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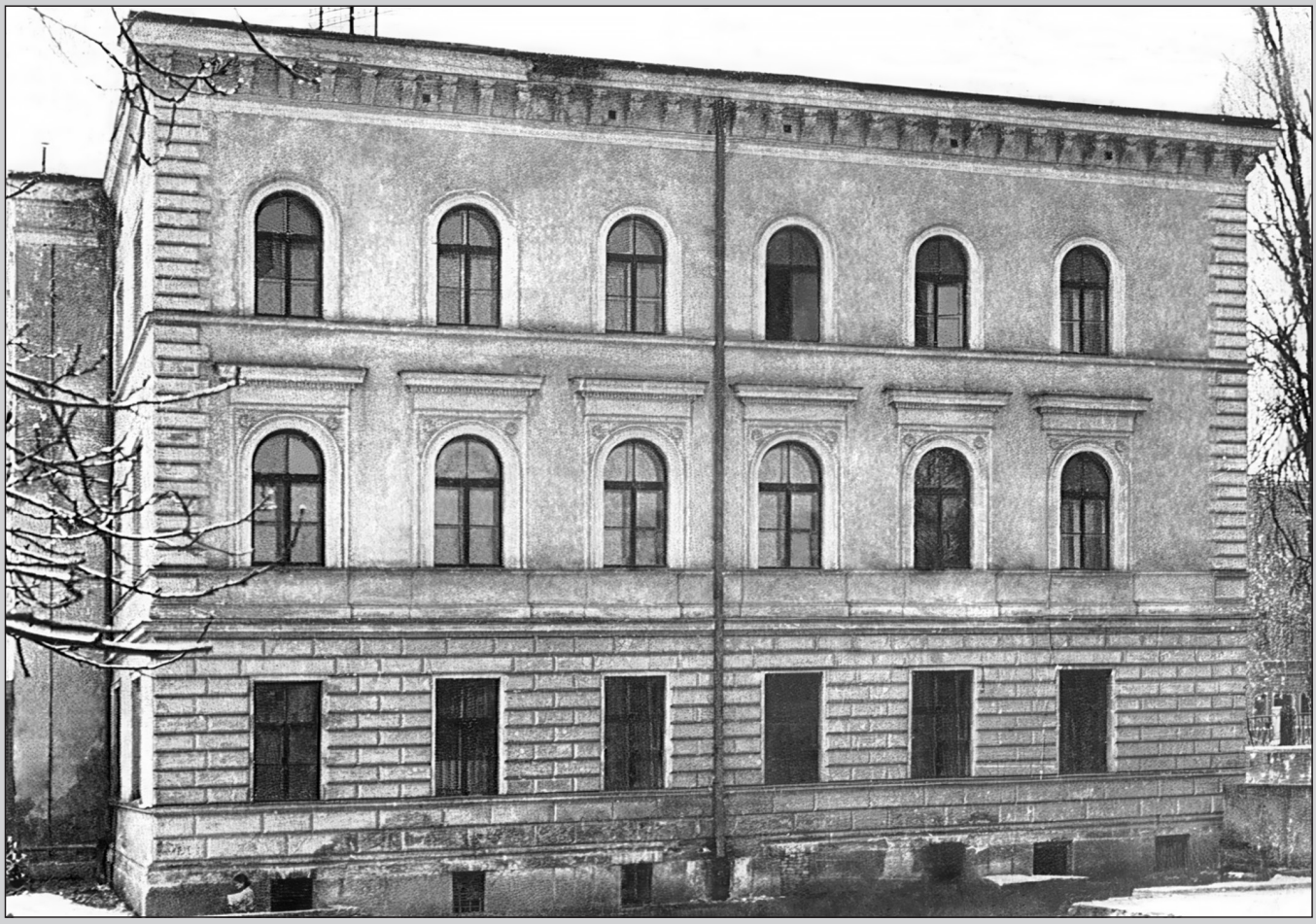

The building in which the Faculty of Medicine in Sarajevo started instruction in 1946.

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Evaluation of BI-RADS 3 Ultrasound Findings: the Frequency and Incidence of Malignant Lesions, and Tumor Size

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Abstract

Objective. The aim of the research was to determine the frequency of BI-RADS category 3 findings in ultrasound examinations in relation to the total number of patients, the frequency of malignant lesions, and their average size at the time of detection in BI-RADS 3 ultrasound findings. **Patients and Methods.** A cross-sectional study was performed on 335 patients (aged 40-75 years) classified in BI-RADS category 3, at the Tuzla Breast Center, University Clinical Center, in the period from March 2017 to November 2020. A total of 13,760 ultrasound examinations were performed, using a Toshiba Xario 100 ultrasound machine with a 12 MHz linear probe. Patients were divided into premenopausal and postmenopausal groups, excluding patients with symptoms and those with previous breast cancer surgery. The images were stored using the Institution's Pictures Activation and Communication System. **Results.** BI-RADS category 3 findings accounted for 27% of all ultrasound examinations (N=3.715). Of these, 9.02% (N=335) underwent recommended short-term follow-up. Malignancy was identified in 1.49% of these cases (N=5), with an average tumor size of 13.6 mm at detection. The malignancy rate did not differ significantly between premenopausal and postmenopausal patients (P=0.412). The overall diagnostic yield for malignancy in BI-RADS 3 findings was low, but clinically significant. **Conclusion.** While the malignancy rate for BI-RADS category 3 findings is low (1.49%), careful monitoring and adherence to follow-up guideline are essential to balance early detection with avoidance of unnecessary biopsies and associated costs.

Key Words: BI-RADS Category 3 ▪ Ultrasound ▪ Tumor Size ▪ Breast Cancer ▪ Follow-up.

Introduction

The role of breast ultrasound has rapidly expanded from simply characterizing the internal contents of the mass to differentiating between benign and malignant breast masses, and as an adjunctive to mammography. It has even been proposed as a screening modality in young women or women with dense breasts (1-4). A BI-RADS category 3 finding should imply a greater than 0% but ≤ 2% probability of malignancy, which is characteristic of a benign finding.

According to the fifth edition of BI-RADS, ultrasound findings classified as category 3 include complicated cysts; microlobulated or oval areas composed of accumulated microcysts; hypoechoic, well-dellineated, oval horizontaly oriented areas without posterior acoustic features or with minimal posterior echo enhancment; hyperechoic areas with a central hypo- to anechoic component and surrounding edema consistent with fat necrosis, though not histologically confirmed; refractive shadowing without an associated lesion; and architectural distortion related to postoperative scars.

Despite the promotion of the BI-RADS lexicon, the main limitations in ultrasound practice are the dependence on operator experience (5). Bowles et al. (6) showed that increased density of breast shadowing and symptomatic patients were more often receive recommendations for short-term follow-up, i.e. biopsy or additional imaging. A likely benign finding is not expected to change

during the proposed follow-up period, but radiologists describing the finding prefer to determine the stability of the finding before recommending routine ultrasound controls. The BI-RADS category 3 finding was created to help reduce the number of false-positive biopsies, while maintaining a high rate of early breast cancer detection. BI-RADS 3 should not be used as a category of uncertainty when the radiologist is not sure if it is a benign lesion that should be monitored, or a biopsy should be performed (7). BI-RADS category 3 findings contain a recommendation for short-term follow-up according to the finding being monitored. Monitoring protocols differ in clinical practice, and controls are usually done at 3, 6, 12 and 24 months. After documenting the stability of the finding for at least two years, the finding can be concluded as BI-RADS 2 (benign). On the other hand, if the findings show suspicious changes, such as an increase in size, or a poorly circumscribed lesion, they should be concluded as BI-RADS 4 or 5 (suspect finding or probably malignant lesion).

The aim of this study was to analyze the proportion of BI-RADS category 3 findings in the ultrasound findings in relation to the total number of patients. The presence of malignant lesions was examined, as well as their size at the time of detection in an ultrasound finding, concluded as BI-RADS category 3.

Patients and Methods

Patients

This cross-sectional study included 335 consecutive patients between the ages of 40 and 75 who underwent ultrasound breast examinations at the Breast Center of Tuzla University Clinical Centar (UCC) in the period from March 2017 to November 2020, and whose results were classified in accordance with the ACR BI-RADS as BI-RADS category 3. Patients included in this study were selected through opportunistic screening, as they presented for routine breast ultrasound examinations, or as secondary readings after initial mammographic examinations. The patients were divided into two categories on the basis of their menstrual cycle status: premenopausal and postmenopausal patients. All symptomatic patients, i.e. patients with clinical symptoms such as palpable lumps, nipple discharge, localized pain or breast skin changes, and patients who had undergone breast surgery due to breast cancer were excluded from the study.

Methods

Thirteen thousand, seven hundred and sixty ultrasound breast examinations were performed at the Radiology and Nuclear Medicine Clinic of Tuzla UCC Public Health Institution, during the period of the study. Ultrasound examinations were performed on a Toshiba Xario 100, Japan, ultrasound machine, with a 12 MHz linear probe. The images obtained were stored in the digital archive of the institution's Pictures Activation and Communication System (PACS). The ultrasound examinations and findings were described by three radiologists with up to ten and more than ten years of experience in using the BI-RADS categorization of findings. There was no double reading and each radiologist independently reviewed their cases without overlap. Out of a total of 335 patients with ultrasound BI-RADS 3 findings, 46 (14%) underwent needle "CORE" biopsy, and 134 (40%) of them underwent Fine Needle Aspiration Cytology (FNAC), based on the following criteria: the patient's own request due to anxiety or fear, or changes in the ultrasound characteristics of the lesion during follow-up, such as: increase in lesion diameter or the appearance of other suspicious features including margins, changes in shape, echotexture, or lesion orientation. FNAC is performed with a fine needle (18, 20 or 22 gauge) while "CORE" biopsy is performed with 14 gauge (G) needles, under local anesthesia - 2% lidocaine, with a Pro-Mag biopsy gun, Argon Medical, USA, under the control of an ALOCA, Japan, ultrasound machine-with a 7.5MHz linear probe. It obtains tissue samples that enable pathohistological diagnosis. At least three biopsy cylinders were taken from each

lesion. Under ultrasound control, the path of the needle was continuously monitored on the screen.

Statistical Analysis

The collected data were stored in a database created in the commercial program Microsoft Access. Quantitative data are presented as number (percentage) and interquartile range (IQR). Statistical tests used include chi-square. The statistical significance of the difference in the frequency of one of the parameters was tested using the standard $\chi 2$ (Chi^2) test. For quantitative comparison of the frequency of occurrence of one of the parameters, the odds ratio (OR) was used, with the corresponding 95% confidence interval (95% CI). Statistical hypotheses were tested at a significance level of five percent ($P<0.05$). Statistical data processing was done using MS Excel 2019 and Epi Info Ver. 7.2.

Results

Out of the total number of ultrasound examinations performed, 3,715 of the findings were concluded as BI-RADS category 3, that is 27% ultrasound findings. In the group of patients whose ultrasound findings were concluded as BI-RADS category 3, 335 (9.02%) complied with the recommendations on short-term follow-up. The average age of patients whose ultrasound findings were concluded as BI-RADS category 3 was 50.25±7.75 years. In the group of 183 (54.63%) premenopausal patients it was 44.96±4.60, and in the group of 152 (45.37%) postmenopausal patients it was 52.90±6.67.

During the monitoring period, the presence of cancer was proven in five of the 335 patients included in the present study, which accounts for 1.49% cancer in relation to the total number of patients included in the study. Carcinomas were detected in two patients after three months of follow-up, in two patients after 6 months of follow-up, and in one patient after 12 months of follow-up. The average age of the patients who were diagnosed with cancer was 45.8, of which 3 (60%) were postmenopausal and 2 (40%) were premenopausal. The malignancy rate did not differ significantly between premenopausal and postmenopausal patients ($P=0.412$).

The carcinomas detected by ultrasound monitoring were of an average size of 13.6 mm, with a range of 8 to 22 mm, and none of the patients were shown to have secondary deposits in the axillary lymph nodes. Of the five malignancies initially classified as BI-RADS category 3, three (60%) were invasive ductal carcinoma and two (40%) were ductal carcinoma in situ. Table 1 provides details on the number, size and histopathological types of cancers detected in our patients, with all invasive cancers corresponding to early breast cancers in the T1 stage.

Of the five cancers detected in ultrasound BI-RADS 3 findings in three patients, hypoechoic oval areas with minimal posterior enhancement of the echo signal were observed; in one patient a refraction shadow without an associated area, and in one patient a microlobulated or oval area composed of microcysts. Out of a total of 335 patients with ultrasound BI-RADS 3 findings, 46 (14%) underwent CORE biopsy, and 134 (40%) of them underwent FNAC.

A malignant lesion was confirmed after FNAC in one patient and in four patients after CORE biopsy. The Chi^2 test determined that the difference in the frequency of FNAC and CORE biopsy findings was not random ($P=0.0003$). The chance

Table 1. Histopathologic Type, Number, and Size of Cancer

Histopathologic type	N*	Size of cancers / the extent of DCIS†
DCIS	2	8 mm; 22 mm
Invasive ductal carcinoma, nuclear grade 3, grade 3	1	19 ×11 mm
Invasive ductal carcinoma, nuclear grade 2	2	11×10 mm; 8×7 mm

*Number of cancers; †Ductal carcinoma in situ.

of using FNAC biopsy is 2.33 (95% CI 1.49-3.69) times higher compared to CORE biopsy. The Chi^2 test did not establish any statistically significant difference in the use of FNAC in the category of pre- and postmenopausal patients (P=0.235). The odds ratio of applying FNAC in these two categories of patients was also calculated: (OR=0.746; 95%CI 0.48-1.16).

The same procedure was carried out with the test patients who underwent a CORE biopsy. No statistically significant difference was found in the use of CORE biopsies in the category of pre- and postmenopausal patients (P=0.087). The odds ratio of the CORE biopsy in these two categories of patients was also calculated: (OR= 0.537, 95% CI 0.28-1.04).

Among the 335 patients who underwent short-term control ultrasound examinations in the study after 3, 6, 12, 18 and 24 months, BI-RADS category 3 findings were assigned to 89 patients (26.57%) for the right breast, for 102 patients (30.45%) for the left breast and for 144 patients (42.98%) for both breasts. In daily clinical practice and in these cases, the first short-term ultrasound control for test patients who were assigned BI-RADS category 3 findings for only one breast was performed for both breasts.

Discussion

The present study highligts the frequency and clinical implications of BI-RADS category 3 ultrasound findings in our patient population. BI-RADS category 3 ultrasound findings constituted 27% of all ultrasound examinations, with a compliance rate for short term follow-up of only 9.02%. These results reveal significant challenges in monitoring BI-RADS 3 lesions and underscore the importance of effective follow-up strategies to prevent delayed cancer diagnosis.

The observed frequency (27%) is higher than that reported in several other studies. The percentage of BI-RADS category 3 findings in ultrasound findings ranges from 0.6% to over 20% (8) in patients at the time of their initial ultrasound scan. In comparison, Nam et al. (9) reported 41.5% BI-RADS 3 lesions, while Kim et al. (10) reported a frequency of 17.3%. The frequency of BI-RADS category 3 lesions is as high as 36.9% in the general population (10). Hooley et al. (11) and Barr et al. (12) reported that BI-RADS category 3 lesions were present in 20% (187 of 935) in ultrasound screening in a high-risk patient population. This variability could be attributed to differences in the study populations, screening protocols, or radiologists interpretations.

