls often present at advanced stages due to limited access to imaging, tumor markers, and trained pediatric surgeons or gynecologists [17, 19, 20].

Results from multiple studies highlight the urgent need for the development of standardized, pediatric-specific diagnostic and treatment guidelines; training programs in minimally invasive and organ-preserving techniques; improved access to diagnostic tools; and integration of psychosocial support and reproductive counseling [13, 17].

Conclusions

Pediatric ovarian tumors pose distinct clinical challenges due to their age-specific presentation, diverse histopathological types, and potential long-term implications for reproductive health. Management requires an individualized, multidisciplinary strategy that integrates early detection, age-appropriate surgical care, fertility preservation, and long-term endocrine and psychological support. Ongoing follow-up is critical for monitoring recurrence risk, pubertal development, and endocrine function. A well-coordinated transition to adult healthcare services is vital to ensure seamless continuity of care.

Competing interests

None declared.

Authors' contributions

All authors participated in the study design and contributed to drafting the manuscript. The authors critically reviewed the work and approved the final version of the manuscript.

Ethics approval

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REVIEW ARTICLE

β-adrenergic agonists and β-antagonists in sport performance: a narrative synthesis of pharmacological effects and anti-doping implications

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ABSTRACT

Introduction. In the context of increasingly intense athletic competition, athletes are motivated to enhance their performance through various methods, including the use of pharmacological substances that act on the adrenergic system. Among these, β2-adrenergic agonists are employed for their metabolic effects and their role in increasing endurance, whereas β-adrenergic antagonists are used in precision sports to reduce tremor and control anxiety. Both classes of substances present potential benefits as well as health risks, and are subject to strict regulations in high-performance sports.

Material and methods. A theoretical study was conducted based on the analysis of specialized scientific literature, aiming to evaluate the impact of β2-adrenergic agonists and β-adrenergic antagonists on athletic performance. Additionally, the current regulations of the World Anti-Doping Agency (WADA) were analyzed.

Results. β2-adrenergic agonists may contribute to the stimulation of muscle protein synthesis, enhancement of energy metabolism, and delay in the onset of fatigue. However, their use is associated with significant cardiovascular and metabolic side effects. β-adrenergic antagonists are effective in reducing tremor and sympathetic activation in precision sports but may decrease overall exercise capacity and induce bradycardia or chronic fatigue. Improper use of these substances can lead to severe sanctions in the context of athletic competitions.

Conclusions. Although β2-adrenergic agonists and β-adrenergic antagonists may offer certain advantages depending on the specific nature of the sport, their use must be strictly medically regulated and comply with anti-doping standards. Careful evaluation of the risk-benefit ratio is essential for safeguarding athletes' health and preserving the integrity of competition.

Keywords: β-adrenoreceptors, β-adrenergic agonists, clenbuterol, salbutamol, β-adrenergic antagonists, propranolol.

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

Although β2-adrenergic agonists and β-adrenergic antagonists are frequently used in athletic contexts, their actual impact on performance in trained athletes remains only partially elucidated. Direct comparisons of their effects based on the type of exer-

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tion and the pharmacological characteristics of these substances are still limited in the specialized literature.

The research hypothesis

To evaluate the impact of β-adrenergics on athletic performance, along with the associated health risks, cardiovascular and metabolic side effects, and the legal and ethical ramifications concerning doping.

The novelty added by the manuscript to the already published scientific literature

The research provides a comprehensive and in-depth understanding of how β-adrenergics influence various aspects of metabolism and sports performance, explains the associated risks, and promotes the responsible use of these substances.

Introduction

Sport performance represents a central objective in today's competitive culture, being determined by a range of physiological, psychological, and technological factors.

Physiological factors include cardiovascular and respiratory capacity, muscle mass and strength, energy metabolism efficiency, and hormonal balance. Psychological factors encompass aspects such as motivation, stress tolerance, concentration, and mental resilience. Technological factors involve the use of advanced equipment, modern performance monitoring techniques, and personalized recovery and nutrition interventions. Within this integrative framework of performance optimization, an increasing number of athletes resort to pharmacologically active substances that can directly or indirectly influence cardiovascular, metabolic, neuromuscular, and central nervous system functions involved in athletic exertion.

Among these, drugs acting on the β-adrenergic system are particularly attractive in the context of high-performance sport. The use of pharmacological substances by athletes as agonists or antagonists of β-adrenergic receptors is a current concern that requires careful evaluation of the benefit-risk ratio [1], in accordance with existing anti-doping regulations. The administration of these compounds must be based solely on a justified medical indication and carried out under specialist supervision, respecting both their mechanism of action on the β-adrenergic system [1] and the regulatory framework governing athletic performance [2].

β2-adrenergic receptor agonists (β2-adrenomimetics) are used therapeutically for their bronchodilator effects, but in sports they have gained notoriety for their anabolic potential, ability to stimulate protein synthesis, and capacity to increase metabolic rate through enhanced lipolysis and glycogenolysis. The use of these substances can lead to the rapid mobilization of energy substrates, delaying the onset of fatigue and increasing muscular endurance during sustained effort. However, their use is associated with significant side effects, such as tachycardia, hypokalemia, left ventricular hypertrophy, and mitochondrial toxicity [3]. Furthermore, systemic administration of β2 agonists is considered doping and is prohibited under World Anti-Doping Agency regulations, except for specified inhalation doses [2].

In comparison, beta-adrenoblockers (β-adrenoblockers), which inhibit the activation of β1 and/or β2-adrenergic receptors, are used in sports that require precision, fine coordination, and emotional control, such as shooting sports or martial arts. These medications reduce sympathetic activity [1], inducing bradycardia and diminishing physiological tremor, thereby facilitating focus and stability in static sports. Although they exert an ergolytic effect in endurance sports, in precision sports they may provide a considerable advantage, which is why their use is restricted by WADA in selected competitions [2].

Recent literature highlights conflicting perspectives regarding the role of these substances in optimizing athletic performance. While some studies support their efficacy in specific physiological contexts, others emphasize the significant health risks and the negative impact on competitive equity [4]. The topic thus remains relevant and controversial, given the lack of clear scientific consensus and the often-divided opinions–factors which justify the need for further analysis.

Material and methods

This study was designed as a narrative review of specialized scientific literature, aiming to analyze the pharmacological mechanisms, physiological effects, and regulatory aspects of β2-adrenergic agonists and beta-adrenoblockers in high-performance sport. The bibliographic search was conducted using electronic databases: PubMed, Scopus, Elsevier, BMJ, Springer, Web of Science, and Google Scholar, which included peer-reviewed scientific articles, clinical studies, systematic reviews, meta-analyses, and official guidelines (WADA, EMA), as well as works detailing the pharmacological mechanisms of action, medical indications, and physiologically relevant effects in the athletic context. The data were thematically classified into two categories: β2-agonists and β-adrenergic antagonists, and comparisons were made based on the type of effects on athletic performance (Table 1).

Statistical methods were not applied, as the study did not include quantitative analysis or meta-analysis. The work is

based on a qualitative and comparative synthesis, with emphasis on the pharmacological significance and clinical relevance of the findings.