Our malignancy rate of 1.49% aligns with the low malignancy rates reported in similar studies, such as Chae et al.(13) (0.7%) and Kim et al. (10) (0.8%). However, our study¨s higher average tumor size of 13.6 mm, compared to the average tumor size of 7.33 mm reported by Kim et al., suggests a potential delay in recognizing changes in the ultrasound descriptors that are monitored.

The low compliance rate (9%) in our study is concerning, especially compared to the significantly higher rates in other studies (e.g. 91.2% in Kim et al.). Possible reasons for non-compliance include patient-related factors (e.g.anxiety, ignorance, financial or logistical barriers). Consistent with Lee at al. (14), our findings confirm that BI-RADS category 3 is a challenging category, often resulting in overuse or misclassification. This aligns with Chae et al. (13), who reported reinterpretation of BI-RADS 3 lesions in 19.3% of cases, which led to translation into BI-RADS 2 (benign finding) or BI-RADS 4 or 5 category findings (suspect finding or probably malignant lesion).

The exceptionaly low compliance rate in our study emphasizes the need for targeted interventions, such as patient education, streamlined follow-up systems, and addressing socioeconomic barriers.

Our results aree with the broader literature that BI-RADS 3 findings are associated with a low malignancy rate but require careful monitoring to balance the risk of under-diagnosis and over-monitoring. For instance: Chae et al.(13) demonstrated similar ultrasound findings but reported higher follow-up complience and smaller tumor size. Kim et al. (10) also found higher follow-up compliance (91.2%) and lower average tumor size (7.33 mm),

suggesting that follow-up adherence may impact early cancer detection.

In the present study, carcinomas detected by ultrasound were detected with an average size of 13.6 mm, in a range from 8 to 22 mm, and none of the patients was shown to have secondary deposits in the axillary lymph nodes. As in the study Chae et al.(13) and Kim et al.(10), in the present study, invasiv cancers were detected in the T1 stage without secondary deposits in the axillary lymph nodes.

In comparison to the study Chae et al. (13) and Kim et al. (10) where the representation of BI-RADS category 3 is significantly higher and the malignancy rate is very low, in the present study the representation of BI-RADS category 3 is low, but the malignancy rate is high.The study points the need to carefully assess each finding in accordance with the BI-RADS classification of findings in order to avoid unnecessary costs of biopsy or short-term controls. The importance of short-term examinations should be explained to every patient with a BI-RADS category 3 finding to encourage them to come for follow-up examinations and potentially reduced anxiety. Radiologists may consider improving communication with patients to potentially increase adherence to follow-up recommendations. The development of systematic approaches to follow-up and support patients with BI-RADS category 3 findings would improve outcomes. Considering that the representation of BI-RADS category 3 is high, there is olso a need for continuous education of radiologists.

Recent advancements highlight the potential benefits of combining imaging modalities to improve diagnostic accurancy. For example, Muthuvel et al. (15) demonstrated the usefulness of combining advanced dynamic contrast-enhanced and diffusion-weighted MRI with ultrasonography to differentiate cancerous from benign lesions in dense breasts, suggesting opportunities for refinement in diagnostic pathways. Additional research is needed to better understand the factors that influence short-term follow-up and to identify the most effective ways to increase short-term follow-up rates. The conclusions of this study emphasize the importance of adequate follow-up and support for patients with BI-RADS 3 findings to increase the likelihood of early detection of malignancy and improve long-term outcomes.

Limitations of Study

One of the limitations of our study is the small sample size. Also, all symptomatic patients were excluded from the study.

Conclusion

The present study confirmed the fact that the probability of breast cancer in BI-RADS 3 lesions is lower than or equal to 2%, and that breast cancers are detected with an average size of about 1,4 cm, without secondary deposits present at the time of detection. The BI-RADS category 3 finding was created to reduce the number of false-positive biopsies, while maintaining a high rate of early detection of breast cancer. Accordingly, careful assessment is required a finding concluded in BI- RADS category 3 in order to avoid unnecessary costs of biopsy or follow-up at short intervals.

What Is Already Known on This Topic:

Ultrasound BI-RADS category 3 findings have a greater than 98% likelihood of being benign. The category is used for lesions that have a high probalitity of being non-cancerous but are not definitively benign. For BI-RADS 3 findings, the recommended menagement typically involves short-interval follow-up imaging rather than immediate biopsy. Follow-up is usually suggested at 6 months, 12 months, and 24 months to ensure stability or resolution of the finding. The purpose of short-interval follow-up is to monitor the lesion for any changes that might indicate malignancy. Stability of the finding over time reinforces its benign nature. If there is any change in the finding during follow-up, such as growth or other suspicious features, the lesion may be upgraded to BI-RADS 4 or BI-RADS 5 warranting further investigation such as biopsy. Follow-up is essential to confirm its nature and ensure there is no malignant transformation.

What This Study Adds:

This study points to the need for a serious approach to every patient with BI-RADS category 3 findings in the sense of an informative conversation that would explain that follow-up is necessary to confirm the stability of the findings, that is, to respond adequately in case of change during follow-up. BI-RADS category 3 findings require a serious and proactive approach, which implies strict adherence to the recommendations given by the American College of Radiology. The decision to classify a lesion as BI-RADS category 3 should not be based on the presence of risk factors.

Authors' Contributions: Conception and design: HF; Acquisition, analysis and interpretation of data: HF and SM; Drafting the article: HF, SM and BI. Revising it critically for important intellectual content: HF and SM.

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Original Scientific Article
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From Rehab to Routine: Ensuring Lasting Lifestyle Changes After Cardiac Rehabilitation – First Experiences from the "Love Your Heart" Program

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Abstract

Objective. The study assesses how well patients follow secondary prevention measures after completing an outpatient cardiac rehabilitation (CR) program. **Materials and Methods.** This research involves 63 patients who completed an outpatient CR program between 2018 and 2020 at the Istrian Health Center in Pula, Croatia. Socio-demographic characteristics, levels of physical activity, adherence to a Mediterranean diet, and compliance with medical recommendations were assessed in a survey to gather data. **Results.** Out of the 63 patients surveyed, 51 responded (34 males, 17 females, average age 68.25 years). After completing a cardiac rehabilitation program, 78.4% maintained appropriate levels of physical activity but showed low adherence to a Mediterranean diet, with an average score of only 3.94 out of 9. Only a small percentage (2%) effectively followed medical recommendations. **Conclusion.** Despite the fact that patients maintain physical activity, there are still challenges regarding adherence to nutritional recommendations and medical advice. Long-term adherence can be improved by utilizing technology, involving family members, and offering education. Future research should focus on identifying obstacles to adherence and developing approaches to achieve lasting behavioral change.

Key Words: Cardiac Rehabilitation ▪ Cardiovascular Diseases ▪ Mediterranean Diet ▪ Physical Activity ▪ Prevention.

Introduction

In developed countries, including Croatia, cardiovascular diseases (CVD) are the primary cause of death, and pose many challenges to public health and the economy (1). Each year CVDs result in 3.9 million deaths in Europe and over 1.8 million in the European Union, thereby representing a significant portion of all mortality rates (45% of all deaths in Europe and 37% in the EU) (2). CVDs were responsible for 39.1% of deaths in Croatia in 2022, with 22,303 people dying from them (3). Global CVD mortality rates declined between 1990 and 2022 according to data from the World Health Organization (WHO) and numerous public health studies, such as Mensha et al (4). However, it is especially concerning that younger demographics, including the working population, are increasingly impacted by CVD, leading to notable economic repercussions (2). The study by Luengo Fernandez et al. in 2023 estimates that productivity losses due to CVD represent 17% of the total, amounting to 48 billion EUR, while informal care expenses amount to 79 billion EUR (5). These data highlight the importance of public health preventive measures in the form of primary and secondary prevention of CVD to reduce morbidity and mortality, and to mitigate the economic impact on the countries' economies. Numerous studies and meta-analyses have shown that cardiac rehabilitation (CR) programs are crucial for prevention, as they significantly reduce mortality rates and recurrent CVD events, while improving quality of life, increasing physical activity levels, and reducing anxiety and depression (6).

In Istria, a coastal tourist region of Croatia, the "Love Your Heart" project was initiated as part of the Adriatic Network for Cardiovascular

Disease Prevention program, funded through the Instrument for Pre-Accession Assistance Adriatic EU Cross-Border Cooperation Program in 2012 (7). It spanned various regions in Croatia, Italy, and Albania within the Adriatic area. One of the primary goals was the establishment of the Center for Cardiovascular Disease Prevention in Istria, aimed at improving the detection of risk factors, providing services to high-risk patients, and efficiently monitoring their condition. In 2018, an outpatient CR program was launched, including activities and educational workshops on CVD, risk factors, and preventive measures. A total of 63 patients completed the program of outpatient CR. After completing the rehabilitation program, patients have the opportunity to join a club for cardiovascular patients, where they can take part in regular Nordic walking training and educational workshops once a week. The program was suspended in 2020 due to the COVID-19 pandemic but was revitalized in 2023.

The effectiveness of rehabilitation and secondary prevention strategies relies heavily on patient adherence to treatment plans. Chen et al., in their meta-analysis, revealed that patients with coronary artery disease were more prone to CVD events and mortality due to their poor adherence to prescribed medications (8). Monitoring patients and ensuring that lifestyle adjustments made during rehabilitation are continued is of significant public health importance. A major impact on society can be achieved by timely detection of complications or recurrence of disease, potential deterioration of quality of life, and the emergence of other diseases. However, the sustainability of these habits varies in relation to the patients' characteristics, socioeconomic status, cultural influences, and regional factors. Regrettably, adherence to these lifestyle changes tends to decrease over time.

This study aims to assess how well patients follow secondary prevention measures after completing an outpatient CR program. This evaluation can aid in identifying areas where the CR program may require modifications to enhance adherence to secondary prevention measures and outcomes, at both regional and global levels.

Patients and methods

Participants

The study was carried out from December 2023 to March 2024. The study included 63 patients who completed an outpatient CR program at the Istrian Health Center in Pula in the period between 2018 and 2020. Three patients passed away between 2020 and 2024, and seven individuals were not available because they changed their contact numbers. Two patients failed to complete the survey. The CR program included patients recovering from myocardial infarction (with or without interventions such as percutaneous coronary intervention – PCI, or coronary artery bypass grafting - CABG), stable angina pectoris, chronic heart failure (stadium NYHA I-II) with a stable clinical condition, and post-valve replacement or repair, and patients with implanted devices such as implantable cardioverter defibrillator (ICD) or pacemakers. Participation required a stable clinical state, as well as the physical and cognitive ability to engage in the program and the motivation to adhere to its recommendations. The exclusion criteria for participation in CR included acute or unstable conditions, such as acute myocardial infarction, unstable angina pectoris, uncontrolled or severe cardiac arrhythmias, or acute heart failure. Patients with uncontrolled concomitant diseases or severe lung disease with respiratory insufficiency were also excluded. In addition, physical and cognitive impairments, such as severe disabilities or significant cognitive impairment, as well as the patient's refusal to participate in rehabilitation were considered exclusion criteria. For patients of working age, outpatient CR was organized five times per week over seven and a half weeks to prepare them for returning to work. Retired or unemployed patients participated three times per week over 12 weeks. The rehabilitation program included aerobic exercises, muscle-strengthening activities, walking on a treadmill, or cycling on a bicycle ergometer for 30–40 minutes as interval training in groups of 1-6 patients, under the supervision of a physiotherapist, with monitoring of blood pressure and pulse, and an electrocardiogram during

treadmill or bicycle-ergometer exercises. Regular workshops on adapting lifestyle habits were also provided. Before joining the rehabilitation program, patients underwent a specialist cardiology examination, and a six-minute walk test was conducted. During the rehabilitation program, patients had regular medical check-ups.

Procedure and Materials

In this study, a customized questionnaire was used to collect information on how patients changed their lifestyles after completing outpatient rehabilitation. In the first part of the survey, information was collected on the basic characteristics of the participants (age, gender, and the indications for rehabilitation). Subsequent sections of the questionnaire included inquiries about the participant's behaviors and physical activity levels after rehabilitation. On the basis of their responses, participants were categorized into three groups: those with a low level of activity (< 50 minutes of physical activity per week), those with moderate physical activity (50-149 minutes of physical activity per week), and individuals with a high level of physical activity (≥150 minutes of physical activity per week).

The third part was the standard questionnaire entitled 'Rate Your Med Diet Score' from Oldways and the Mediterranean Foods Alliance, which contains nine standard questions (9). This questionnaire was not specifically created for cardiovascular patients but is very easy to use and easily understood by patients of various cultural and educational backgrounds. In this section, the health benefits of a Mediterranean diet were highlighted, with each positive response being worth one point. The findings were classified into four groups (0-3 points – "Time to turn your life around" – your diet is not following the ideal Mediterranean diet; 4-5 points "A good start, but you can do better if you value your health." – your diet includes some elements of the ideal Mediterranean diet; 6-7 points – "You're doing well. What would help you to add another point or two?" – your diet has a lot in common with the ideal Mediterranean diet; 8-9 points – "Long life! Your eating habits follow the Med Diet very closely" – your diet is largely in line with the ideal Mediterranean diet.)

The final part of the questionnaire featured four questions aimed at gauging respondents' views on adherence to recommendations, such as not smoking, monitoring blood pressure daily following the prescribed medication intake, and attending regular cardiac checkups. A point was awarded for each affirmative response. Poor adherence to these habits was implied by one positive answer, two positive answers were associated with the attitude of moderate adherence to medical recommendations, while three or more positive answers implied good adherence to medical recommendations.

Ethical Considerations

The study was approved by the Ethics Committee of the Istrian Health Centers (approval number: 2168/01-59-49-01-1/801-23-176). The Law on the Protection of Patients' Rights (Official Gazette 169/04, 37/08) and the Law on the Protection of Personal Data (Official Gazette 103/03-106/12) were adhered to in order to protect the rights of the participants and their data. The study also followed the principles of the Declaration of Helsinki (1964-1973). Participation in the study was voluntary and anonymous. Participants were informed in advance about the aims of the study. All participants signed a consent form.

Statistical Analysis

The data obtained were analyzed using Statistica 13.3 (TIBCO Software Inc.). The computer program Microsoft Office Excel was used for the graphical and tabular presentation of the results for better visualization.

The socio-demographic data are presented in absolute frequencies and as percentages. Descriptive statistical methods were used to determine the respondents' views on the continuity of lifestyle changes after CR, including the determination of the mean and standard deviation. The reliability of the "Rate Your Med Diet Score"

questionnaire was assessed by calculating the Cronbach's alpha coefficient. Multivariate regression analysis was used to determine the influence of independent variables from Table 1, such as the BMI, height, oxygen saturation, and blood pressure of the participants in the results of the 6-minute walk test. The normality of the scores obtained was tested using the Kolmogorov-Smirnov test. As the data were not normally distributed, a non-parametric chi-square test was used to test the differences between the groups of patients obtained depending on their diet. Also, on the basis of the results obtained, the most frequent physical activity levels after rehabilitation were determined using the Chi-square test. Statistical significance was assessed at the level of $P \leq 0.05$, with 95% confidence limits.