Results

The qualitative analysis of the specialized literature revealed a significant number of findings regarding the impact of β2-agonists on athletic performance. The main hypothesis, that these substances could contribute to performance enhancement through metabolic and neuromuscular mechanisms, was supported by several lines of research.

a) Stimulation of muscle protein synthesis

The use of β2-agonists leads to an increase in protein synthesis in skeletal muscle, contributing to hypertrophy and muscle recovery, especially during strength training. This effect is genetically mediated through the activation of PGC-1α and other pathways involved in myogenesis [5-11].

b) Reduction of protein degradation and mitochondrial protection

β2-agonists not only stimulate protein synthesis but also reduce the rate of muscle protein degradation. Additionally, they increase mitochondrial protein synthesis and the expression of PGC-1α mRNA, which is involved in mitochondrial biogenesis [12-16].

c) Increase in energy metabolism

Activation of β2 receptors leads to the mobilization of fatty acids through lipolysis, increased glycogenolysis, and stimulation of metabolic pathways for ATP production. These mechanisms support intense and prolonged physical effort [8, 17-21].

d) Improvement of fatigue resistance

Increased resistance to fatigue is explained by central nervous system stimulation, enhanced muscle perfusion, mobilization of energy resources, and a reduced perception of pain. All these effects contribute to sustaining long-term performance [13, 14, 18, 22-24].

e) Modulation of muscle contraction

β2-agonists produce positive ionotropic and lusitropic effects on slow-twitch muscle fibers without significantly altering myofibrillar sensitivity to Ca^{2+}. These effects occur only at high concentrations of β-adrenergic agonists [25-29].

f) Reported limitations and adverse effects

Prolonged use or high doses are associated with severe adverse effects, such as tachycardia, mitochondrial toxicity, cardiac impairment, and metabolic disorders. The risks are particularly pronounced in the case of clenbuterol [18, 30-32].

g) Confirmation of anti-doping regulations

According to WADA 2025, most β2-agonists are prohibited, with some exceptions for inhaled forms. Explicit guidelines are provided for salbutamol, formoterol, and vilanterol [2].

After analyzing the pharmacological and physiological effects of β2-adrenergic agonists on athletic performance, data regarding β-adrenergic antagonists were also synthesized, especially in the context of sports requiring precision, fine coordination, and emotional control. These substances, by blocking β1 and/or β2 receptors, reduce sympathetic activity [3], induce bradycardia, and attenuate physiological tremor, thereby contributing to performance stabilization under competitive stress conditions.

a) Reduction of tremor and anxiety

Propranolol is used off-label (outside of the approved indication) for the management of performance anxiety, usually being administered approximately one hour before a sports event. It reduces tremor and associated somatic symptoms such as tachycardia and palpitations, and is frequently used in sports contexts [33]. A recent randomized, placebo-controlled clinical study demonstrated that propranolol exerts a general reduction effect on neuronal activity in the motor cortex, regardless of the specific tremor context. This finding indicates that propranolol's action is not limited to peripheral effects – such as the reduction of heart rate or muscular tremor through β-adrenergic receptor blockade – but also involves a central, neurophysiological component. More precisely, the drug directly influences the reactivity of neuronal networks in the motor cortex, an essential area for the planning and execution of voluntary movements. This central mechanism contributes to the stabilization of fine motor control and can explain the efficacy of propranolol in reducing tremor both in neurological disorders (such as Parkinson's disease) and in performance anxiety situations, where excessive activation of the nervous system may interfere with motor precision [34].

b) Efficacy in precision sports

In contrast to endurance sports, where β-adrenergic antagonists may negatively affect performance, in precision sports they can bring significant benefits.

Precision sports like shooting, archery, or billiards require fine motor control and high psychophysiological stability – factors that can be positively influenced by blocking adrenergic receptors. It is considered that the beneficial effect of metoprolol in this context derives from its ability to selectively block β1-adrenergic receptors. By reducing sympathetic activity, metoprolol contributes to the attenuation of physiological tremor and to the stabilization of fine movements, which are essential for precision. The study conducted by Kruse et al. (1986) showed an increase in pistol shooting performance by approximately 13% compared to placebo, in the absence of significant changes in monitored cardiovascular parameters (such as heart rate or oxygen saturation), suggesting that the observed benefits are more likely attributed to the neuromuscular control of tremor rather than a hemodynamic effect. This mechanism confers a specific therapeutic value to metoprolol in static sports that require coordination and precision [35]. However, the efficacy of β-adrenergic antagonists can vary considerably depending on the type of sporting discipline and the pharmacological profile of the administered substance. Similar to metoprolol in sport shooting, the use of propranolol or bisoprolol has also been analyzed in archery. Nevertheless, the study conducted by Ergen et al. (2021) did not demonstrate significant performance improvements under simulated conditions, suggesting that the favorable effect of

β-adrenergic antagonists may strictly depend on the type of activity, dosage, and the application context [36].

c) Impact in endurance sports

Unlike precision sports, where β-adrenergic antagonists may have a favorable effect on fine motor control, in endurance disciplines their effects are predominantly negative. β-adrenergic antagonists reduce heart rate and cardiac output during physical exertion, which limits tissue oxygenation capacity and may lead to an increased perception of fatigue. As a result, oxidative performance declines in dynamic aerobic activities like running and cycling. The study conducted by Priel et al. (2021) confirms these observations, highlighting an impairment of cardiorespiratory parameters under the influence of β-adrenergic antagonists, along with reduced exercise tolerance compared to subjects not receiving treatment [37].

d) Adverse effects and contraindications

The use of β-adrenergic antagonists, particularly non-selective ones, is frequently associated with adverse reactions such as bradycardia, arterial hypotension, persistent fatigue, bronchospasm, and an increased risk of depressive symptoms [1, 3]. These effects can become limiting for professional athletes, affecting both physical performance capacity and their overall psychological state. Moreover, recent guidelines emphasize the risks associated with the use of these medications in certain comorbidities, such as bronchial asthma, diabetes mellitus (due to the risk of masking hypoglycemia symptoms), or slow-onset hypoglycemic episodes that are difficult to detect. These contraindications highlight the need for careful patient selection and close monitoring of treatment effects in the context of sports performance [38].

Table 1. Comparison between β2-adrenergic agonists and β-adrenergic antagonists in professional sports

Characteristics	β2-adrenergic agonists	β-adrenergic antagonists
Main mechanism	Agonistic effect on β2 receptors → sympathetic stimulation	Antagonistic effect on β1/β2 receptors → sympathetic inhibition
Main effects	Bronchodilation, stimulation of protein metabolism, mobilization of energy	Decrease in heart rate, tremor reduction, anxiolytic effect
Targeted sports	Endurance sports, strength competitions, bodybuilding	Precision sports: shooting sports, cue sports, golf
Possible benefits	Increase in muscle mass, delayed onset of fatigue	Improvement of fine motor control
Risks/adverse effects	Tachycardia, cardiotoxicity, hypokalemia	Bradycardia, fatigue, bronchospasm, depression
Anti-doping status (WADA)	Systemic forms prohibited (except for metered-dose inhalers)	Prohibited in certain precision sports
Examples	Clenbuterol, salbutamol, formoterol	Propranolol, metoprolol, bisoprolol

Note: *WADA – World Anti-Doping Agency*

Discussion

The analysis of specialized literature highlights that, while β2-adrenergic agonists show positive effects in animal experiments and occasionally in humans, the evidence regarding performance improvement in trained athletes remains limited [39]. These substances mimic the action of catecholamines on adrenergic receptors, being frequently used in the treatment of asthma and other respiratory diseases [40], but also with the aim of increasing muscle mass and physical performance.

According to the 2025 World Anti-Doping Code, the use of β2-adrenergic agonists is prohibited, except for certain inhaled doses of salbutamol, salmeterol, formoterol, and vilanterol, accompanied by a Therapeutic Use Exemption.

β2-adrenergic agonists act by:

- ***Enhancing physical performance*** by increasing heart rate, contractility, and inducing bronchial dilation [41].
- ***Stimulating protein synthesis***, leading to muscle growth and improved recovery [9].
- ***Accelerating energy metabolism*** by promoting fat and glucose mobilization.
- ***Influencing cognitive functions and mood*** by acting on central β-adrenoreceptors.

The anabolic effect of β2-agonists is supported by the enhancement of protein synthesis, the redistribution of body composition ("repartitioning effect"), and the influence on genes involved in myogenesis [5-8, 10, 11].

At the mitochondrial level, β2-agonists can stimulate the expression of PGC-1α, which is involved in mitochondrial biogenesis [13-16]. An increase in mitochondrial and muscle protein synthesis has been observed following 7 days of administration [12], but validation of these results in athletes requires further investigation, as currently available evidence comes predominantly from animal models.

At the metabolic level, β2-agonists increase the release of fatty acids through activation of hormone-sensitive lipase (HSL), stimulate glycogenolysis, and contribute to sustaining intense physical effort [17, 21]. Clenbuterol use is also associated with severe adverse effects (tachycardia, hypokalemia, chest pain, myocardial injury) [18, 30, 32].

Regarding fatigue, β2-agonists can stimulate the release of neurotransmitters (dopamine, noradrenaline), reduce pain perception, and improve muscle blood flow [22]. Although these effects may enhance sustained physical effort, the benefits in humans vary.

Muscle contractility is influenced by β2-agonists in slow-twitch (type I) fibers by improving relaxation and Ca^{2+} handling, but these effects are not sustained at physiological concentrations [26-29]. In cases of chronic administration, the increased level of cAMP stimulates adenosine production, which may antagonize the positive effects [25, 27].

In conclusion, the use of β2-adrenergic agonists may provide metabolic and muscular benefits but involves con-

siderable systemic risks, especially at high doses or with prolonged administration. Evidence for their effectiveness in sports remains inconsistent, requiring further research alongside compliance with anti-doping regulations.

β-adrenergic antagonists offer a clear pharmacological contrast, acting as antagonists of β1 and/or β2 receptors. In precision sports, their benefits lie in reducing physiological tremor, controlling anxiety, and inducing a state of calm necessary under competitive pressure. Their effectiveness is well documented, with performance improvements of up to 13% in sports such as competitive shooting [35]. However, these effects can vary significantly depending on the type of sport discipline, dose, and the selectivity of the β-adrenergic antagonists used [36].

On the other hand, in endurance sports, β-adrenergic antagonists can have an ergolytic effect. By reducing heart rate and cardiac output, they limit the capacity for sustained effort and increase the sensation of fatigue [37]. These effects are relevant in activities such as running and cycling, where cardiorespiratory efficiency is essential.

There are several adverse effects: bradycardia, hypotension, chronic fatigue, bronchospasm (especially with non-selective beta-blockers), and risk of depression. β-adrenergic antagonists can also mask symptoms of hypoglycemia, which poses an additional risk for athletes with diabetes, as they delay the recognition of a dangerous drop in blood glucose levels [38]. Contraindications include severe asthma, atrioventricular block, and certain forms of heart failure [1, 3].

Therefore, the inclusion of β-adrenergic antagonists in the analysis of sports performance highlights a complex reality: although they can offer clear advantages in precision sports, their use is limited by significant side effects and anti-doping regulations. An individualized assessment based on the type of activity, the athlete's physiological profile, and medical context is essential for an ethical and safe decision regarding the use of these pharmaceutical substances.

Conclusions

This study provides an integrative perspective on the pharmacological and physiological implications of β2-adrenergic agonists and β-adrenergic antagonists in sports performance. By synthesizing current scientific evidence and regulatory guidelines, it underscores the importance of a nuanced, discipline-specific approach to these substances. The added value lies in clarifying their differential impact depending on the sport type, offering a scientifically grounded basis for informed decisions in both clinical and anti-doping contexts.

Note: Parts of this review have been adapted and extended from previously published work (Pogonea I. et al., Farmacist.ro, 2025), with additional analysis regarding β-adrenergic antagonists [42].

Competing interests

None declared.

Authors' contributions

IP – conceptualization, designed the study, critically revised the manuscript. TC – conceptualization, writing original draft. TT – designed the study, critically revised the manuscript. AJ – validation, project administration. SS – supervision, data curation. AT – data collection and analysis. VC – data analysis and interpretation. All the authors approved the final version of the manuscript.

Ethics approval

Not needed for this study.

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Provenance and peer review

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REVIEW ARTICLE

Digital planning in orthodontics. Applicability of the Kau, Pan, Gallerano index in contemporary orthodontics

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ABSTRACT

Introduction. Digital planning in modern orthodontics is crucial in contemporary era, as it allows dentists to expand the limits of patients' diagnosis and clinical treatment. Using tools such as 3D scanners, cone beam computed tomography with various software gives clinicians the opportunity to achieve more precise diagnoses and accurate predictions of treatments. In this way, digital imaging offers a precise localization of impacted canines which allows for the integration and applications of the Kau, Pan, Gallerano index.

Material and methods. To assess the applicability of the Kau, Pan, Gallerano index and digital planning in orthodontics, this review evaluates key studies that explore various aspects of the topic. Scientific databases such as Cochrane Library, Medline, Scopus, Medicus, NCBI, PubMed, and Google Scholar were used to find the necessary articles. Research methods like analysis, synthesis, systematization and description were applied.

Results. After analyzing the available data, this review presents the benefits and opportunities of digital orthodontics. The findings demonstrate that the Kau, Pan, Gallerano index offers high diagnostic accuracy by effectively categorizing various types of impactions. Moreover, the analysis reveals a strong correlation between the Kau, Pan, Gallerano index scores and successful treatment outcomes, showing that the index with digital technologies improves diagnostic accuracy and treatment planning.

Conclusions. The reviewed literature collectively indicates that this index is a valuable tool in contemporary digital orthodontics. Its interdisciplinary connection with digital planning which provides strong predictive value, and its comprehensive assessment approach make it an important component in modern orthodontic practice. Future research should continue to explore the advantages and disadvantages of modern technologies used in orthodontic diagnosis and treatment planning, thus validating their efficiency and their applicability across diverse complex orthodontic cases.

Keywords: digital orthodontics, the Kau, Pan, Gallerano index, inclusion, impacted canines, digital planning, 3D scanner, cone bean computed tomography, predictive value.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

While the KPG index is a validated 3D classification tool for assessing impacted maxillary canines, its integration with digital orthodontic technologies – such as CBCT, intraoral scanning, and advanced treatment planning software – has not been comprehensively evaluated in the literature. Specifically, the extent to which digital workflows enhance the index's diagnostic precision, predictive value, and clinical utility remains underexplored. Moreover, comparative data on its superiority over other orthodontic indices in digital environments is limited or absent.

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The research hypothesis

The integration of the Kau, Pan, Gallerano index with advanced digital orthodontic tools (e.g., CBCT imaging, 3D visualization platforms, intraoral scanners) significantly enhances diagnostic accuracy, facilitates more efficient and individualized treatment planning, and improves clinical outcomes in the management of impacted canines.

The novelty added by the manuscript to the already published scientific literature

This manuscript introduces a novel perspective by systematically evaluating the clinical applicability of the KPG index in the context of modern digital orthodontics. It establishes the index's superiority over traditional assessment tools by demonstrating improved accuracy in localization, classification, and treatment planning of impacted maxillary canines. This synthesis advances the scientific literature by validating the clinical efficacy of a digitally integrated KPG index as a next-generation tool in contemporary orthodontics.

Introduction

The field of orthodontics has undergone significant transformations with the advent of digital technology. Digital dentistry, encompassing a wide range of tools such as digital imaging, 3D printing, and computer-aided design/computer-aided manufacturing (CAD/CAM) systems, has revolutionized diagnostic procedures, treatment planning, and patient outcomes [1, 2]. The integration of these digital advancements into orthodontic practice has not only improved the precision and efficiency of treatments but also enhanced patient experiences through more accurate and less invasive methods.

One of the significant challenges in orthodontics is the diagnosis and management of impacted canines [3]. Impacted canines occur in approximately 1-3% of the population, with maxillary canines being more frequently affected than mandibular canines. Accurate diagnosis is crucial as these impactions can lead to complications such as root resorption of adjacent teeth, cyst formation, and aesthetic concerns. Traditional 2D diagnostic tools, such as panoramic radiographs, often fall short in providing precise localization and orientation of impacted canines, leading to potential misdiagnosis or treatment delays. Studies have shown that panoramic radiographs can misinterpret the position of impacted canines in up to 50% of cases, emphasizing the need for more reliable diagnostic tools.

Cone Beam Computed Tomography (CBCT) has emerged as a valuable tool in this context, providing three-dimensional imaging that allows for precise localization and assessment of impacted canines. Alongside CBCT, another diagnostic tool gaining prominence is the KPG index. The KPG index is designed to assess and predict the outcomes of orthodontic treatments, offering a comprehensive approach that combines both aesthetic and functional parameters. With its robust diagnostic framework, it assists clinicians in predicting the complexity of cases involving impacted canines, facilitating more targeted and effective interventions. When these tools are combined, the diagnostic accuracy and treatment planning for impacted canines are further enhanced. As orthodontics increasingly embraces digital technology, the KPG index stands out for its potential to integrate seamlessly with these tools, thereby improving its diagnostic and predictive capabilities [4].