Results

The research involved 51 respondents (N=51). Of these participants, 34 were men (66.7%) and 17 were female (33.3%) (Table 1). The average age of the respondents was 68.25±8.54 years. The youngest participant was aged 49, while the oldest was 86 years old. The most common indication for CR was myocardial infarction (64.7%).

Table 1. Characteristics and Baseline Values of the Study Population

Characteristics	N (%)
Gender	
Male	34 (66.7)
Female	17 (33.3)
Diagnosis	
Myocardial infarction	33.7 (64.7)
Chronic ischemic heart disease	13 (25.5)
Other diagnoses*	5 (9.8)
Baseline values of the patients	
Age average (years)	68.3±8.5§
Average height (cm)	173.5±8.95§
Average weight (kg)	81.6±12.8§
BMI† (kg/m^2)	27.1±3.11§
LDL‡ (mmol/L)	2.71±0.94§
Triglycerides (mmol/L)	1.71±0.58§
Oxygen saturation (%)	97.5±0.63§
Systolic blood pressure (mmHg)	126.8±4.8§
Six-minute walk test (m)	320.85±88.81§

*Aortic valve replacement, Electro-stimulator placement, Heart failure; †Body mass index; ‡LDL cholesterol; §M±SD.

The Patients' Consistency in Engaging in Physical Activity after Finishing the CR Program

According to the analysis, most respondents continued to engage in physical activity after completing the outpatient CR program. According to the survey data, one-fourth (27.4%) of the respondents reported walking 1 to 2 times per week, while over one-third (33.4%) walked three to four times per week, and nearly forty percent (39.2%) walked five or more times per week. Furthermore, one-fifth (19.6%) walked less than 30 minutes, and around thirty percent (31.3%) for over an hour. It was also found that 54.9% of the respondents participated in a different type of physical activity alongside walking. In conclusion, using the Chi-square test it was determined that according to the time spent engaged in physical activities, 16% of the respondents were classified as having low levels of physical activity, 6% were classified as having moderate physical activity, and a significant 78% were classified as having a high level of physical activity, (χ^2=47.412, P=0.000). Furthermore, in the fourth iteration, the multivariate regression analysis (R=0.727, P=0.000) established a statistically significant relationship between the subject's height (b*=0.468, P=0.003) and the initial blood pressure measured (b*=0.351, P=0.047) in the results of the 6-minute walk test. In addition, the statistically significant differences (t=8.553, P=0.000) between the results of these tests at the beginning (320.85±88.81 m) and the end of CR (527.83±137.36 m) were utilized.

Application of Healthy Eating Rules Adopted During Outpatient CR

To assess compliance with the Mediterranean diet, a study used the "Rate Your Med Diet Score" questionnaire. The calculated value of the Cronbach's alpha coefficient (α=0.75) indicates

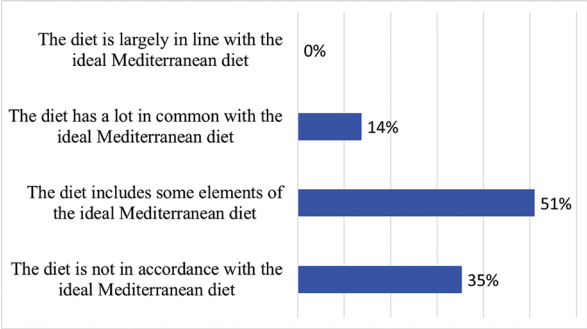

Figure 1. Percentage distribution of the respondents by eating habits.

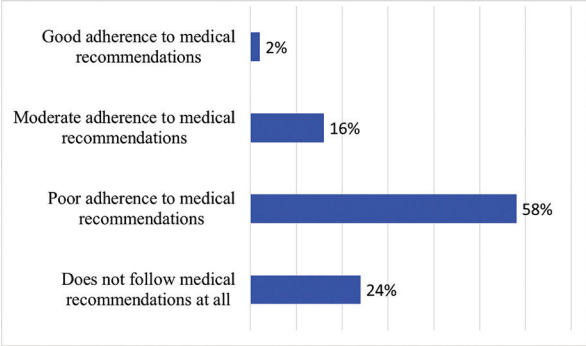

Figure 2. Percentage representation of respondents according to categories on adherence to medical recommendations.

the good reliability of the scale. The results of the Kolmogorov-Smirnov statistical test showed that the data were not normally distributed (P<0.01).

It is worth noting that the ideal Mediterranean diet pattern was not followed by any of the respondents. Percentage distribution of the respondents by eating habits is depicted in Figure 1. The majority of respondents only partially adhere to the rules of the Mediterranean diet.

To assess compliance with the Mediterranean diet, a study used the "Rate Your Med Diet Score" questionnaire. The calculated value of Cronbach's alpha coefficient (α=0.75) indicated the good reliability of the scale. The results of the Kolmogorov-Smirnov statistical test showed that the data were not normally distributed (P<0.01).

However, a difference was found in the frequency of the application of healthy eating rules during CR between the defined categories (χ^2=10.706, P=0.005). Moreover, each respondent's average score was calculated as 3.94±1.58 points. This indicates that the sampled individuals either did not strictly follow the Mediterranean diet or only included some elements from it in their food intake

Adherence to Medical Recommendations

Representation of respondents according to categories on adherence to medical recommendations is shown in Figure 2. Moreover, the data analysis revealed the existence of significant differences between these categories (χ^2=38.961; P=0.000). Unfortunately, only a minimal number of respondents adhere to the medical recommendations, while three quarters of them do not or only partially follow the recommendations they received during the CR.

Discussion

This study aimed to assess how well participants adhered to the rules and maintained the lifestyle changes learned as part of a CR program. CR is a crucial aspect of secondary prevention in cardiovascular care, which aims to improve the prevention of heart problems by encouraging healthy habits, adherence to medication routines, and regular check-ups with healthcare providers (10). The participants in this study were predominantly elderly, with an average age of 68 years. This age range is important because the risks and complications tend to increase with age. Twice as many men as women took part in the study. A higher number of men was certainly to be expected, as numerous studies to date have shown a higher prevalence of CVD in men than in women, especially in younger age groups, where the protective role of estrogen in the development of CVD has been confirmed (11, 12). In addition, it is important to consider that men are more likely to engage in high-risk behaviors such as smoking or excessive alcohol consumption, and be affected by socioeconomic inequality (13-15). The most common indication for outpatient CR among participants was myocardial infarction. This finding

reflects the continued prevalence of ischemic heart disease, which, as noted in the article by Wu et al, is the most common form of CVD worldwide (16). Recent studies show that ischemic heart disease remains one of the leading causes of death, highlighting the need for effective secondary prevention strategies (17). On the other hand, the significant positive effect of CR on the cardiorespiratory status of patients with heart failure is indisputable. A study by Acanfora et al. confirmed the benefits of CR in patients with heart failure and reduced left ventricular ejection fraction (18).

However, outpatient CR is not yet fully developed in the Republic of Croatia, partly because patients often opt for inpatient CR after cardiovascular events. There are only two facilities offering inpatient cardiac rehabilitation in Croatia, and their capacity is insufficient to meet the needs of the population. Outpatient CR, which serves as a complement to inpatient CR, could significantly reduce the burden on the healthcare system. However, it is currently only offered in three public and one private facility. The CR center in Istria offers outpatient CR treatment and is primarily intended for stable, low-risk patients. This is mainly due to the still prevailing belief of both patients and healthcare professionals that rehabilitation after a cardiovascular event should take place in an inpatient setting. This explains the predominance of simple, less complicated patients with ischemic heart disease in this study. At the same time, this limitation highlights the need for wider availability and easier access to outpatient rehabilitation services and the need to change public awareness of the role of outpatient CR.

This study emphasizes existing knowledge about the importance of participants adhering to physical activity recommendations, as the benefits of regular physical activity in reducing cardiovascular risk are well-known (19). According to the guidelines of the European Society of Cardiology, it is recommended to exercise at least 150-300 minutes per week at moderate intensity (20). The results of the study suggest that participants understood the importance of physical activity and its positive impact on reducing the morbidity and mortality associated with cardiovascular disease. This is supported by the fact that the patients improved their 6MWT results. Subsequent analysis revealed that the patient's height influenced these results, which is understandable given the widely accepted ratio of stride length to height, which varies according to age, gender, fitness level, and exercise style. Despite the results related to physical activity, the study shows that maintaining a healthy diet is a challenge. None of the participants fully adhered to the secondary prevention guidelines for the Mediterranean diet, which is known for its cardiovascular benefits (21). Only about half of the participants adhered to certain aspects of these recommendations. With an average adherence rate of 3.94 out of a possible 9 points, poor adherence to the prescribed diet plan highlights the need for targeted interventions and ongoing support to address this common problem in secondary prevention (22). The average LDL cholesterol value determined is significantly higher than the values recommended by the professional associations, with only two participants reaching the target values. This result is all the more worrying as the study was conducted in a Mediterranean region where the Mediterranean diet should be a common lifestyle. It underscores the need to examine regional socio-demographic, economic, cultural, and other factors that may contribute to such unhealthy eating habits in the population. These findings serve as a basis for planning targeted public health initiatives.

Regarding adherence to medical recommendations, only one participant showed high adherence, while eight participants showed moderate adherence. This reflects a general problem with adherence to recommendations in areas such as taking medication, attending regular check-ups, and monitoring vital signs. Nonadherence to prescribed medications can lead to adverse outcomes, including increased hospitalization and mortality, especially in the elderly and patients with comorbidities (23). Although it is a widespread assumption that women are more likely to adhere to medical recommendations and medication regimens, the results of previous studies are quite

controversial and full of contradictions, as shown by the studies of Venditti et al. and Olmastroni et al. which indicate poorer adherence to recommendations among women. This can certainly be influenced by various factors, such as socio-demographic, cultural, psychological, or treatment-related factors (24, 25).

Further research should be conducted to determine which specific medical recommendations patients do not adhere to, how long they maintain the habits acquired during cardiac rehabilitation before they falter, and what the exact causes of nonadherence are. These factors may help to develop more effective follow-up programs and interventions to improve long-term adherence to secondary prevention strategies. Recent studies have investigated various strategies to improve adherence to lifestyle changes and medical recommendations in CR. Mobile health applications and remote monitoring technologies have proven to be effective in promoting adherence to exercise, diet, and medication plans (26). Long-term adherence and outcomes could be improved by incorporating technology into CR. Involving patients' families and their social environment in the rehabilitation process through family-centered interventions can provide important support and encouragement and help patients adhere to lifestyle changes and medical advice (27). Patients may benefit from refresher sessions or follow-ups that emphasize the importance of maintaining activities, dietary changes, and medication regimens. These sessions provide an opportunity to address barriers to adherence, motivate the patients, and suggest ways to maintain this behavior. Behavioral interventions, such as motivational interviewing and behavioral therapy, have been shown to improve adherence to lifestyle changes and medical advice (28). These approaches strengthen patients' motivation, self-confidence, and problem-solving skills. Integrating these approaches into CR programs helps to overcome barriers, improve treatment adherence, and maintain behavior change. Addressing socioeconomic factors, such as access to healthy food, safe places to exercise, and medical care, and through policies and community initiatives, can reduce disparities in adherence and treatment outcomes.

Limitations of the Study

The small sample size of the study makes it difficult to generalize the results to a wider population and reduces the study's ability to detect smaller but potentially significant effects. With such a limited number of participants, the likelihood of bias that could affect the generalizability of the results is also higher. Since the study focused on an outpatient rehabilitation program in Istria, Croatia, the conclusions may not be relevant for people in other regions. Another limitation is the gender imbalance in the sample, in which there were twice as many men as women, which could bias the results and make them less representative of both genders. The use of questionnaires in research also brings challenges as they rely on patient self-report, which is often unreliable. Patients may answer in a way they think is socially acceptable or in a way they believe the researcher wants to hear, rather than giving honest answers. In addition, the use of predetermined questionnaires limits the researcher's ability to ask follow-up questions that could clarify responses, which may lead to misinterpretation. The study also did not examine how factors such as socioeconomic status or cultural background might affect treatment adherence, leaving important influences on behavior unaddressed.

Conclusion

This study emphasizes the importance of secondary prevention and lifestyle changes in reducing the impact of CVD. While adherence to physical activity after rehabilitation is promising, there are still gaps in adherence to dietary habits and medical interventions. Targeted interventions, the use of technology, and family involvement can improve adherence. Tailored programs, education, and behavioral strategies such as motivational interviewing will support lasting lifestyle changes. Policies need to address socioeconomic barriers and expand access to outpatient CR. Further research

is needed, taking into account regional population characteristics. Integrating evidence-based practices will strengthen program effectiveness and help patients maintain healthy behaviors, reduce relapse, and improve quality of life.

What Is Already Known on This Topic:

CVD is one of the most common causes of death worldwide and in Europe. CR programs are considered important secondary prevention strategies that reduce mortality and recurrent events, and improve quality of life. Despite their effectiveness, adherence to CR and associated lifestyle changes often declines over time. Factors such as socioeconomic status, cultural influences, and regional characteristics have a significant impact on adherence. Public health initiatives, such as the Croatian "Love Your Heart" project, demonstrate the potential of community-based programs to combat CVD through prevention and rehabilitation. However, maintaining healthy habits and ensuring long-term adherence remain key challenges in optimizing CVD outcomes.

What This Study Adds:

This study evaluates adherence to secondary prevention measures following an outpatient CR program in Istria, Croatia. It shows promising results in sustained physical activity. However, adherence to the Mediterranean diet and medical recommendations remains low: no participant fully adhered to the dietary guidelines and only 2% strictly followed the medical advice. The findings highlight the need for tailored interventions to address barriers, improve long-term adherence, and refine CR programs. These findings contribute to improving secondary prevention strategies at regional and global levels.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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APPENDIX

Questionnaire

Assessing Long-Term Adherence to Physical Activity, Diet, and Medical Advice Following Cardiac Rehabilitation

Dear Participant,
Thank you for taking the time to complete this questionnaire. Your responses will help us assess how well patients adhere to the recommended prevention measures after completing the outpatient cardiac rehabilitation program. All your information will be kept confidential and used only for this study. Your responses will help improve patient care and rehabilitation programs.
Please take your time to answer each question honestly. There are no right or wrong answers — this is simply to understand your habits and adherence to the recommendations after completing the cardiac rehabilitation program.
If you have any questions or need assistance while filling out the questionnaire, please feel free to ask for help.
Thank you for your participation!

• Gender: _____

• Age (years): _____

• Indication for cardiac rehabilitation: _____

Here Are Some Questions about Dietary Habits

*(For each question, please answer **Yes** or **No** based on your daily dietary habits.*
If you're unsure about a particular question, please try to answer as accurately as possible based on your typical eating habits over the past few weeks.)

1. I eat two or more cups of vegetables a day
   a) Yes b) No
2. I eat two or more pieces of fruit a day
   a) Yes b) No
3. I eat two or more whole grains a day
   a) Yes b) No
4. I drink ½ to 1 drink wine a day for women, 1 to 2 for men (but no more)
   a) Yes b) No
5. I eat fish 2 or more times a week
   a) Yes b) No
6. I eat 2 or more servings legumes/beans a week
   a) Yes b) No

7. I eat a handful of nuts most days
   a) Yes b) No
8. I eat lots of olive oil and few other fats
   a) Yes b) No
9. I eat 2 servings or fewer red or processed meat a week
   a) Yes b) No

We Will Briefly Discuss Your Physical Activity

(The first set of questions asks about your walking habits. Please indicate how many times per week you go for a walk, as well as how long you typically walk each time.
The second part asks whether you engage in other physical activities (e.g., jogging, swimming, cycling). If you do, please indicate the frequency and duration of those activities.)

1. How many times a week do you go for a walk?
   a) 1-2 times or less b) 3-4 times c) 5 and more times
2. How much time do you spend walking?
   a) less than 30 min b) 30-60 min c) more than 60 min
3. Do you engage in any other physical activity?
   a) No b) Yes: (Please specify which activity)
4. If your answer to the previous question was YES, how often per week do you engage in the specified activity?
   a) 1-2 times or less b) 3-4 times c) 5 and more times
5. How much time do you spend engaging in the specified physical activity?
   a) less than 30 min b) 30-60 min c) more than 60 min

Finally, Here Are a Few Questions about following Medical Recommendations

(The last set of questions concerns adherence to medical recommendations such as smoking cessation, daily blood pressure monitoring, medication adherence, and follow-up visits with a cardiologist.
For each question, please select the answer that best represents your current habits.)

1. Have you quit smoking after the onset of cardiovascular disease?
   a) Yes b) No c) I have never smoked
2. Do you measure your blood pressure every day?
   a) Yes b) No
3. Do you regularly take all prescribed medications?
   a) Yes b) No
4. Do you regularly check with a cardiologist?
   a) Yes b) No

Literature Review
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Anatomical Variations of the Auditory Cortex and their Clinical Significance, Prevalence and a Review of the Literature

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Abstract

Objective. This literature review aims to present anatomical variations in the primary and secondary auditory cortex, and an analysis of the categories (or classifications) of variation. **Background.** Many types of variation of the auditory cortex have been described, but there is a need to classify these variations and reveal their clinical implications. **Methods.** A detailed search in PubMed medical database was conducted, from October 2023 to November 2024 using the terms« ("anatomical") AND ("variations" OR "categories" OR "types" OR "differences") AND ("human" OR "man") AND ("auditory cortex" OR "auditory cortex" [MeSH]) and 44 articles were found. Applying the inclusion and exclusion criteria, 29 articles were finally selected. **Results.** A number of asymmetries have been recorded related to the size of the cortex between the two hemispheres, the number of Heschl's gyri and Heschl's gyrus surface variations, the distribution of gray and white matter, and the surface and size of the secondary auditory cortex. Reviews, and all prospective and retrospective studies, as well as case reports, were eligible for this review. **Discussion.** Variations werefound amongst patients of reduced auditory ability and the non-hearing impaired, musicians, amateur musicians, and non-musicians, right and left-handed people, men and women, and people with high and low pitch perception ability. Furthermore,variations were detected in people with dyslexia and learning disabilities. **Conclusion.** Asignificant number of anatomical variations of the auditory cortex have been recorded, andcloser research will contribute to our comprehension of the origin of some discrepancies in everyday clinical practice.

Key Words: Auditory Corte ▪ Heschl's Gyrus ▪ Planum Temporale ▪ Anatomical Variation.

Introduction

The auditory cortex consists of the primary and secondary auditory cortex. The Heschl's gyrus (HG) is foundon the superior surface of the temporal lobe, and demarcated anteriorly by the first transverse sulcus and posteriorly by Heschl's sulcus, containing the primary auditory cortex (medial 2/3 of the gyrus) (1). In addition, the HG is bounded laterally by the fissure of Sylvius, and includes 1-3 gyri per hemisphere. The Planum Temporale (PT) is located posterior to the HG, hosting the secondary auditory cortex (2). More specifically, the posterior 2/3 of the HG belongs to the primary auditory cortex, while the anterolateral part is associated with auditory stimuli perception ability and pitch pattern perception ability (3). Therefore, the HG is a brain structure involved in the processing of speech, and is adjacent to the Wernicke area (2).

The PT is detected on the medial surface of the superior temporal gyrus, posterior to the HG, and it contains the Wernicke area, which is involved in the processing of speech (4). Regarding the anatomical interaction of HG and PT, since there are 1-3 gyri per hemisphere, the HG (Primary Auditory Cortex) is considered to be H1, while H2 and H3 are considered to appertain to the PT. This distinction (H1, H2, H3) is due to the anatomical position of Heschl's sulcus which separates the HG from the PT. The PT is defined posteriorly by

the posterior branch of the fissure of Sylvius (5). To sum up, the HG houses the primary auditory cortex (PAC) and receives stimuli from the medial geniculatenucleus of the thalamus. These stimuli are expedited to the adjacent PT. In the PT, auditory stimuli are correlated with the visual stimuli coming from the occipital lobe (6).

As a general rule, the volume of theleft HG seems to be positively related to the ability to process sound, while an increase in the left PT is associated with increased activity during language comprehension (2). In addition, as far as the functionality of the auditory cortex in relation to the cerebral hemisphere (right or left) is concerned, the left PAC is mainly related to speech processing, while the right PAC to processing of extra linguistic elements (7). Also, the left hemisphere is specialized in processing auditory stimuli which occur in a short temporal integration window, while the right hemisphere is specialized in auditory processing for a longer temporal integration window (8). The neurons of the right auditory cortex are more strongly synchronized at specific frequencies compared to the neurons of the left cortex in a homologous area. Moreover, the processing of spectral information takes place in the right hemisphere, while the processing of temporal information occurs in the left hemisphere (9). Finally, voicing and aspiration differences between stop consonants are processed differently between the two hemispheres (10), and an asymmetry in tonotopic organization was observed (9). This might implicate hemispherical specialization.

Consequently, the aim of our project was the complete presentation of anatomical variations in the primary and secondary auditory cortex, and an analysis of categories of people who display variations (for instance according to occupation, handwriting, possible learning disabilities, etc.).

Materials and Methods

The topic of our narrative project focuses on anatomical variation of the auditory cortex. Database PubMed was the source of our investigation. We conducted an advanced search of the PubMedblockchain.The search strategy included the following keywords:(“anatomical”) AND (“variations” OR “categories” OR “types” OR “differences”) AND (“human” OR “man”) AND (“auditory cortex” OR “auditory cortex” [MeSH]). Initially, 44 articles were found, while afterusing a date filter for the period 2000-2024, 38articles remained. Of these primary38 articles, five were related to the topic of our paper. From a secondarysearch ofthereferences of these fivearticles, 29 more were added, of which four articles were duplicates. However, one main search article was not used, as a reference article. Consequently, for the composition of our paper, 29 articles were used (34-5=29). The main search articles were 18, 22, 24, and 28. Eligible articles were identified by a search for the period from October 2023 to November 2024.

Language restrictions were not applied (only articles in English were found in the database).Two investigators (KP and DC), working independently, searched the literature and extracted data from each eligible study. Manuscripts that did not state the names of the authors were excluded. In addition, we checked all the references of relevant reviews and eligible articles that our search retrieved, so as to identify potentially eligible conference abstracts. Titles of interest were further reviewed by the abstract. Finally, reference lists of eligible studies were manually assessed in order to detect any potentially relevant article (“snowball” procedure).

The inclusion criteria encompassed: (a) clinical trials, comparative studies, and anatomical reviews, (b) articles published in the English language, (c)research involving human-only studies, (d) reviews of variationassociated with the auditory cortex, (e) a limitation regarding the year of the publication.

Results

Article Selection and Study Demographics

The search strategy retrieved 38 articles for full-text evaluation.All of the articles from the search in database were in English.Twenty-nine studies were deemed eligible and were included in the

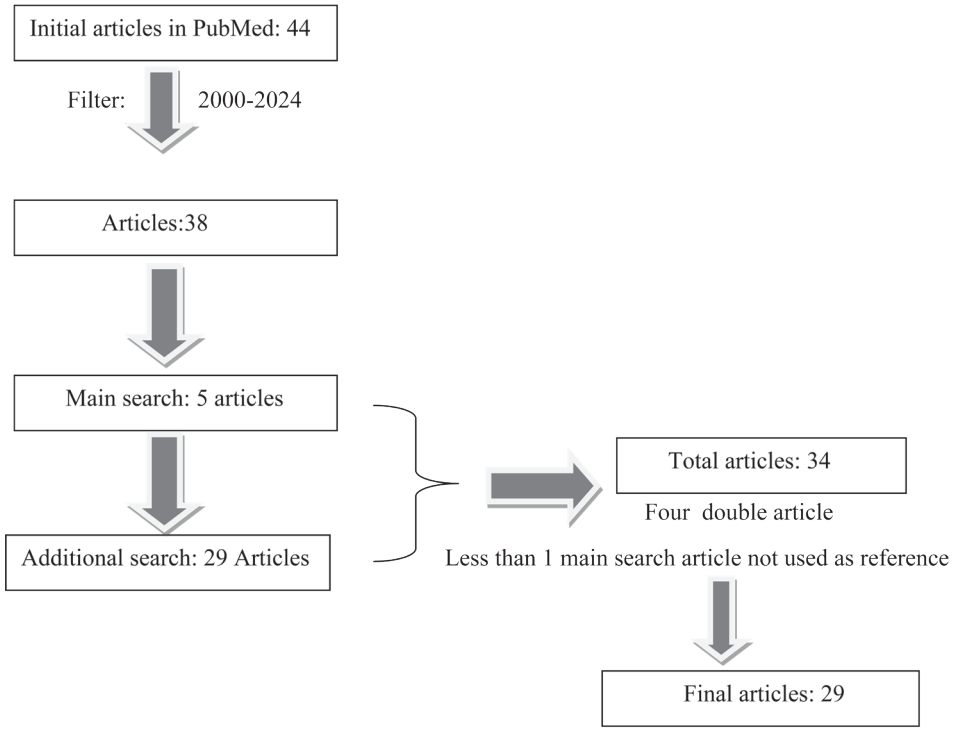

Figure 1. Search strategy.

analytic cohort. According to our literature review, variations included the size of the cortex between the two hemispheres, the numberand the surface area of the HG, the distribution of gray and white matter, and the surface and size of the secondary auditory cortex.

Furthermore, the narrative study focused on the correlation betweenvariations and specific categories of people, for example, patients with reduced auditory ability and the non-hearing impaired, musicians/amateur musicians/non-musicians, right and left-handed people, and men and women. Also, variationsweredetected in people with high and low pitch perception ability, and in people with dyslexia and learning disabilities.

The search strategy is depicted in Figure 1.

Discussion

Variation of the Auditory Cortex

HG, which house the PAC, may exist as single, as partially duplicated (CSD: common stem duplication), as completely posteriorly duplicated (CPD), and as multiply duplicated (MD) HG,according to a study about the gyrification patterns and surface areas of HG in 430 healthy volunteers mapped with magnetic resonance imaging (2). Also, there are variations in the HG as far as the volume is concerned, the surface at specific parts, the distribution of white and gray matter, and the distance from the midline. As far as HG duplication is concerned, one study referred to the developmental trajectory of its morphology and the function of the superior temporal cortex in children, adolescents, and young adults. It showed that, morphologically, the superior temporal cortex shows cross-sectional variability in the number of HG, including the absence and complete duplication of the HG in both hemispheres (11). In fact, the frequency of duplications ranges from 20% to 60%, depending on the distance from the midline, according to a study reporting variations in frequency and location of the sulcal boundaries of HG in volumetric magnetic resonance imaging scans of 105 normal controls

aged 5-65years(12). Finally, CSD is more frequent than CPD, especially in the right hemisphere (12). As for the PT, the variations found were related to asymmetries in volume, the angle of the PT, its surface, the number of minicolumnstructures, and the length of the PT. In addition, variationsweredetected in the quantity of gray matter, in the ratio of white/gray matter, and in the distance fromthe midline.

Classification of Variations

Significant variations were observed in both the primary and secondary auditory cortices between the two hemispheres. First of all, a comparative study involving 42 infants, 8–19 months of age, 26 with normal hearing (NH) and 16 with sensorineural hearing impairment, using high-resolution 3D magnetic resonance imaging, showed that the left auditory cortex was larger than the right auditory cortex (13). The study's measurements of the volume of the PT and HG on the MRI scans of epilepsy patients indicated that there was more gray matter in the right hemisphere, while there was more white matter in the left hemisphere in the area of the auditory cortex(14). Generally, according to a study in 17 neurologically normal adults, surface asymmetry was found in the HGs as they were larger in the left hemisphere ($P<0.01$). The study concerns minicolumn spacing and number, and regional cortical volume and surface area measurements in the primary auditory region (HG) and the posterior auditory association region (PT) (15). Two experiments in a study, one regarding magnetic resonance (MR) scans of normal healthy volunteers, and the other MR data on normal, right-handed volunteers, showed that the PAC volume was larger in the left hemisphere compared to the right onedue to the larger volume of white matter ($P<0.0005$). The left PAC was found to be on average further forward and towards the midline. This fluctuation was about 5-8 mm (16). Nevertheless, regarding the surface of the HG, in a study conducted of 430 healthy brains, in the right hemisphere a medial decrease in the cortical surface area (CSA) and a lateral increase in the CSA in the CSD compared to a single HG were found (17).

The PT was observed to have significant asymmetry between the two hemispheres. More specifically, the left PT was larger in the left hemisphere than in the right by 0.9 cm ($P<0.001$) (left hemisphere 3.6 cm, right hemisphere 2.7cm), on the basis of a study involving 100 adult human brains, obtained postmortem and free of significant pathology (4). In fact, a study of the brains of 21 individuals free of known neurological and neuropathological abnormalities showed that the left PT was thin and elongated, while the right PT was short and thick, with a significant difference. This means that the left and the right PTs have approximately the same volume, although their surface area varies due to this morphology (18). Nevertheless, according to a comparative study of 76 young,nativeEnglish-speaking adults, the women's PT was symmetrical in both hemispheres, while men's PT was observed to be larger in the left hemisphere (19). In addition, as far as the surface area was concerned, there was a reduction in the CSA on the PT in instances of duplication in the left hemisphere (17). The right PT showed a greater inclination than the left ($P<0.01$) (14), i.e., the angle created by the gyrus was more acute. The left PT was larger than the right in 65% of brains, with greater frequency in right-handed people, according to a review study (20), while the right PT was larger than the left in only 11% of brains (4). As far as microanatomy is concerned, a greater distance between minicolumn structures was observed in the left PT ($P<0.03$) (15). Some authors argue that a greater quantity of gray matter is found in the left PT, compared to the right PT ($P<0.05$) (14). However, there was a greater amount of white matter in the total left auditory cortex, compared to the right.