This review aims to evaluate the current applicability and effectiveness of the KPG index in contemporary orthodontic practice, with a particular focus on its integration with digital dentistry. By synthesizing findings from several key studies [5-7], this review will provide a comprehensive overview of the KPG index's utility, validation, and comparative advantages, as well as its clinical applications. Additionally, the review will explore the novel contributions of digital dentistry to the KPG index, highlighting the potential for advanced digital tools to augment the index's functionality and improve overall orthodontic outcomes.

Material and methods

To assess the applicability of the KPG index, this review evaluates studies that explore various aspects of the index. The studies were selected based on their relevance, methodology, and contributions to the understanding of the KPG index in orthodontics. Peer-reviewed articles were selected from databases including Wiley Online Library, PubMed, Scopus, and Thieme Connect. The chosen articles focus on the validation, comparative analysis, and clinical application of the KPG index. Comprehensive searches were conducted using keywords such as "KPG index", "orthodontic diagnostics", "digital dentistry", "CBCT", and "impacted canines". Boolean operators and filters were applied to refine the search results and ensure the inclusion of relevant studies. Relevant data such as study design, sample size, statistical methods, and key findings were extracted from different articles. This information

was synthesized to provide a comprehensive overview of the current state of research on the KPG index. Studies were included if they directly assessed the KPG index or compared it with other orthodontic indices, provided empirical data on its effectiveness, and were published in reputable journals. The fundamental part is to understand how this index influences and facilitates the undertaken treatments and the planning.

Results and discussion

The synthesis of these studies reveals several key findings. The KPG index demonstrates high diagnostic accuracy, effectively categorizing various types of malocclusions. For example, the studies found in orthodontic literature reported that the KPG index could accurately identify the severity of malocclusions in over 90% of cases [8]. This high level of accuracy is crucial for ensuring appropriate treatment planning and interventions. Besides, there is a strong correlation between KPG index scores and successful treatment outcomes. Studies have also shown that patients with higher KPG index scores tended to have better treatment outcomes, with a success rate of approximately 87%. This predictive capability makes the KPG index a valuable tool for orthodontists in planning and adjusting treatment strategies based on anticipated outcomes [9]. The KPG index offers a more comprehensive assessment compared to other indices, integrating both aesthetic and functional parameters. The comparative study published by Fox NA *et al.* found that the KPG index provided a more holistic evaluation of orthodontic cases compared to the PAR and ICON indices [10]. This comprehensive assessment ensures that both aesthetic and functional aspects of malocclusion are addressed in treatment planning. The KPG index enhances clinical decision-making, patient communication, and overall treatment efficiency. The case studies highlighted how the KPG index facilitated more accurate diagnoses and treatment plans. For instance, the index helped in predicting the complexity of cases involving impacted canines, leading to more effective interventions. Additionally, the clear and quantifiable treatment goals provided by the KPG index improved patient understanding and satisfaction. The integration of digital tools such as CBCT and intraoral scanners with the KPG index enhances its diagnostic and predictive capabilities. Digital models and treatment simulation software allow for precise manipulation and analysis of dental structures, leading to more accurate and efficient treatment planning. The use of CBCT provides detailed 3D imaging, improving the localization and assessment of impacted canines, which is a significant advantage over traditional 2D diagnostic methods [11]. The application of the KPG index in clinical settings has led to improved treatment outcomes. The studies reviewed reported high patient satisfaction rates and better overall treatment results. For example, patients whose treatments were guided by the KPG index showed a significant reduction in treatment time and an increase in treatment success rates [12]. This is attributed to the index's ability to provide a detailed and accurate assessment of malocclusions, facilitating more effective treatment planning.

Furthermore, digital orthodontics has proven to be instrumental in the practical application of the KPG index. The use of digital tools such as intraoral scanners, CBCT, and CAD/CAM systems has streamlined the diagnostic and treatment planning process, making it more precise and efficient. Digital models generated from intraoral scans provide accurate 3D representations of the dental arches, which can be easily manipulated and analyzed. This allows for better visualization and understanding of the malocclusion, leading to more effective treatment planning [11]. Treatment simulation software further enhances this process by allowing clinicians to visualize and predict treatment outcomes, facilitating better communication with patients. This integration of digital tools with the KPG index has led to more accurate diagnoses, improved treatment planning, and better overall patient outcomes. For instance, the detailed 3D imaging provided by CBCT has improved the localization and assessment of impacted canines, which is a significant advantage over traditional 2D diagnostic methods. The case studies reviewed highlighted the practical benefits of using the KPG index in conjunction with digital tools, demonstrating its effectiveness in enhancing diagnostic accuracy, treatment planning, and patient communication.

The KPG index represents a significant advancement in orthodontic diagnostics and treatment planning, particularly when integrated with digital dentistry tools. The robustness of the index in assessing both aesthetic and functional parameters, combined with its predictive capabilities, makes it a valuable asset in modern orthodontic practice. As digital technologies continue to evolve, the integration of platforms such as Diagnocat, BlueSkyPlan, and Dolphin further enhances the utility of the KPG index, enabling orthodontists to achieve more precise diagnoses, personalized treatment plans, and improved patient outcomes.

Visualization of impacted canines, especially using specialized software like BlueSkyPlan, Dolphin, and Diagnocat, offers significant advantages in orthodontics and dentistry. Here are some key benefits of visualization that are represented in the context of impacted canines:

- **Accuracy and detail**: programs such as BlueSkyPlan and Dolphin allow the creation of three-dimensional models of dental structures based on CBCT data. This provides a more precise understanding of the position and orientation of impacted canines, crucial for accurate treatment planning.
- **Treatment planning**: visualization in these programs enables orthodontists and dentists to conduct detailed treatment planning. They can virtually manipulate teeth, determine optimal locations for orthodontic traction, and develop personalized treatment plans for each patient.
- **Outcome prediction**: with visualization in Dolphin and other programs, virtual modeling and outcome

prediction can be performed and by analyzing it improves case complexity assessment and clearer goals. This helps patients and dentists better understand expected changes after treatment and discuss various intervention options.

- **Interactivity and education**: programs like Diagnocat offer interactive tools for education and patient interaction. They help visualize complex concepts and procedures, improving education and understanding among medical professionals and patients.
- **Enhanced patient engagement**: visualization allows patients to better understand their dental issues and treatment plans. This improves patient engagement in decision-making and confidence in upcoming treatments, so the KPG index as shown in involves higher satisfaction for patients and reduced treatment time.

These benefits make software visualization such as BlueSkyPlan, Dolphin, and Diagnocat essential tools for orthodontists and dentists, particularly when dealing with complex cases of impacted canines.

The Kau, Pan, Gallerano index serves as a comprehensive tool in orthodontics, aiding in the assessment of various malocclusions, including impacted canines. However, several considerations arise when applying the index to such cases, as noted by multiple authors in the field. Authors highlight the index's subjective nature in evaluation, which can lead to variability in scoring interpretations among practitioners. This subjectivity poses challenges in cases like impacted canines, where precise measurements are critical for treatment planning.

Moreover, the predictive value of the KPG index in determining outcomes for impacted canines may be limited, as emphasized in discussions by orthodontic researchers [12]. Factors such as root resorption, surgical complications, and patient compliance play significant roles but may not be fully captured by the index alone. Additionally, the complexity inherent in assessing severe impactions and variations in root positions adds further nuances to its application.

Furthermore, the dependency of the KPG index on radiographic data, such as CBCT scans, is crucial yet introduces potential issues related to image quality and positioning during imaging. These concerns are pertinent in cases requiring detailed anatomical assessments, such as impacted canines, where precise radiographic data is essential for accurate diagnosis and treatment planning.

In addressing these aspects, orthodontic literature underscores the need for clinicians to integrate the index's findings judiciously with clinical expertise and patient-specific considerations. This holistic approach ensures that the KPG index contributes effectively to treatment planning while accommodating the complexities and variations encountered in cases of impacted canines.