Individuals with congenital deafness have a lower amount of white matter associated with HG and PT than those with NH, according to a study comparing 53 prelinguallyhearing-impaired-subjects with 51 non–hearing-impaired control subjects (21, 22). Thus, the auditory cortex ofhearing-impaired individuals had significantly

less white matter than that of hearing individuals. Since the amount of gray matter is the same inpeople with impaired hearing and with NH, the white-to-graymatter ratio was lower in the auditory cortex ofhearing-impaired people than in those with NH(2). Also, in general, the left superior temporal gyrus white matter was lower in thehearing-impaired people than inthose with NH(13). In non-hearing-impaired human beings, the PT shows volume asymmetry,which it does not in thehearing-impaired, to a statistically significant degree (P<0.0002).Furthermore, the angle in the PT is more acute in the right than in the left hemisphere in thehearing-impaired (P<0.07), but there is no differentiation in those with NH (P>0.05), as indicated by a clinical trial involving 12 right-handedhearing-impaired adults and 10 right-handed hearing adults (22) (23). Moreover, 42 infants aged 8-19 months, 26 with normal hearing (NH), and 16 with impaired hearing (IH) were involved in a relative study. SixteenIHinfants had increased gray matter and decreased white matter in the anterior HG (aHG) compared to the NH infants, while the typical pattern of asymmetry between the right and left hemispheres (L>R) was absent (13). These differences might cause auditory deprivation (13).

Professional musicians and musically trained children were reported to have increased gray matter volume in the HG and its repetitions, according to a study of 41 experienced musicians (24). More specifically, professional musicians showed 130% more gray matter (in volume) on the anterior and medial surface of the HG (amHG) than non-musicians (P<0.0001). Professional musicians showed 37% more gray matter in the HG overall compared to non-musicians, and 67% more gray matter in the aHG (P<0.001) (1). In general, a study comparing 20 professional male musicians and 20 amateur male musicians with a matched control group of 40 male non-musicians showed that more gray matter was observed in the musicians, particularly in the left HG (25). The activity in the PAC of musicians was 102% greater, compared to non-musicians, according to a clinical trial involving 37 right-handed adults with NH, 12 of whom were non-musicians, 12 were professional musicians, and 13 were amateur musicians (1). In addition, professional musicians showed 30% more white matter overall in the HG compared to non-musicians (P<0.05) (1). Regarding HG repetitions, the vast majority of musicians (90%) showed HG repetitions (CSD, CPD, MD) either in one (39%), with higher frequency in the left hemisphere, or both (51%) hemispheres. In 27% of musicians, multiple duplications of up to four curves of helices were found (24). When it comes to amateur musicians, they showed 60% more gray matter in the anteromedial and anterior sections of the HG (amHG and aHG) than non-musicians (P<0.01). Furthermore, there was no statistically-significant variation in the white matter volume between amateur musicians and non-musicians (1). Finally, no significant changes in the PT were observed in any of the comparisons (25).

In the right-handers, the right hemisphere had a higherprevalenceof CPD than CSD by 10%, while the left hemisphere had a higher prevalenceof CSD than CPD by 200% (2). Concerning both hemispheres, a duplication of HG appeared in 37% (approximately) of the left hemispheres and 49% (approximately) of the right hemispheres (24). Specifically: L1/R1: 36.2%, L1/R2: 27.2%, L2/R1:15.1%, L2/R2:21.5%. Moreover, in right-handed individuals, in the case of duplication, the aHG showed a significant reduction of 22% in the left hemisphere and 11% in the right hemisphere, regardless of the type of duplication (P<0.001). However, if there was duplication, the total area of the HG increased significantly in both hemispheres by 46% in the right hemisphere and 79% in the left hemisphere, to a statistically significant degree. Furthermore, in right-handed people, the posterior HG (postHG) surface area of the left hemisphere increased by 64% in the CSD compared to CPD.The surface of the right hemisphere increased by 42% (P<0.001), and the aHG surface area was greater in the left hemisphere in individuals with L1/R1 and L1/R2, while in individuals with L2/R1 and L2/R2, it was greater in the right hemisphere. Regarding overall HG surface area (totHG), L2/R1 and L1/R1 showed greater surface

area in the left hemisphere and L1/R2 in the right hemisphere, whereas L2/R2 showed no statistically significant variation between the two hemispheres (2).In left-handed people, the rates of variation in HG repetitions were: L1/R1: 49%, L2/R2: 19.2%, L1/R2: 39.4%, L2/R1: 30.8%. The L1/R1 variant was more frequent than L2/R2 (49% and 19.2% respectively). In addition, there were more individuals with duplication in the right hemisphere than those with duplication in the left (2). When the right-handed and the left-handed people were compared, it was observed that L1/R1 was more frequent in left-handed people than in right-handed people (49% and 36.2% respectively), to a statistically significant degree. L1/R2 was more frequent in the right-handers (48.7%) than in the left-handers (39.4%). L2/R2 and L2/R1 showed no statistically significant difference between the two hands used for writing. To a statistically significant degree, it appears that the percentage of left-handers who had the same number of HG helical structures in both hemispheres was higher than that of the right-handers (68.2% and 57.7% respectively) ($P<0.001$). Additionally, both aHG and totHG in the right hemisphere had a significantly larger surface area, by 28 mm2 and 20 mm2, respectively, in the left-handed compared to the right-handed subjects, with $P<0.05$. In fact, the right aHGwas larger in the left-handers regardless of the existence of duplications in the right HG. In the left aHG and totHG, there was no statistically significant difference in the surface section depending on the dominant writing hand. The aHG showed less asymmetry between the two hemispheres in the left-handers compared to the right-handers, while the total HG showed a significant increase in the right hemisphere in the left-handers, with $P<0.05$ (2). Finally, the PT showed statistically significantly less asymmetry between the two hemispheres in the left-handers compared to the right-handers according to a study examining 52 healthy volunteers (26).

Given that the female brain is smaller in volume than the male brain, in women the superior temporal gyrus has 17.8% more volume than in men, to a statistically significant degree ($P<0.04$), while the PT has 29.8% more volume in women than in men ($P<0.04$). These data relate to the overall volume of these brain zones. The HG in both men and women occupies the same volume in each of the two hemispheres [avg 2mL and 1.7 (men) 1.8 (women) in the left and right hemispheres, respectively] as demonstrated by a study involving 10 males and 11 females free from neurological or neuropathological abnormalities (27). However reverse asymmetry towards the right side (larger right hemisphere than left), compared to the known asymmetries towards the left side, was more frequent in women according to a study using an observer-independent cytoarchitectonic method (28). Finally, in terms of microanatomy, the PT minicolumn number showed greater asymmetry in men than in women, although the mean PT volume as a function of brain weight was greater in women (15).

Anatomical differences in the HG and PT were also observed in other categories of individuals. For example, in individuals with dyslexia, two HGs were observed at the inner level. The more aHG branched at some of the convolutions, while the more posterior HG remained as it was. In contrast, in individuals without dyslexia, one HG was observed in the internal plane, which moved anterolaterally and branched at some convolutions and therefore producedmore than one helix (anteriorly, not medially), according to a study of individuals with dyslexia from professional families and unaffected first- and second-degree relatives (6). In addition, while in women the PT is more symmetrical in both hemispheres and in men it shows increased size in the left hemisphere, in men with dyslexia there may be opposite asymmetry or an absence of asymmetry (19). The HG duplications (CSD, CPD, MD) increased in families with learning disabilities, while children with reduced comprehension-attention had smaller HGs and enlarged PTs, as indicated by a study investigating 111 children without developmental disorders and 21 children with attention deficit (hyperactivity) disorder AD(H)D (29). Furthermore, anatomical differences were also observed according to the ability to intonate syllables. The results presented

were for native English speakers learning a foreign language and trying to render the language with correct pitchpatterns. Thus, the gray matter of the left HG of those with slightly better intonation ability had a larger volume compared to that of those with greater difficulty in voice coloration (P<0. 05), while there was a small increase in the white matter that needs further research (P=0.047). Indeed, a study involving 17 young adult native speakers of American English who reported having no audiological, cognitive, neurological, or linguistic deficits, showed that the gray matter of the left HG shrank in volume in people who did not practice voice intonation, but increased in people with increased intonation ability (3). Finally, young children with poor vocal skills had significantly lower asymmetry than their peers with better vocal performance (12). In terms of age, the number of HG duplications in the right hemisphere was consistent across all age groups (11). Therefore, age does not affect the presence of HG duplications. Moreover, it seems that there are more HG duplications in boys with genetic resistance to thyroid hormones(12). As for deviation from the midline of the brain, an average deviation of 3 cm was found in the position of the auditory cortex grooves from their normal position. However, despite the variety of asymmetries, they showed firmness in both the right and left hemispheres: the frequency of duplication increases as the distance from the midline increases. Age, gender, and handedness are not strong enough factors to influence the diversity of asymmetries in terms of distance from the midline (12). Finally, in terms of microanatomy, more minicolumnswere found in HG than in the PT (P<0.01) (15).

Conclusions

There are several types of variations in both the primary auditory cortex (HG) and the PT, and they occur in different categories of people. Variationseven occur between the two hemispheres, with the left auditory cortex being overall larger than the right (13). Variations in the auditory cortexareseen betweenhearing-impaired and hearing people, while in musicians there is an increase in the gray matter volume of the HG (1). Variationsin the HG in individuals with good and reduced intonation ability suggest that the HG play an important role in intonation ability. In addition, reduced asymmetry was observed in females, left-handed people, and children with reduced language manipulation skills in some areas of the auditory cortex, while excessive asymmetry was linked to early musical ability (12). Moreover, variation associated with HG duplication may be related to the functions processed jointly within each hemisphere: temporal processing and speech processing on the left, and spectral and musical processing on the right (17). Finally, asymmetry is also related to the volume of cortical fibers carrying information to and from the auditory cortex (16), and this in turn affects hemispheric specialization.

What Is Already Known on This Topic:

The auditory cortex consists of the primary and secondary auditory cortex. According to the literature, many types of variation have been described which are correlated to certain categories of individuals and cognitive functions. Variations should be acknowledged because they can define and explain some crucial clinical issues.

What This Study Adds:

The study records anatomical variations of auditory cortex and categories those variations in correlation with their clinical significance. It is of paramount importance to understand how the plurality of anatomical variationsof the auditory cortex contributes to our comprehension of the origin of pathologies in the auditory cortex found in clinical practice in the field of neuroanatomy and neurosciences. The study focuses on the difference betweenhearing-impaired and normal hearing people.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Narrative Review
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Unlocking the Potential of Radiofrequency Ablation in Treating Hepatocellular Carcinoma Among Elderly Patients: A Literature Review

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Abstract

Objective. This study aimed to thoroughly assess and evaluate recent studies comparing radiofrequency ablation (RFA) and surgical resection in older patients with hepatocellular carcinoma (HCC). **Methods.** We searched the databases PubMed, Scopus, and Cochrane for articles published up to 31 October 2024. This review included studies comparing RFA and surgical resection in individuals with HCC aged 65 years or older. The exclusion criteria were non-human research, case reports, editorials, and studies involving patients with liver metastases or cholangiocarcinoma. **Results.** We found four retrospective cohort studies. The derived data showed no difference in one-year survival rates. However, the RFA group exhibited a better disease-free survival rate and a lower mortality rate than the surgical resection group. **Conclusion.** RFA outperformed surgical resection in terms of overall and disease-free survival rates while showing no appreciable variation in the occurrence of complications. However, this study underscores the need for more extensive research utilizing larger sample sizes, particularly in low- and middle-income countries.

Key Words: RFA ▪ Surgical Resection ▪ HCC ▪ Elderly Patients ▪ Literature Review.

Introduction

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and one of the most common and aggressive forms of cancer worldwide (1, 2). The prevalence of HCC varies geographically, with higher rates in regions such as East Asia and sub-Saharan Africa, largely due to the endemic nature of hepatitis B virus infections in these areas (2, 3). However, in developed regions such as Europe, the United States, or Japan, the hepatitis C virus (HCV) has been identified as a leading etiological factor for HCC (3). The relationship between HCC and HCV infection is well established. Studies conducted between 1992 and 2000 indicated that over 70% of patients with HCC tested positive for HCV, highlighting a strong connection between the virus and the onset of liver cancer. The process through which HCV causes HCC involves chronic inflammation and liver damage that can eventually lead to cirrhosis and, subsequently, the onset of cancer (4, 5).

The demographic profile of HCC patients is changing, with a notable increase in the number of older patients, especially in Japan. This trend can be attributed to several factors, including advancements in healthcare that have increased life expectancy, an aging population, and the increasing age of individuals infected with HCV. As these individuals age, their risk of developing HCC increases due to the prolonged latency period between HCV infection and the onset of liver cancer. The aging HCV-infected population poses a significant public health challenge, as it increases the number of older HCC patients and complicates treatment. Compared with younger patients,

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older patients frequently encounter additional health concerns and may have a reduced capacity to endure aggressive treatments. This trend has resulted in an increased demand for research into HCC treatments that are more suitable for elderly patients. Such research focuses on advancing less invasive therapies, personalized treatment strategies that consider the patient's overall health and specific cancer traits, and exploring new therapeutic agents with reduced side effects (6).

Physicians primarily treat HCC with transcatheter arterial embolization, percutaneous ablation therapy, and surgical resection. While surgery has long been viewed as the preferred method, its application is frequently limited by factors such as underlying liver cirrhosis or the existence of multiple tumors (7). Liver transplantation can be highly effective in certain cases. However, the limited supply of donor organs constrains the availability of liver transplantation (8). Radiofrequency ablation (RFA) has gained significant global popularity among non-surgical options. RFA is especially effective for early-stage HCC, providing a minimally invasive and highly curative treatment that is now recognized as a standard option alongside liver resection. However, recent guidelines have not fully recommended the use of RFA in place of surgical resection, which may be due to the lack of studies supporting its efficacy and safety (9, 10).

Elderly patients often present with multiple health concerns and are generally considered at higher risk for major surgical procedures (11). Recent studies have demonstrated that RFA can be performed in older HCC patients with satisfactory efficacy (12-15). However, the findings of various studies still differ. Peng et al. indicated that RFA demonstrated superior efficacy compared with hepatic resection in patients with HCC ≥3 cm (15) Guangzhou, China. Written informed consent was obtained from each patient before treatment. As an initial treatment, 89 patients were treated by RFA and 91 patients by HR. The survival curves were constructed by the Kaplan-Meier method and compared by log-rank test.\nRESULTS: The 1-, 3-, and 5-year overall survivals were 93.2%, 71.1%, and 55.2% for the RFA group and 88.8%, 62.8%, and 51.9% for the HR group, respectively (P = .305. Furthermore, a recent study by Kim et al. revealed that RFA exhibits a non-inferior therapeutic effect compared to resection (12). Yoo et al. published a systematic review of this topic encompassing a literature search up to March 2022, which also discusses that RFA has shown similar results to surgical treatment for earlier stages of HCC and is of peak interest as it may be a better management option for elderly patients who are more prone to surgical complications (14). However, recent studies have provided new evidence, so an updated meta-analysis is warranted.

Consequently, this study aimed to review and synthesize the latest research comparing RFA and surgical resection in older patients with HCC.

Methods

Information Sources and Search Strategy

We conducted a literature search using the databases PubMed, Scopus, and Cochrane. This study focused on identifying research that compares the efficacy and safety of RFA and surgical resection in elderly patients with HCC . The main outcomes assessed were efficacy, measured by overall survival or disease-free survival rates, and safety, evaluated based on postprocedural complications. We used the following key terms : "Elderly", "Hepatocellular Carcinoma", "Radiofrequency Ablation", and "Surgical Resection". We used the term 'elderly' as a key term since it aligns with commonly used terminology in gerontological research (≥65 years old). Figure 1 shows the study's PRISMA flowchart.

Study Eligibility Criteria

The two authors established the eligibility criteria. We defined the following inclusion criteria: (1) clinical trials as the research design; (2) RFA or surgical resection as the intervention; (3) elderly patients (≥65 years old) with HCC as the study population; (4) surgical resection as the comparison; and (5) outcomes, including safety

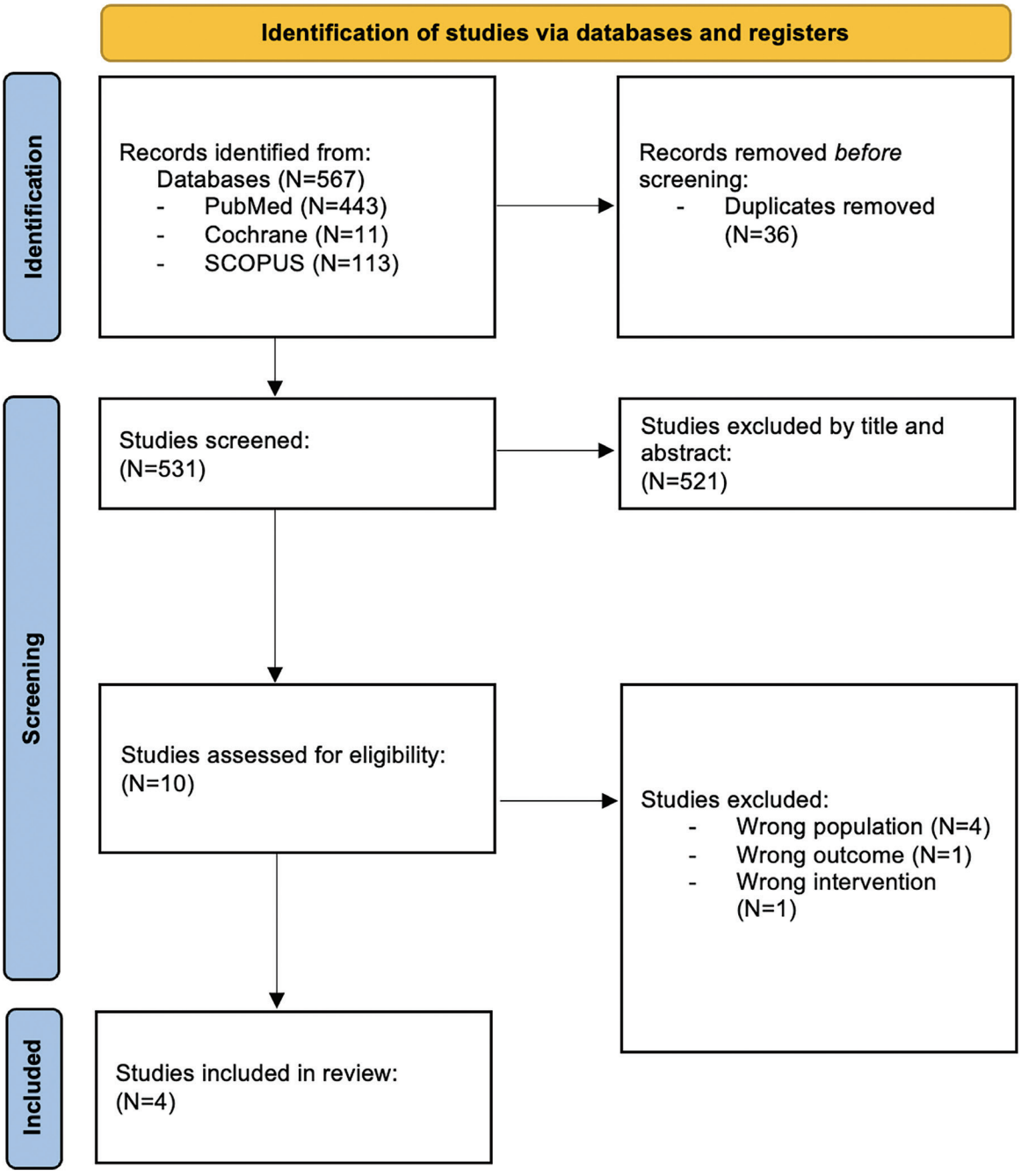

Figure 1. Flow diagram of the literature search strategy.

from complications and efficacy on the overall survival rate or the disease-free survival rate. We established the following exclusion criteria: non-human research (1), case reports (2), editorials (3), patients with liver metastases (4), and cholangiocarcinoma. . Table 1 summarizes the research eligibility criteria.

Table 1. Study Eligibility Criteria

Category	Inclusion Criteria	Exclusion Criteria
Participants	Elderly patients (≥ 65 years old) with HCC	Studies with patients <65 years old, patients with liver metastases, and cholangiocarcinoma
Intervention	RFA or surgical resection	-
Comparison	Surgical resection	-
Outcome	Safety from complications and efficacy on the overall survival rate or the disease-free survival rate	-
Study design	Clinical trials	Non-human research, case reports, and editorials

Data Extraction

The authors thoroughly documented the main conclusions and pertinent data from all the studies included in this review. The following information was acquired: (1) author and publication year; (2) study attributes, including design and setting; (3) study population, including sample size and average age; (4) intervention; and (5) study results, which include the evaluated parameters and significance (P-values). The two authors of this study created the data extraction criteria .

Results

Study Characteristics

This research included four retrospective cohort studies : two conducted in Italy, one in South Korea, and one in China. The studies were conducted between 2013 and 2024. Older individuals with HCC diagnosed with Child-Pugh class A or B, without evidence of hepatic metastases, participated in the included trials. The patients were categorized into two groups: the first group received RFA treatment, whereas the second group underwent surgical resection. In both groups, the assessed outcomes were effectiveness and the safety profile. Survival rates at one, three, and five years were used to assess effectiveness. Following each surgery, issues related to the safety profile were reported. We present a summary of the included studies in Table 2.

Overall Survival Rate

The data derived from the studies show no discernible difference in one-year survival rates between the two intervention groups. Despite variations in study design and patient populations, the collective findings suggest that both interventions do not confer a meaningful benefit in extending survival at the one-year mark. For example, Kim et al. (12) report a one-year overall survival rate of 95.6% for RFA and 98.6% for resection—a difference of only 3%. Similarly, Filippo et al. (16) report rates of 86% for RFA and 89% for resection. However, the three-year survival rate shown by Peng et al. (15) shows a significant association between RFA and enhanced survival outcomes, with RFA at 71.1% and resection at 62.8%. Peng et al.'s findings show that patients who underwent RFA had notably higher survival three years post-treatment than the surgery group. There were only two studies that assessed the 5-year overall survival rate. Both studies indicate that individuals who underwent surgical procedures may have significantly reduced survival probabilities over a five-year period.

Disease-free Survival Rate

Two studies were conducted to ascertain disease-free survival rates. The findings of the studies reveal a robust and statistically substantial impact of RFA compared to the surgical approach, which significantly enhanced the 1-year and 3-year disease-free survival rates. This minimally invasive technique improves overall survival and demonstrates a

Table 2. A Summary of the Included Studies

Author, year	Study location	Study design	Study population			Efficacy				Complications
			Characteristics	Sample size (N)	Mean age, years (SD)	Parameter	RFA (%)	Resection (%)	P	
Peng et al., 2013 (15)	Guangzhou, China	Retrospective cohort	Older people aged over 65 One HCC lesion with a diameter of 5.0 cm, or three HCC lesions with a diameter of less than 3.0 cm each No radiologic indication of an invasion into the main branches of the portal or hepatic vein No metastases outside the liver Lesions that are apparent on ultrasonography and that have a reasonable and secure passage from the lesion to the skin Class A or B Child-Pugh illness	RFA: 89 LR: 91	RFA: 70.4 (4.9) LR: 68.7 (3.3)	1-year survival rate 3-year survival rate 5-year survival rate	93.20 71.10 55.20	88.80 62.80 51.90	0.305	90-day mortality rate: RFA: 0% LR: 1.1% Pain (P=0.025) Grade 1 (RFA: 19, LR: 10) Grade 2 (RFA: 13, LR: 29) Grade 3 (RFA: 1, LR: 9) Fever (>38.5) (P=0.014) Grade 1 (RFA: 24, LR: 25) Grade 2 (RFA: 2, LR: 17) Ascites (P<0.001) Grade 1 (RFA: 0, LR: 5) Grade 2 (RFA: 0, LR: 8)
Conticchio et al., 2020 (13)	Multicenter: France, Spain, Switzerland, Italy	Retrospective cohort	Older people aged over 70 A single HCC with a diameter of less than 3 cm and no signs of extrahepatic illness or significant portal/hepatic vein branch invasion Completed LLR/RFA A or B Child-Pugh class	RFA: 98 LLR: 86	RFA: 75 (5.48) LLR: 75.7 (4.91)	1-year disease-free survival (DFS) 3-year disease-free survival (DFS) 1-year overall survival (OS) 3-year overall survival (OS)	66.40 38 90.10 67	89.90 67 96.30 90	0.001 0.001	90-day mortality rate (P=1.0) RFA: 3% LLR: 2% Liver failure (P=0.03) RFA: 1% LLR: 8% Ascites (P=0.15) RFA: 2% LLR: 6% Postoperative complications RFA: 15% LLR: 31%

Continuation of Table 2.

Author, year	Study location	Study design	Study population			Efficacy				Complications
			Characteristics	Sample size (N)	Mean age, years (SD)	Parameter	RFA (%)	Resection (%)	P	
Filippo et al., 2023 (16)	Multicenter: France, Spain, Switzerland, Italy	Retrospective cohort	Older people aged 80 and above HCC in BCLC stage 0/A, with a maximum diameter of 5 cm A maximum of three tumor nodules Lack of metastases outside the liver There is no radiological proof that the tumor has invaded the main portal or hepatic vein branches A or B Child-Pugh class	RFA: 37 LR: 65	RFA: 82.2 (2.5) LR: 82.4 (2.4)	1-year disease-free survival (DFS)	59.40	87	0.007	90-day mortality rate (P=0.707) RFA: 2% LR: 6% Liver failure (P=0.048) RFA: 0% LR: 12% Ascites (P=0.013) RFA: 0% LR: 15% Postoperative complications (P<0.001) RFA: 11% LR: 49%
						2-year disease-free survival (DFS)	50.70	76		
						3-year disease-free survival (DFS)	42.40	60.50		
						1-year overall survival (OS)	86	89	0.144	
						2-year Overall Survival (OS)	68	87		
						3-year overall survival (OS)	60.50	77		
Kim et al., 2024 (12)	South Korea	Retrospective cohort	Older people aged 65 and above Tumor size smaller than 3 cm HCC in BCLC stage 0/A	RFA: 225 LR: 141	RFA: 70 (4.44) LR: 68 (4.44)	1-year overall survival (OS)	95.6	98.6	<0.001	N/A
						3-year overall survival (OS)	82.7	91.5		
						5-year overall survival (OS)	67.9	83.8		

HCC=Hepatocellular carcinoma; RFA=Radiofrequency ablation ; LR=Liver resection; LLR=Laparoscopic liver surgery; BCLC=Barcelona Clinic Liver Cancer.; DFS=Disease-free survival; OS=Overall survival.

notable advantage in achieving disease-free intervals compared to surgical interventions.

Safety Profiles

According to Peng et al. (15), the surgical resection group exhibited a mortality rate of 1.1%, as one patient died during the same hospital stay. In contrast, no in-hospital mortality was reported for the RFA group. Additionally, according to Conticchio

et al. (13), the RFA group' s 90-day mortality rate was slightly higher than that of the surgical resection group (3% vs. 2%, respectively). However, Filippo et al. (16) discovered that the RFA group exhibited a lower mortality rate (2% vs. 6%) than the surgical resection group.

Discussion

RFA is a minimally invasive treatment option for various liver tumors, including HCC. By generating heat through radiofrequency waves, it induces coagulative necrosis, effectively destroying cancer cells. RFA involves inserting a small needle electrode into the tumor utilizing ultrasonic or CT scan guidance. Once installed, radiofrequency radiation is applied, causing ions to rapidly vibrate and generate heat, which destroys tumor cells within minutes. When a patient is not eligible for a liver transplant or has small, early-stage HCC that cannot be treated with surgery, RFA is highly effective. Additionally, it can be applied to individuals with multiple tumors or in conjunction with other therapies. RFA offers several benefits over conventional surgery, including reduced postoperative discomfort, shorter hospital stays, and faster recovery times (17).

Furthermore, Peng et al. (15) also found that 26 patients in the RFA group and 42 patients in the surgical resection group developed post-treatment fever, defined as an axillary temperature exceeding 38.5 °C after treatment (P=0.014). Thirty-three patients in the RFA group and forty-eight patients in the resection group required analgesics (P=0.025). Ascites, cardiac issues, liver failure, transient ischemic attacks, pulmonary infections, hepatobiliary disorders, pleural effusions, postoperative hemorrhage, and acute renal injury were among the other significant side effects that the hepatic resection (HR) group encountered. One patient in the RFA group was diagnosed with hepatobiliary disease and pleural effusion. The RFA group experienced significantly shorter hospital stays (8.01 days ± 2.70 days versus 13.50 days ± 4.05 days, P<0.001) than the HR group.

This study indicates that RFA is more effective than surgical resection in survival and disease-free survival rates for older patients with HCC. These findings are consistent with those of previous studies on non-elderly populations. According to Huang et al., resection and RFA demonstrate comparable efficacy regarding long-term survival rates and tumor recurrence in HCC patients (18). Numerous studies have demonstrated the effectiveness and safety of surgical interventions for HCC in older adults, with most indicating similar survival rates and safety profiles to those of younger patients (19-21). However, research specifically examining these aspects of RFA in older populations is limited. In a cohort of 1 000 patients treated with RFA, Tateishi et al. found no statistically significant difference in the 3-year survival rate between patients older than 68 years (76%) and those younger than 68 years (79.2%) (22).

In addition to the effectiveness of RFA , it can also be used in multi-modality with laparoscopy and thoracoscopy, which has been associated with a shorter hospital stay and a less invasive procedure. However, further studies may be needed to consider it the standard treatment for HCC (23). Although RFA has demonstrated beneficial effects, it is currently recommended primarily for patients who are ineligible for surgery due to comorbidities or a high risk of surgical complications. There are suggestions to combin e RFA with transcatheter arterial chemoembolization to prevent local and distant tumor rec urrence in patients with HCC with a diameter of >3 cm. Hence, RFA alone is not recommended for patients with HCC with a diameter of >3 cm, as it is related to poor prognosis of distant tumor recurrence, even though patients with good liver function (Child-Pugh class A) might be eligible for RFA treatment (24).