The integration of digital orthodontics and the KPG index not only enhances diagnostic precision and treatment planning but also accelerates treatment timelines and improves treatment outcomes. By leveraging advanced digital tools and comprehensive assessment frameworks, orthodontists can streamline various aspects of orthodontic care.

Conclusions

This review underscores the enhanced clinical applicability of the KPG index within a digitally integrated orthodontic framework. Our synthesis demonstrates that the index, when utilized alongside advanced imaging and planning technologies, provides a structured and reliable approach to the assessment and management of complex cases, particularly impacted canines. When combined with advanced imaging modalities such as cone-beam computed tomography (CBCT) and 3D visualization software, the KPG index enables precise three-dimensional localization of impacted maxillary canines, including accurate assessment of crown and root positions in relation to adjacent anatomical structures (e.g., lateral incisors, nasal floor, and cortical bone boundaries). The added value of this integration lies in its capacity to support precise diagnosis, informed decision-making, and optimized treatment efficiency, thereby reinforcing its role in modern evidence-based orthodontic practice.

Competing interests

None declared.

Authors' contributions

VT, AB, and IB conceptualized the study, designed the review structure, and conducted the comprehensive literature search and analysis. VT led the drafting of the manuscript and coordinated the integration of digital orthodontic applications with the KPG index. AB contributed to the synthesis of clinical evidence and critical revision of the manuscript. IB performed data extraction from selected studies and assisted in comparative evaluation of diagnostic indices. All authors contributed to the interpretation of findings, critically reviewed the manuscript for intellectual content, and approved the final version for publication.

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CASE STUDY

Refractive lens exchange in a patient with high myopia and myopic astigmatism: a clinical case report

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ABSTRACT

Introduction. Refractive lens exchange is increasingly utilized for visual rehabilitation in patients with high myopia unsuitable for corneal refractive procedures. While effective, refractive lens exchange with intraocular lens implantation significantly improves visual acuity in young patients with high myopia and astigmatism, while maintaining a low risk of postoperative retinal complications, provided that thorough preoperative vitreoretinal assessment is conducted. However, it carries potential risks, notably retinal complications.

Case presentation. We present a case involving a 45-year-old female with high axial myopia and myopic astigmatism who underwent bilateral refractive lens exchange with the implantation of monofocal intraocular lenses. Comprehensive preoperative assessments included optical biometry, tonometry, fundus examination, and visual acuity measurements. Postoperative recovery was uneventful in the right eye. However, the patient developed a retinal detachment in the left eye approximately one year post-surgery. This complication was successfully managed with pars plana vitrectomy, endolaser photocoagulation, and silicone oil tamponade, resulting in an improvement of visual acuity to 0.3.

Conclusions. Refractive lens exchange can be an effective intervention for patients with high axial myopia but carries a risk of retinal detachment. Thorough preoperative evaluation, meticulous surgical technique, and patient counseling are essential. Prompt detection and management of complications like retinal detachment are crucial for preserving visual outcomes.

Keywords: refractive lens exchange, myopia, astigmatism, retinal detachment, vitrectomy.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Although refractive lens exchange is increasingly used in young patients with high myopia and astigmatism, the long-term safety and optimal patient selection criteria remain unclear, particularly regarding the risk of retinal detachment.

The research hypothesis

Refractive lens exchange with intraocular lens implantation significantly improves visual acuity in young patients with high myopia and astigmatism, while maintaining a low risk of postoperative retinal complications, provided that thorough preoperative vitreoretinal assessment is conducted.

The novelty added by manuscript to the already published scientific literature

This clinical case demonstrates that even in young myopic pa-

tients, refractive lens exchange can achieve excellent outcomes when retinal health is thoroughly evaluated preoperatively. It also underlines the importance of early detection and prompt management of retinal detachment, which, although rare, remains a significant risk.

Introduction

Refractive Lens Exchange (RLE) is becoming an increasingly popular procedure, especially for individuals who are not suitable candidates for keratorefractive surgeries. It is particularly considered for patients with high degrees of myopia or hyperopia. However, the widespread availability of phacoemulsification devices and the growing number of surgeons trained in this technique have led to a reduction in the minimum age at which RLE is performed [1].

Case presentation

A 45-year-old female patient, B., presented to the *Eye Microsurgery* Ophthalmologic center in Moldova, with complaints of decreased visual acuity in the left eye (OS) for the past 9–10 days. Ophthalmologic history revealed that the patient had undergone clear lens phacoemulsification with monofocal IOL (Alcon Acrysof IQ) implantation in both eyes one year earlier. The ophthalmological examination findings are presented below in Table 1.

Table 1. Physical examination and instrumental data

	OD	OS
UDVA	0.35	0.01
BDVA	0.75	0.01
Autorefraction	-2.00 D sph / -0.75 D cyl ax 5	-1.50 D sph / -1.25 D cyl ax 43°
Tonometry	17 mmHg	18 mmHg
Biometry	29.97 mm	28.3 mm
Biomicroscopy	Normal conjunctiva, white pearly sclera, clear smooth cornea, medium-depth anterior chamber, round reactive pupils, normal iris stroma, centered IOLs.	Normal conjunctiva, white pearly sclera, clear smooth cornea, medium-depth anterior chamber, round reactive pupils, normal iris stroma, centered IOLs.
Fundus Examination	Flat, well-defined optic disc, mild peripapillary atrophy, C/D = 0.4, diminished macular reflex, moderately narrowed arteries and dilated veins, mobile vitreous opacities.	Superior sectorial retinal detachment, macula off (Figure 1)

Note: *OD - Right eye; OS - Left eye; UDVA - Uncorrected distance visual acuity; BDVA - Best corrected distance visual acuity; IOL - Intraocular lens; C/D - Cup/disc ratio.*

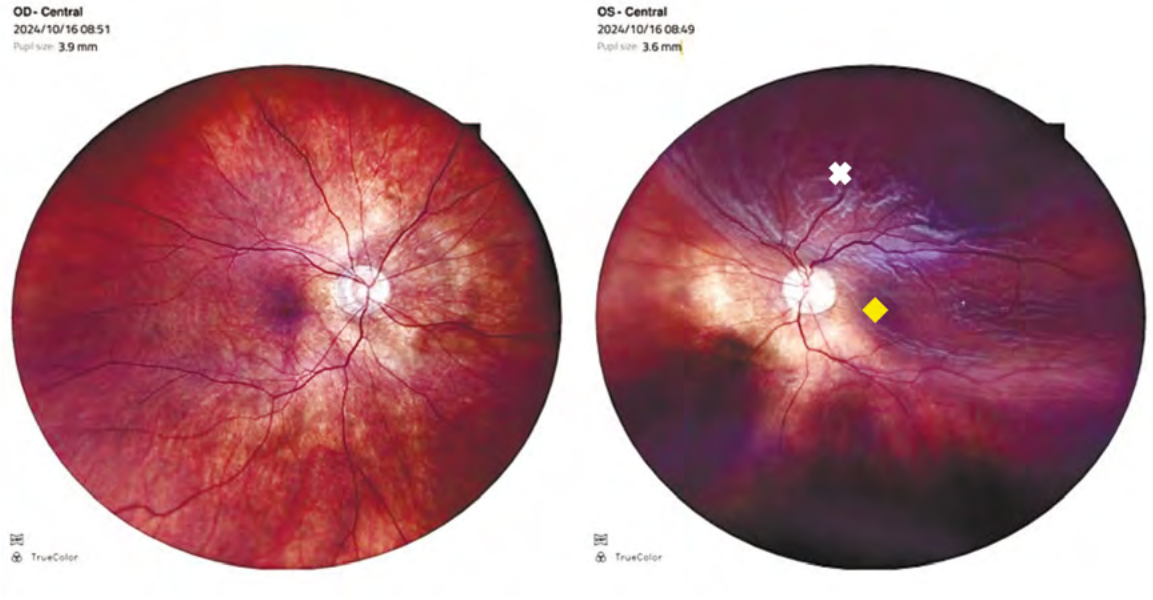

Fig. 1 Fundus photo at initial visit
OD – Retina is attached, peripapillary atrophy, macular reflex is attenuated.
OS – Superior retinal detachment (white cross), macula off (yellow rhombus)

An ultrasound examination of the left eye was performed shown on Figures 2 & 3.