Strengths and Limitations

This review has both advantages and disadvantages. Direct comparisons were possible because of significant similarities in the outcomes of the included studies. However, the authors acknowledge that this study has several limitations. The included studies exhibit a considerable degree of heterogeneity. Therefore, future research should focus on conducting randomized controlled trials with larger sample sizes to generate more robust clinical recommendations.

Conclusion

In conclusion, RFA treatment offers similar overall and disease-free survival rates compared to surgical resection. Furthermore, there is no identifiable difference in the incidence of complications between the two therapies. This study recommends further research with larger sample sizes to achieve more definitive conclusions, particularly in low- to middle-income countries or regions with predominantly older populations.

What Is Already Known on This Topic:
Hepatocellular carcinoma (HCC) can be treated by transplantation, surgical resection, transcatheter arterial embolization, or percutaneous ablation therapy. Although surgery has traditionally been considered the preferred approach, its use is frequently limited by considerations such as underlying conditions. Radiofrequency ablation (RFA) has gained significant global popularity among non-surgical options.

What This Study Adds:
RFA treatment achieves comparable rates of overall and disease-free survival.

Authors' Contributions: Conception and design: DAA and DD; Acquisition, analysis and interpretation of data: DAA and DD; Drafting the article: DAA and DD; Revising it critically for important intellectual content: DAA; Approved final version of the manuscript: DAA and DD.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Series
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Multimodal Anesthesia-Analgesia for Patients with Huntington's Disease: A Case Series

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Abstract

Objective. The aim of this article is to demonstrate that the anesthetic challenges faced by patients with Huntington's disease (HD) undergoing major surgery, can be successfully managed using modern, opioid-sparing, multimodal strategies. **Case Report.** We present two case studies involving HD patients who received general anesthesia. The first patient also suffered from alcohol use disorder (AUD) and underwent thoracoscopic pleural biopsy. The second patient was scheduled for laparoscopic hemicolectomy. Due to the unavailability of ultrasound and excessive choreic movements, locoregional anesthetic techniques were not feasible. Both patients were successfully managed using similar opioid-sparing, multimodal anesthetic-analgesic strategies, and had uncomplicated postoperative courses. In both patients, a dexmedetomidine infusion was used, and both reported a brief amelioration of their chorea postoperatively. **Conclusion.** This is the first reported case of a patient with Huntington's disease with concurrent AUD undergoing general anesthesia using modern, opioid-sparing, multimodal, anesthetic-analgesic strategies. Even when the advantages of locoregional anesthesia are not available, HD patients can be safely and effectively treated using modern anesthetic methods that minimize opioid use and its associated side effects.

Key Words: Alcohol Use Disorder ▪ Chorea ▪ Dexmedetomidine ▪ Huntington Disease ▪ Non-opioid Analgesic.

Introduction:

Huntington's Disease (HD) is a rare condition affecting 5-7 per 100,000 people, characterized by the typical adult onset of irreversible motor, psychiatric and cognitive symptoms, and progressive neurodegeneration (1). The literature on the anesthetic management of patients with HD is scarce, mostly in the form of individual case reports (2-4), with most focusing on the safe use of various pharmacological agents, summed up in the review by Kivela et al. (1). Some papers illustrate the use of remifentanil (3) and dexmedetomidine (4), for improved patient outcome.

The objective of this article is to illustrate that HD patients undergoing major surgery can be effectively managed by implementing modern, opioid-sparing, multimodal strategies.

Case Reports:

We report the successful anesthetic management of two HD patients using different multimodal anesthesia-analgesia strategies. A 69-year-old male patient, weighing 78 kg, (BMI 25.45 kg/m^2), underwent thoracoscopic pleural biopsy and talc pleurodesis due to a large cyst on the left hemithorax. A 82-year-old female patient, weighing 61 kg, (BMI 26.4 kg/m^2) was scheduled for laparoscopic right hemicolectomy due to colon cancer. Both reported no family history of HD and it was confirmed by genetic testing. Their chorea mainly affected their upper body, causing moderate gait problems, and the female patient also reported depression treated with escitalopram, and dysphagia with frequent episodes of choking. Both patients displayed mood swings, treated with quetiapine

and benzodiazepines, respectively. Neither patient reported autonomic dysfunction upon evaluation using orthostatic Heart Rate (HR) and Blood Pressure (BP) tests. Patients reported excessive chorea in the upper body, rendering an epidural catheter placement perilous, due to probable accidental removal. Due to the unavailability of ultrasound, loco-regional anesthetic techniques were not feasible.

The male patient reported alcohol use disorder, which further complicated his anesthetic management. He also reported chronic obstructive pulmonary disease (COPD), stage 2B, noting poor compliance with his treatment. During preoperative evaluation, he exhibited wheezing. He was prescribed methylprednisolone 12.5 mg IV b.i.d, nebulized ipratropium and budesonide b.i.d., in order to reduce the incidence of postoperative pulmonary complications. His premedication included bromazepam 1.5 mg b.i.d., esomeprazole 40 mg b.i.d., domperidone 10 mg b.i.d., and he was instructed to take his quetiapine. He reported a history of abdominal aortic aneurysm repair surgery, after which he was on clopidogrel. The clopidogrel had not been discontinued, but the patient initially withheld that information. He admitted taking clopidogrel 3 days before, during the insertion of the intraarterial cannula. After careful consideration of the patient's bleeding risk, together with the patient's surgeon, it was decided to continue with the surgery due to the patient's advanced condition, as he had rapidly lost 7 kg in the month prior.

The patient reported a preoperative BP of 130/80 mmHg, HR of 89 bpm and SpO_2 of 96% in room air. Standard ASA monitors were used and an arterial 20G cannula was inserted in the right radial artery. Anesthesia was induced with fentanyl 2μg/kg, lidocaine 1mg/kg, propofol infusion using the Target Controlled Infusion (TCI) Schneider protocol set at 7μg/ml, and rocuronium 1 mg/kg for a modified rapid sequence induction. The patient was intubated with a left-sided Robertshaw double-lumen tube and placed in the right lateral decubitus position. One Lung Ventilation was then initiated, with Volume Control Auto-flow, Tidal Volume at 4 ml/kg ideal body weight, and respiratory rate at 16 bpm titrated by arterial CO_2. After a lung recruitment maneuver, the patient's PEEP was set at 6 mmHg, and FiO_2 at 40%, titrated by arterial PO_2. Anesthesia was maintained by TCI propofol infusion set at 4μg/ml, supplemented with a dexmedetomidine 0.5 μg/kg loading bolus over 10 minutes, followed by an infusion set at 1μg/kg/h. Pain management included parecoxib 40 mg, paracetamol 1g, dexamethasone 8mg, magnesium sulphate 2.5 g, morphine 2 mg and two additional boluses of fentanyl, for a total dose of 300 μg. An infusion of vitamin B complex was administered to prevent Wernicke's encephalopathy. After a 5-minute episode of hypertension that resolved with the administration of Clonidine 60 μg, the patient became hemodynamically stable, with BP and HR not deviating more than 20% from baseline. The operation lasted 50 minutes. Postoperative pain was managed with tramadol IV 100 mg t.i.d. and paracetamol IV 1 g t.i.d. Lorazepam 1 mg q.i.d. was prescribed in order to prevent acute alcohol withdrawal.

The female patient was premedicated with esomeprazole 40 mg b.i.d., and domperidone 10 mg b.i.d., and was instructed to take her escitalopram. She reported a preoperative BP of 136/73 mmHg, HR of 54 bpm, and SpO_2 of 99% in room air. Standard ASA monitoring was established and an arterial 20G cannula was inserted in her right radial artery. Anesthesia was induced with fentanyl 2μg/kg, lidocaine 1mg/kg, propofol 2mg/kg and rocuronium 1mg/kg, for a modified rapid sequence induction. Anesthesia was maintained by a dexmedetomidine 0.5μg/kg loading bolus over 10 minutes, followed by an infusion set at 0.7μg/kg/h, a lidocaine 0.5 mg/kg loading bolus followed by an infusion set at 0.7 mg/kg/h and desflurane set at approximately 1 MAC. Post-induction, mild bradycardia (47 bpm) with mild hypotension were treated with 2 boluses of ephedrine 10 mg. During the initiation of abdominal insufflation with CO_2 the patient suffered a brief episode of severe bradycardia (minimum Heart Rate: 32 bpm) that quickly resolved after pneumoperitoneum deflation and administration of atropine 0.5 mg. Insufflation was then gradually restarted without issues, with

no further instability. She was placed supine, in a 10° Trendelenburg position. Pain management included dexketoprofen 50 mg, paracetamol 1g, dexamethasone 4mg, ketamine 30 mg, magnesium sulphate 2.5 g, morphine 3mg and additional fentanyl, for a total dose of 200 μg. The operation lasted 110 minutes. Postoperative pain was managed with a PCA pump with morphine, ketamine and midazolam for 3 days, alongside paracetamol IV 1 g t.i.d.

Additionally, ondansetron 4mg was administered to both patients for nausea prevention. Their wounds were infiltrated with ropivacaine 0.75%, 1 mg/kg. Dexmedetomidine and lidocaine infusions were discontinued ten minutes before the end of surgery. Their surgeries were uncomplicated, and both were extubated following a bolus of sugammadex 2 mg/kg. They were observed in the post-anesthesia care unit, reporting minimal pain (Visual Analogue Scale = 1-2). Initially their chorea improved, but returned to baseline after approximately 1h. Both had uneventful postoperative courses and were soon discharged from hospital.

Discussion

HD is an autosomal dominant disease, caused by a trinucleotide (CAG) repeat expansion in the huntingtin gene on chromosome 4, producing a mutant protein, with an extended polyglutamine repeat (5). Microglial activation triggers an inflammatory response in these patients, causing neurodegeneration and cellular apoptosis, leading to HD's clinical manifestations (5). One of its most characteristic motor symptoms is chorea, which consists of involuntary, excessive movements that progress from facial twitches to whole-body movements. With disease progression, chorea gives way to bradykinesia, rigidity and akinesia. Due to pharyngeal muscle involvement, patients have an increased risk of choking and aspiration (3-5). The use of prokinetics that act on central dopamine receptors, such as metoclopramide, should be avoided, as they can exacerbate chorea (1). Conversely, domperidone, characterized by lower brain exposure (6), has not been reported to cause adverse effects in HD patients.

Autonomic Dysfunction is reported in some sources (7) as a frequent complication of HD; however, it has not yet been reported for patients with HD undergoing anesthesia (1). HD patients' response to succinylcholine is contradictory in population studies, with some describing atypical cholinesterase genotypes in HD patients, and others showing similar genotype distribution to normal controls (1). Increased sensitivity to barbiturates and benzodiazepines among patients with HD is noted in older reports, however, the dosages described were likely excessive (1).

The literature on the anesthetic management of patients with HD is quite scarce and mostly in the form of individual case reports (3, 4, 8) and retrospective reviews (1). Total Intravenous Anesthesia (TIVA) techniques with propofol and remifentanil have been demonstrated in HD patients, with the rapid recovery of airway reflexes reported.

In our study, both patients received dexmedetomidine, resulting in brief postoperative chorea alleviation, in accordance with other case reports (4, 8). Both patients were managed using modern opioid-sparing, anesthetic-analgesic strategies, and had uneventful postoperative courses. Such approaches may help reduce post-thoracotomy pain syndrome incidence (9). By using dexmedetomidine, intravenous lidocaine, magnesium, ketamine, dexamethasone, paracetamol and Non-Steroidal Anti-Inflammatory Drugs, it is possible to effectively stabilize the sympathetic nervous system and reduce inflammation and postoperative pain, whilst minimizing the use of opioids and their associated side-effects (10). Opioid-sparing approaches offer additional advantages in the context of Alcohol Use Disorder (AUD), due to the expected higher effective dosage of opioids and associated side-effects (11). We suggest that these modern anesthetic approaches can be used safely in HD patients presenting for major surgery, with added benefits such as chorea amelioration.

Conclusion

To our knowledge this is the first case report of HD patients under general anesthesia using multimodal, opioid-sparing anesthetic strategies. Such tailored approaches may enhance patient outcomes, while reducing opioid-related side effects.

What Is Already Known on This Topic:

HD is a rare disorder and thus anesthetic management of HD patients is primarily documented in individual case reports. Most of these focus on the safe use of various pharmacological agents, with insufficient data to propose an ideal anesthetic strategy. Since 1966, fewer than 40 HD patients have been documented as having received general anesthesia. Moreover, these incidences of general anesthetics relied on older, opioid-based, anesthetic techniques.

What This Study Adds:

In contrast to previous reports, both patients in this study were managed using a modern clinical approach, with opioid-sparing, anesthetic-analgesic strategies, and had an uneventful postoperative course. Even when locoregional anesthesia is not feasible, tailored approaches, with newer anesthetic techniques, can optimize patient outcomes while minimizing opioid use and the related side-effects.

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Informed Consent: Informed consent was obtained from the patients for the publication of this case report.

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Case Report
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Immunosuppressive-Free Renal Allograft Function After Allogeneic Hematopoietic Stem Cell Transplantation: A Case Report

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Abstract

Objective. We describe a rare case of satisfactory renal allograft function without immunosuppressive therapy following allogeneic hematopoietic stem cell transplantation (alloHSCT). **Case Report.** The patient was a 64-year-old male who had undergone a kidney transplant from a sibling donor in 2007. After 16 years, he required alloHSCT for acute myeloid leukemia (AML), with the same sibling serving as the donor for both transplants. HLA was a 50% match. Post-alloHSCT, immunosuppressive therapy was discontinued, and the renal allograft function remained stable. The patient later developed severe complications and succumbed to infection. Insights into the precise tolerance mechanisms were limited because laboratory evaluation for chimerism was not performed. **Conclusion.** There is potential for immunosuppressive-free renal allograft function after alloHSCT. This case underscores the significant risk of infection-related mortality. To achieve the best outcome, rigorous patient selection, tailored conditioning regimens, robust infection prevention strategies, and the possibility of combined transplantation for carefully selected patients are needed.

Key Words: Kidney Transplantation ▪ Allogeneic Transplantation ▪ Immunosuppression.

Introduction

The best way to treat chronic kidney failure is a kidney transplantation from a living or deceased donor. Successful maintenance of graft function requires immunosuppressive therapy, the use of which has unwanted effects. These include: susceptibility to infections, the rise of malignant diseases, de novo diabetes, dyslipidemia, neurotoxicity, and chronic allograft nephropathy due to the impact of immunosuppressive drugs (1). If the patient somehow develops transplantation tolerance, there is no need for immunosuppressive therapy.