The patient was diagnosed with OS: Rhegmatogenous retinal detachment, macula off; OU: Pseudophakia (postoperative high myopia), status post RLE and vitreous body degeneration.

Vitrectomy with endolaser photocoagulation and silicone oil tamponade was performed in the left eye (OS).

Postoperative follow-up (after 2 weeks) revealed OS BDVA of 0.3 and fundus examination (OS) with showed the retina reattached (Figure 4).

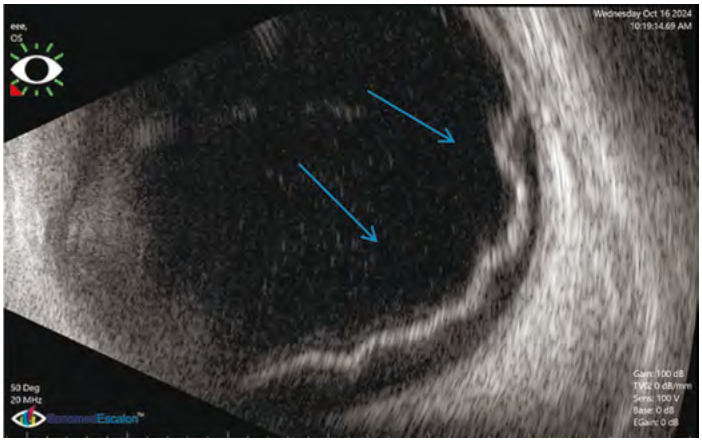

Fig. 2 OS B-scan
OS Retinal detachment (blue arrows).

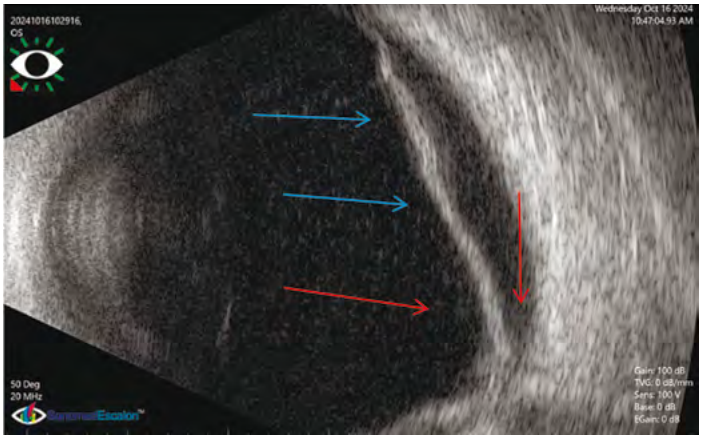

Fig. 3 OS B-scan
OS Retinal detachment (blue arrows), optic disc (red arrows).

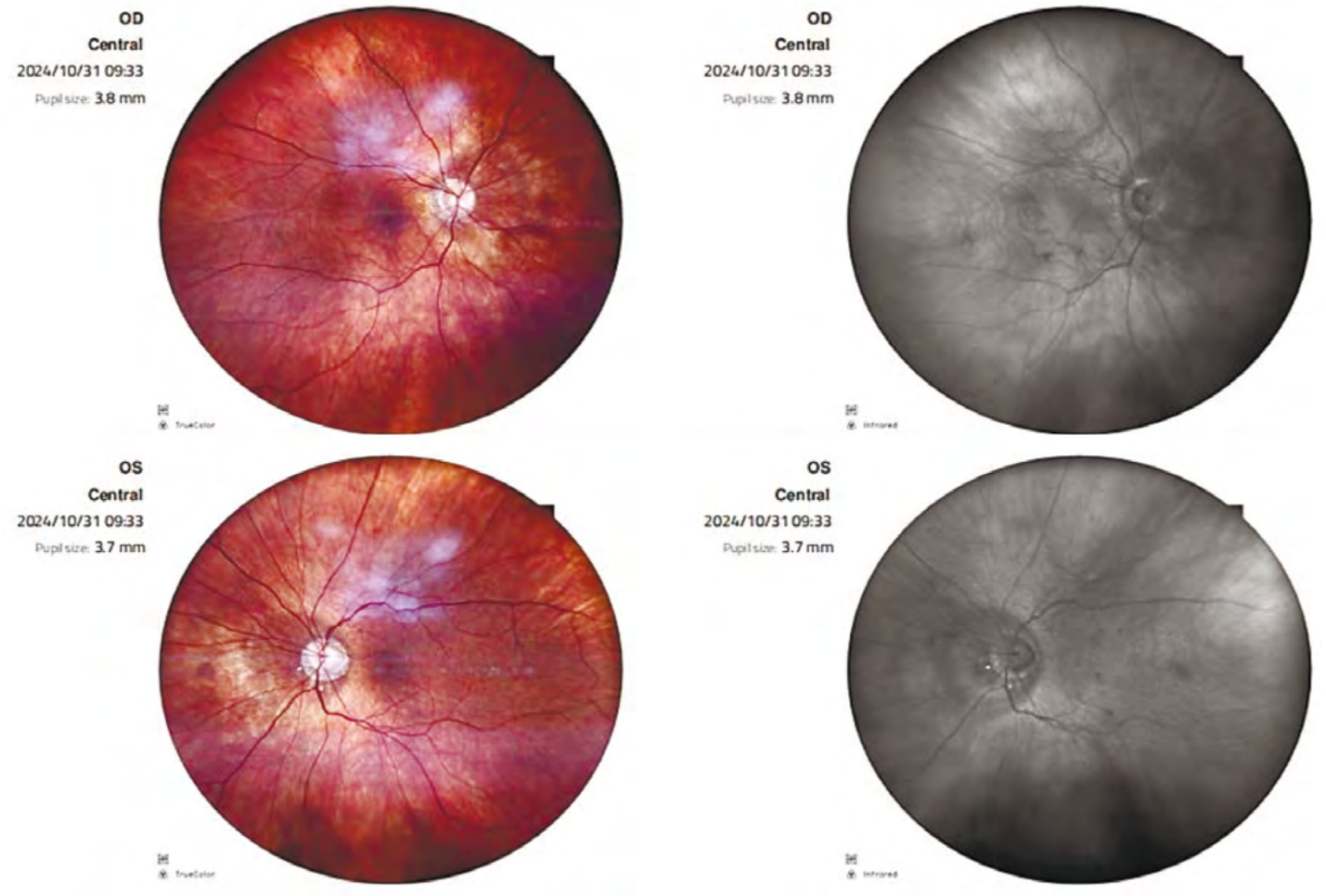

Fig. 4 Fundus photo OS Retina attached under silicone oil

Discussion

One of the main considerations when evaluating refractive lens exchange (RLE) is the timing of posterior vitreous detachment (PVD). Patients with high myopia tend to develop PVD at a younger age compared to those without significant myopia.

Retinal detachment (RD) after RLE in highly myopic patients has been reported with an overall prevalence between 1.5% and 8.1%, depending on follow-up duration in various studies [1]. For comparison, the annual incidence of RD in the general population with high myopia is around 0.102%.

RD after RLE may be linked both to a predisposing factor (a higher prevalence of peripheral retinal lesions in highly myopic eyes) and a triggering factor (vitreous changes induced by the surgical procedure). Younger patients, especially those with an axial length greater than 26 mm (typically associated with myopia > -6.00 D), are at increased risk. The group with the highest risk includes patients under the age of 50 with an axial length exceeding 28 mm [1].

According to several sources, myopic eyes have a threefold higher risk of RD compared to the general cataract population [2].

Retinal complications – particularly in highly myopic eyes after refractive surgeries like RLE are primarily attributed to two main causes:

- a higher incidence of predisposing peripheral retinal lesions in myopic eyes, and
- the hypothesis that refractive surgery may induce certain iatrogenic factors increasing the incidence of such pathology [1, 3].

To prevent retinal detachment, careful preoperative fundus examination with scleral depression is necessary to assess the vitreous body. During surgery, minimal disturbance of the intraocular environment is essential. Some authors recommend bimanual microincision phacoemulsification (BMMI) or small-incision lens extraction in myopic eyes [1, 4].

During lens implantation, a transient drop in intraocular pressure (decompression effect) can lead to changes in the vitreous, particularly in eyes with existing vitreous degeneration. Protein alterations identified in pseudophakic eyes often coincide with structural changes in the vitreous body, which may contribute to the development of retinal complications postoperatively.

Preoperative determination of retinal detachment risk – especially in myopic eyes with axial length over 26.0 mm and spherical equivalent greater than -6.00 D – is of high importance [1, 5]. In young myopic patients, clear lens extraction may trigger vitreous changes and retinal traction not commonly seen in older patients undergoing age-related cataract surgery [1].

Conclusions

RLE is an intraocular surgical procedure that must be performed with precision and high accuracy. The indication for this intervention is the presence of a significant refractive error in the absence of cataract, and it requires a risk-benefit approach, taking into account the patient's age, refractive condition, and preoperative status. It is important to inform patients about the possible complications.

Competing interests

None declared.

Authors' contributions

SP conducted the preoperative and postoperative assessment of the patient and collected the clinical data and performed the literature review. IT structured the article, drafted the conclusions. AP reviewed and revised the manuscript and recommended relevant literature to support the conclusions. All authors reviewed the manuscript critically and approved the final version.

Informed consent for publication

Obtained.

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Provenance and peer review

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4.	taking responsibility and being accountable for all aspects of the work	

All persons who have made substantial contributions to the work (technical editing, writing assistance, general administrative support, financial and material support) **but do not meet all four criteria for authorship** are listed in *Acknowledgments* section and have given us their written permission to be named.

☐ Yes
☐ No
☐ We have not received substantial contributions from non-authors.

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Please thick the box if the following statements are correct to the best of your knowledge:

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Reported research was approved by institutional/national ethics committee:

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☐ No
☐ Not applicable

If yes, please state the name of the approving ethics committee, the protocol number and the date of evaluation: _____

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☐ Yes
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MJHS Moldovan Journal of Health Sciences
REVISTA DE ȘTIINȚE ALE SĂNĂTĂȚII DIN MOLDOVA

[Revised January, 2025]

GUIDE FOR AUTHORS

Moldovan Journal of Health Sciences is an open access, double blind peer reviewed medical journal, published quarterly. The journal accepts for publication **original research papers**, **review articles**, **case reports**, **letters to the editor**, **In memoriam** and **book reviews**.

The *Moldovan Journal of Health Sciences* follows the ***Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals*** set out by the **International Committee of Medical Journal Editors (ICMJE)** and publication ethics practices suggested by the **Committee on Publication Ethics (COPE)**.

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- The manuscripts should be written in **English**;
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ABSTRACT AND KEYWORDS	> Structured abstract > Keywords > Clinical trial registration information
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The Introduction section should: provide a context or background for the study that would allow readers outside the field to understand the purpose and significance of the study; define the problem addressed and explain why it is important; include a brief review of recent literature in the field; mention any relevant controversies or disagreements existing in the field; formulate research hypothesis and present the main and secondary assessed outcomes; conclude with the research' propose and a short comment whether the purpose has been achieved. The Introduction should not contain either results or conclusions.

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The *Materials and methods* section should present in sufficient details all carried out procedures. Here should be described protocols and supporting information on the used methods. It will include study design, subjects' recruitment procedure, clear description of all interventions and comparisons and applied statistics (*Selection and Description of Participants, Technical Information, Statistics*). The Methods section should be sufficiently detailed such that others with access to the data would be able to reproduce the results. For studies on humans or animals a statement about ethical approval and informed consent of study subjects should be include. Please specify date and number of Ethics Committee (EC) decision, chair of the EC as well as institution within EC is organized.

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Authors must present results logically using text, tables, and figures, giving the main or most important findings first. Results should be explained (not justified or compared in this section) and include fundamental statements related to hypothesis behind the study.

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The data should be interpreted concisely without repeating materials already presented in the *Results* section. Describe the impact, relevance and significance of the obtained results for the field. The results are compared with those from previous publications and draw potential future research directions. Discussions should include important interpretations of the findings andresults compared with previous studies. In addition, study limitations and potential bias should be mentioned.

> Conclusions

This section should conclude laconically entire study, and highlight the added-value brought on the studied issue. The conclusions should not provide new information or double (repeat) those presented in the *Results* section.

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In-text reference citations should be numbered consecutively, identified by Arabic numerals in square brackets [ ]. References should be listed at the end of the manuscript and numbered in the order in which they are first mentioned in the text. Every reference cited in the text is also present in the reference list (and vice versa). Journals titles should be abbreviated according to the Index Medicus.

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Examples of references

Journal article

Belîi A, Cobâleţchi S, Casian V, Belîi N, Severin G, Chesov I, Bubulici E. Les aspects pharmacoéconomiques dans la gestion de la douleur périopératoire [Pharmaco-economic aspects of perioperative pain management]. Ann Fr Anesth Reanim. 2012;31(1):60-6. French. doi: 10.1016/j.annfar.2011.09.008.

Book

Razin MP, Minaev SV, Turabov IA. Detskaia khirurgiia [Pediatric surgery]. 2nd ed. Moscow: Geotar-Media; 2020. 696 p. Russian.

Chapter in a book

Steiber AL, Chazot C, Kopple JD. Vitamin and trace element needs in chronic kidney disease. In: Burrowes J, Kovesdy C, Byham-Gray L, editors. Nutrition in kidney disease. 3rd ed. Cham: Humana Press; 2020. p. 607-623.

Conference paper

Ojovan V. Medical rehabilitation of children with type 1 diabetes: medical bioethical and psychosocial aspects. In: MedEspera: 9th International Medical Congress for Students and Young Doctors, 12-14 May 2022, Chisinau, Republic of Moldova: Abstract book. Chișinău; 2022. p. 77.

Website reference

World Health Organization (WHO). Therapeutics for Ebola virus disease [Internet]. Geneva: WHO; 2022 [cited 2022 Sep 5]. Available from: https://www.who.int/publications/i/item/9789240055742

TABLES AND FIGURES

Tables should be numbered consecutively with Arabic numerals, and be cited in text. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Tables must be submited as editable text and not as images. Each table should be completely informative in itself, and the data presented in it do not duplicate the results described elsewhere in the article.

The label "Table 1" and a short descriptive title should be provided above the table. Legends, notes, and any abbreviations used in the table should be explained below the table in a footnote. Applied statistical tests and the type of presented data should be also mentioned. Please follow the example:

Table 1. Intra-anesthetic and immediately post-extubation adverse events

	Experimental Cohort (n=100)	Control Cohort (n=100)	p
Dysrhythmia	6.0%	30%	0.49
Hemodynamic instability	7.0%	1.0%	0.034
Prolonged awakening*	11.0%	4.0%	0.19
PONV post-intubation	8.0%	27.0%	0.007
Strong pain on awakening	17.0%	19.0%	1.0

Note: *Unusually slow awaking, after that cerebral concentration of the anesthetic reach the under hypnotic level.
Used statistical analysis: Fisher's exact test.

Figures (photographs or radiographs, drawings, graphs, bar charts, flow charts, and pathways) should be submitted in a suitable format for print publication. Figures should be either professionally drawn and photographed, or submitted as photographic-quality digital prints. Figures' quality should assure the visibility of details. The following file formats are accepted: ".jpeg", ".tiff", ".eps" (preferred format for diagrams), ".ppt", ".pptx" (figures should be of the size of a single slide), with a resolution of at least 300 dpi.
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4. agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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KARDATUXAN 20 mg: *Adulți:* Prevenirea accidentului vascular cerebral și a emboliei sistemice la pacienții adulți cu fibrilație atrială non-valvulară cu unul sau mai mulți factori de risc, cum sunt insuficiența cardiacă congestivă, hipertensiunea arterială, vârsta ≥75 ani, diabetul zaharat, accidentul vascular cerebral sau atacul ischemic tranzitoriu în antecedente. Tratamentul trombozei venoase profunde (TVP) și al emboliei pulmonare (EP) și prevenirea recurenței TVP și a EP la adulți. *Copii și adolescenți:* Tratamentul tromboembolismului venos (TEV) și prevenirea recurenței TEV la copii și adolescenți cu vârsta mai mică de 18 ani și cu greutatea peste 50 kg după cel puțin 5 zile de la tratamentul anticoagulant parenteral inițial. **Contraindicații:** KARDATUXAN 2,5 mg, 10 mg, 15 mg, 20 mg: Hipersensibilitate la substanța activă sau la oricare dintre excipienții; Hemoragie activă, semnificativă din punct de vedere clinic; Leziune sau afecțiune considerată a prezenta un risc semnificativ de sângerare majoră. Aceasta poate include ulcerația gastrointestinală curentă sau recentă, prezența neoplasmelor maligne cu risc crescut de sângerare, leziune recentă la nivelul creierului sau măduvei spinării, intervenție chirurgicală recentă cerebrală, spinală sau oftalmică, hemoragie intracraniană recentă, varice esofagiene cunoscute sau suspectate, malformații arterio-venoase, anevrism vascular sau anormalități vasculare intraspinale sau intracerebrale majore;Tratament concomitent cu orice alte anticoagulante, de exemplu, heparină nefracționată, heparină cu greutate moleculară mică (enoxaparina, dalteparina, etc.), derivate de heparină (fondaparina, etc.), anticoagulante orale (warfarina, dabigatran etexilat, apixaban etc.) exceptând situațiile specifice de schimbare a tratamentului anticoagulant sau când heparina nefracționată este administrată la dozele necesare pentru a menține deschis un cateter venos central sau arterial; Tratament concomitent al SCA cu terapie antiplachetară la pacienții cu accident vascular cerebral anterior sau accident ischemic tranzitoriu (AIT); Tratament concomitent al BAC/BAP cu AAS la pacienții cu accident vascular hemoragic sau lacunar în antecedente sau cu orice accident vascular cerebral în interval de o lună; Afecțiune hepatică asociată cu coagulopatie și risc hemoragic relevant din punct de vedere clinic, incluzând pacienții cu ciroză clasele B și C ale sistemului de clasificare Child Pugh; Sarcina și alăptarea.**Atenționări și precauții speciale pentru utilizare:** KARDATUXAN 2,5 mg: La pacienții cu SCA, eficacitatea și siguranța rivaroxaban 2,5 mg de două ori pe zi au fost investigate în administrare concomitentă cu medicamentele antiplachetare AAS în monoterapie sau AAS plus clopidogrel/ticlopidină.La pacienții cu BAC/BAP cu risc crescut de evenimente ischemice, eficacitatea și siguranța rivaroxaban 2,5 mg de două ori pe zi au fost investigate în asociere cu AAS. La pacienți după procedura recentă de revascularizare a membrului inferior din cauza BAP simptomatice, eficacitatea și siguranța rivaroxaban 2,5 mg de două ori pe zi au fost investigate în asociere cu medicamentul antiplachetar AAS în monoterapie sau AAS plus clopidogrel pe termen scurt. Dacă este necesar, tratamentul combinat antiplachetar cu clopidogrel trebuie să fie de scurtă durată; tratamentul combinat antiplachetar pe termen lung trebuie evitat. KARDATUXAN 10 mg, 15 mg, 20 mg: Se recomandă supravegherea din punct de vedere clinic pe toată durata perioadei de tratament. Risc hemoragic: pacienții trebuie monitorizați cu atenție pentru semnele de sângerare. Se recomandă să fie utilizat cu precauție în condiții cu risc crescut de hemoragie. Administrarea Kardatuxan trebuie întreruptă dacă apare hemoragie severă. Alți factori de risc hemoragic: rivaroxaban nu este recomandat la pacienți cu risc crescut de sângerare. Pacienți cu proteze valvulare:Rivaroxaban nu trebuie utilizat pentru tromboprofilaxie la pacienții care au trecut recent printr-o înlocuire percutanată de valvă aortică (TAVI).Pacienți cu sindrom antifosfolipidic: Rivaroxaban nu sunt recomandate la pacienții cu antecedente de tromboză, care sunt diagnosticați cu sindrom antifosfolipidic.Pacienții cu EP instabilă hemodinamic sau pacienții care necesită tromboliză sau embolectomie pulmonară: Kardatuxan nu este recomandat ca alternativă la heparina nefracționată la pacienții cu embolie pulmonară care sunt instabili hemodinamic sau care pot beneficia de tromboliză sau embolectomie pulmonară, deoarece siguranța și eficacitatea rivaroxaban nu au fost stabilite în aceste situații clinice. KARDATUXAN 10 mg: Recomandări privind dozele înainte și după proceduri invazive și intervenții chirurgicale, altele decât intervențiile chirurgicale elective pentru substituția șoldului sau a genunchiului: Dacă este necesară o procedură invazivă sau o intervenție chirurgicală, trebuie oprită administrarea Kardatuxan 10 mg cu cel puțin 24 ore înainte de intervenție, dacă este posibil, precum și în funcție de opinia clinică a medicului. Dacă procedura nu poate fi amânată, trebuie evaluat riscul de sângerare comparativ cu gradul de urgență al intervenției. KARDATUXAN 15 mg, 20 mg: Recomandări privind dozele înainte și după proceduri invazive și intervenții chirurgicale: Dacă este necesară o procedură invazivă sau o intervenție chirurgicală, trebuie oprită administrarea Kardatuxan 15 mg sau Kardatuxan 20 mg cu cel puțin 24 ore înainte de intervenție, dacă este posibil, precum și în funcție de opinia clinică a medicului. Dacă procedura nu poate fi amânată, trebuie evaluat riscul de sângerare comparativ cu gradul de urgență al intervenției. **Reacții adverse:** KARDATUXAN 2,5 mg, 10 mg, 15 mg, 20 mg: Reacțiile adverse raportate cel mai frecvent la pacienții la care se administrează rivaroxaban au fost hemoragiile. Hemoragiile raportate cel mai frecvent au fost epistaxisul (4,5%) și hemoragia de tract gastrointestinal (3,8%). Frecvente (≥1/100 și <1/10): Anemie (inclusiv parametri de laborator corespunzători); Amețeli, cefalee; Hemoragie oculară (incluziv hemoragie conjunctivală); Hipotensiune arterială, hematom; Epistaxis, hemoptizie; Sângerare gingivală, hemoragie la nivelul tractului gastrointestinal (incluziv hemoragie rectală), dureri gastrointestinale și abdominale, dispepsie, greață, constipație, diaree, vărsături; Creștere a valorilor serice ale transaminazelor; Prurit (incluziv cazuri mai puțin frecvente de prurit generalizat), erupție cutanată tranzitorie, echimoză, hemoragie cutanată și subcutanată; Dureri la nivelul extremităților; Hemoragie la nivelul tractului urogenital (incluziv hematurie și menoragie), insuficiență renală (incluziv creșterea creatininei serice, creșterea ureei serice); Febră, edem periferic, scădere a tonusului general și a energiei (incluziv fatigabilitate și astenie); Hemoragie după o procedură (incluziv anemie postoperatorie și hemoragie la nivelul plăgii), contuzie, secreții la nivelul plăgii. **Data și numărul autorizației de punere pe piață:** 2,5 mg - Nr.29430; 10 mg –Nr. 29431; 15 mg – Nr.29432; 20 mg – Nr.29433 din 30.11.2023. **Statutul legal:** cu prescripție medicală. **Data revizuirii textului:** Aprilie 2024. *Acest material publicitar este destinat persoanelor calificate să prescrie, să distribuie și/sau să elibereze medicamente. Pentru informații complete vă rugăm să consultați rezumatul caracteristicilor produsului. Informații detaliate privind acest medicament sunt disponibile pe site-ul Agenției Medicamentului și Dispozitivelor Medicale (AMDM) http://nomenclator.amdm.gov.md/*

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