Transplantation tolerance is the stable acceptance of a transplanted organ without ongoing immunosuppression. It represents a critical milestone in transplant medicine. Several mechanisms for achieving tolerance exist, such as durable hematopoietic chimerism and regulatory T-cell therapy. The coexistence of donor and recipient hematopoietic cells within the recipient's immune system is hematopoietic chimerism. In this situation, an environment where the transplanted organ is recognized as "self" is created. Combined kidney and alloHSCT is a unique therapeutic strategy that may induce transplantation tolerance. At the same time, the long-term complications of immunosuppressive therapy are avoided. This type of transplantation, combined kidney and alloHSCT transplantation, is rarely performed and is typically limited to patients with concurrent renal and hematologic conditions. The first described cases were in patients with multiple myeloma.

Patients with combined kidney and alloHSCT transplantation have highly variable outcomes that are influenced by different factors, such

as: pre-transplant conditioning regimens, HLA compatibility, and recipient health status (2, 3). Aggressive conditioning regimens can lead to renal allograft damage and infections; malignancies remain significant risks.

As far as we know, there have previously not been any patients with combined transplantation of kidney and alloHSCT in Bosnia and Herzegovina.

We present the first case of a patient who underwent allogeneic hematopoietic stem cell transplantation 16 years after a kidney transplant from a living relative.

Case Report

We present the case of a male patient, 64 yrs old, whose primary renal disease was unknown, and who spent five years on hemodialysis before a living related kidney transplant was performed in 2007. After his kidney transplantation, he was regularly monitored by a nephrologist, and his immunosuppressive regimen consisted of cyclosporine (100 mg and 75 mg) and mofetil mycophenolate 250 mg 2×4. He was on steroid-free protocols of immunosuppressive therapy after careful evaluation. His levels of cyclosporine were kept in the range of 100-150 μmol/l during regular checkups. He did not want to switch to tacrolimus therapy because of personal reasons.

On January 2023 the patient was admitted to hospital due to malaise, weight loss and profuse sweating. A complete hematological work-up was performed: bone marrow aspiration puncture with myelogram, immunophenotype, and cytogenetic analysis, showing that it was a case of acute myeloid leukemia/AML M4-M5/FAB. It was mutually agreed that the patient should be prescribed induction chemotherapy according to the Idarubicin/Cytarabin 3+7 protocol. A previous assessment of renal allograft function was performed. He was discharged on day 23 of therapy and recovered. Aspiration puncture control was performed, and disease remission confirmed. After that, the first consolidation with high-dose cytarabine was prescribed. A nephrologist was consulted for correction of the immunosuppressants. HLA typing was performed for the recipients in Sarajevo, and for the donor procedure, it was performed in a transplantation center abroad. The results of HLA typing are shown in Table 1. The patient was transferred to a center abroad for allogeneic bone marrow stem cell transplantation. This was performed in May 2023 with a preconditioning regimen consisting of cyclophosphamide and busulfan, but data about the intensity and dosage of the preconditioning regimen were impossible to obtain.

Table 1. HLA Typing for Donor and Recipient before AlloHSCT

HLA Locus	Donor Alleles	Recipient Alleles
HLA-A	02	02, 26
HLA-B	50	38, 57
HLA-C	06	06, 12
HLA-DRB1	04	11, 13
HLA-DQB1	03	03, 06

After the procedure, his immunosuppressive maintenance therapy for kidney allograft was halted, and he was followed up at our center from September 1, 2023. According to the nephrologist's recommendations, he was not on immunosuppressive therapy for his renal allograft. His immunosuppressive therapy before and after alloHSCT is shown in Table 2.

Table 2. Immunosuppressive Therapy before and after AlloHSCT

Time Point	Medication	Dosage
Pre-alloHSCT	Cyclosporine	100 mg morning /75 mg evening
Pre-alloHSCT	Mycophenolate Mofetil	250 mg twice daily
Post-alloHSCT	None	N/A

On one occasion after that, in November 2023, he was hospitalized at our center, due to bicytopenia and a drop in hemoglobin values. A hematological work-up was performed, and no relapse of the disease was confirmed during this hospitalization. His laboratory results are shown in Table 3.

Table 3. Laboratory Results from January to December

Laboratory values	January	September	November	December
Creatinine (μmol/l)	95	89	112	97
Urea (mmol/L)	10.8	6.2	5.3	18
Uroprotein (g/d)	0.17	0.47	-	2.2
Creatinine clearance (ml/min)	110	95	-	94
White blood count (109/L)	68	3.8	5.76	3.4
Red Blood count (109/L)	2.92	2.55	2.17	2.81
Hemoglobin (g/L)	91	99	88	88
Platelet (109/L)	83	130	75	33
CRP (mg/L)	27.7	11.4	74.2	112

Three weeks later, he was re-hospitalized due to febricity. Upon admission, blood samples, as well as urine and sputum were obtained. Physical inspection revealed ascites. Diagnostic ultrasound (US) and computed tomography were performed. The ascites was evacuated several times. According to the US scan, a polypoid mass on the stomach was verified, but gastroscopy did not confirm this due to the patient's deteriorating condition. Color Doppler ultrasound of the renal allograft showed normal findings. Trends of laboratory values are shown in Figure 1. Urine output was satisfactory.

Viral testing, including cytomegalovirus (CMV) PCR and Epstein-Barr virus (EBV) PCR, were performed with negative results. Microbiology results showed the presence of Klebsiella pneumoniae ESBL and Acinetobacter baumannii in his sputum and Escherichia coli in his urine. Broad-spectrum antibiotic therapy, including carbapenems and colistin, was introduced. Despite the therapy, the patient's condition continued to deteriorate, and three weeks after admission and 24 weeks after the alloHSCT, he died. No post-mortem examination was conducted.

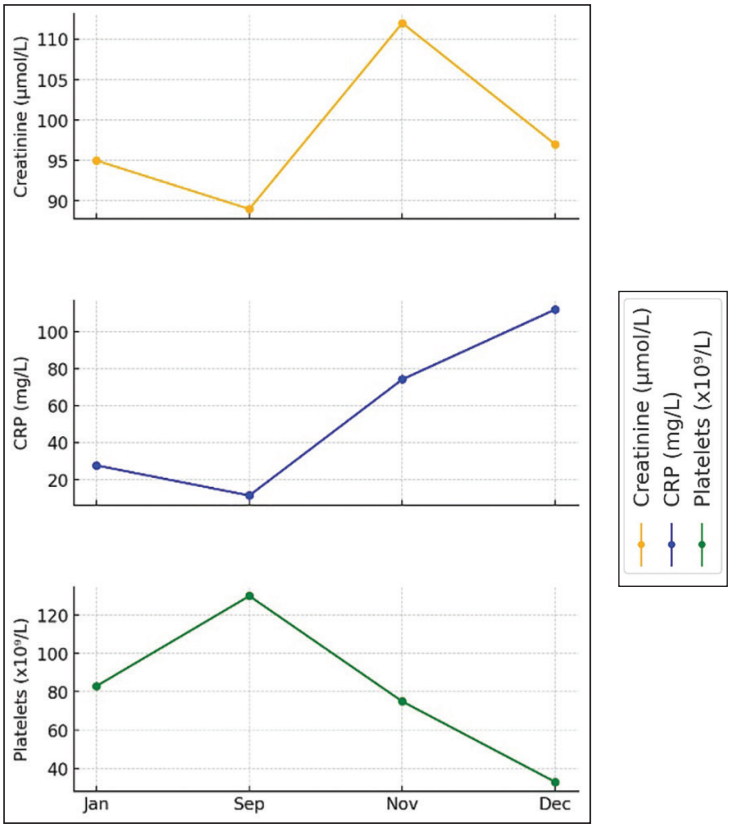

Figure 1. Trends of key laboratory values from January to December 2023.

Discussion

Proper function of a renal allograft is possible without immunosuppression in patients after bone marrow transplantation and is the result of newly established transplantation tolerance. That is: "the lack of destructive immune responses to a transplanted organ in the absence of immunosuppression", as first described by Oven et al. (4) and later by Medawar et al. (5). So far, the

most promising ways to achieve successful transplantation tolerance in humans are regulatory cell therapy and hematopoietic chimerism. We present a case highlighting the convergence of kidney transplantation and alloHSCT as a therapeutic strategy. Also, this case emphasizes the challenges associated with long-term outcomes, such as infection-related complications.

However, to achieve tolerance and durable chimerism, it is necessary to determine the state in which donor hematopoietic cells are present in the recipient organism (6). This may be achieved by allogeneic bone marrow transplantation (BMT) or allogeneic hematopoietic stem cell transplantation (HSCT) (7). To accomplish any chimerism, a preconditioning regimen is necessary. Preconditioning consists of myeloablative regimens in which high-dose total irradiation is used in combination with chemotherapeutic drugs. Still, this approach carries the most significant risk of developing complications: infections and graft versus host disease (GvHD). In the absence of malignant disease, the risk of GvHD is not justified, so different non-myeloablative regimens have been developed (8).

Solid organ transplantation is a unique challenge. Immunosuppression could lead to the development of end-stage organ failure and malignancies, which require a second transplant. One to three percent of patients with liver and kidney, 3–9% of heart and lung transplant patients, and up to 10% of intestinal or multi-organ transplant patients develop post-transplant lymphoproliferative disease (PTLD). Acute myeloid leukemia develops in 0.2% of recipients of solid organs (9). Our patient belonged to the small group of patients who developed acute myeloid leukemia after 16 years of immunosuppressive therapy.

The outcome of patients with combined transplantations depends on several factors: 1) the histocompatibility of the transplanted organs, 2) the sensitivity of the solid organ graft to conditioning the regime necessary for HCT and the effects of long-term immunosuppression, infection complications, and relapse rate after HCT (10). The donor of hematopoietic stem cells (HSC) usually has an HLA that matches the recipient, not the transplanted organ. Therefore, alloHSCT can lead to graft rejection. If the donor of the stem cells and the solid organ kidney are the same, tolerance may develop.

The most studied mechanisms of transplantation tolerance involve hematopoietic chimerism and regulatory T-cell (Treg) activity. In this case, the donor was the same for the renal allograft and the hematopoietic stem cells. This resulted in a shared immune environment. In a situation where both donor and recipient hematopoietic cells coexist, mixed chimerism develops, where the immune system recognizes the renal graft as "self," thereby preventing rejection. Expansion of donor-derived Tregs may suppress alloreactive T-cell responses. That further contributes to tolerance. Although Treg function was not evaluated in this case, existing studies suggest their critical role in preventing graft rejection in immunosuppression-free conditions. Also, preconditioning regimens may lead to the depletion of alloreactive T-cells or render them inactive, reducing the possibility of rejection. Despite the absence of immunosuppressive therapy, the stable renal function in this patient post-alloHSCT suggests the successful integration of these mechanisms. It should be stressed that the lack of solid evidence, such as laboratory confirmation of chimerism or Treg profiling, limits definitive conclusions. Future cases should include these evaluations to better explain the mechanisms underlying tolerance.

Studies with combined transplantation are scarce, and results are sporadically reported (11). This reflects differences in patient selection, transplant protocols, and post-transplant management. The European Society for Blood and Marrow Transplantation reported on 28 patients with combined transplantation, of whom 12 patients had a transplanted kidney. Patients with a transplanted kidney were more prone to organ failure compared to those with liver transplants. The overall survival (OS) was 75% at 3 months, 60.2% at 12 months, 45.1% at 36 and 40.1% at 60 months. The European Society for Blood and Marrow Transplantation study highlighted infection-related complications

as the leading cause of mortality. It was mainly within the first year post-transplant. These findings align with our case, where the patient died due to sepsis six months after alloHSCT. Also, the same study noted that loss of graft function was the second most common cause of mortality, emphasizing the challenges of maintaining long-term renal allograft function in patients with combined transplantation (12). Preserved renal graft function without immunosuppressive therapy, observed in our patient, underscores the potential for tolerance in such cases. However, the lack of chimerism testing in this case limits a more profound understanding with complete evidence.

A study from Japan reported on 19 patients with combined hematopoietic stem cell transplantation (HSCT) and solid organ transplantation, where nine patients had a kidney transplant. Eight of the kidney transplant patients experienced renal dysfunction during and after HSCT, with a worsening of renal function within one year after transplantation. All of them required dialysis, and three of them experienced rejection of the renal allograft. Five of the nine patients died within the first year after HSCT. In this study, 5-year OS in patients undergoing alloHSCT was 41.7% for non-malignant diseases but just 23.1% for those with a malignant disease. Median survival time was 10.7 months for all patients (13). In our case, the patient did not need any form of renal replacement therapy, and he was without any sign of malignancy, which is different from the Japanese study. His survival was shorter than the average median survival time reported by Japanese authors: 7 months vs. 10.5 months (13). This difference may be attributed to several factors, such as the conditioning regimen (cyclophosphamide and busulfan) used in our case, and HLA matching. The Japanese study highlighted a high incidence of rejection in kidney recipients, a complication not observed in our patient. This could be explained by the fact that the same donor for both the kidney and alloHSCT facilitated a degree of immune tolerance.

The latest French study presented excellent overall survival of 94% for a 5-year period, with excellent kidney graft survival (14). The survival rates and complications reported in European and Japanese studies show critical differences in patient outcomes based on geographic regions, transplant protocols, and post-transplant care. The French study demonstrates the potential for excellent outcomes with proper patient selection and optimized care, contrasting data from European and Japanese studies, which reported high early mortality due to infections and graft rejection.

The patient we have presented died 24 weeks after allogeneic HSCT transplantation due to infection complications. This result correlates with previous findings from The European Society for Blood and Marrow Transplantation. His kidney allograft function remained stable without immunosuppressive therapy (12). This highlights the critical need for robust infection prevention and management strategies in the early post-transplant period.

The main limitation of our case is the absence of laboratory analyses to confirm chimerism, such as B and T lymphocyte testing. Those tests would provide direct evidence of tolerance mechanisms. In this case, the absence of an established infection monitoring protocol limited the ability to manage complications preemptively. Strategies, such as routine viral load and microbiological testing with prophylactic antimicrobial therapies, could mitigate this risk in future cases. Also, no postmortem examination was performed to identify the exact cause of death. Without autopsy, there was no possibility to determine the extent of residual disease and infection. This article contributes to the body of evidence by demonstrating the feasibility of maintaining stable renal graft function without immunosuppression, even with early mortality due to infection.

Conclusion

In conclusion, we have provided a report of a rare case of a patient with acute myeloid leukemia who successfully underwent alloHSCT from a related donor 16 years after having received a related kidney transplant. Later, he died due to infectious complications. Our experience, in this case, supports the findings from European data of the high

risk of death in the first year post-alloHSCT due to infective complications. This case underscores the need for personalized approaches in cases like this. We recommend several key procedures for future practice in similar cases. First, donor-recipient compatibility should be maximized. Preferably, the same donor should be used for kidney and alloHSCT transplants. Infection management should include comprehensive screening for latent infections (e.g. CMV, EBV), a prophylactic strategy, and a rigorous prophylactic antimicrobial regimen (e.g., fungal, bacterial, and viral infections). Routine microbiological surveillance should be standard practice to enable early infection detection and intervention. Post-transplant monitoring should be done weekly during the first three months, and it is essential to address complications promptly. Chimerism monitoring is obligatory to understand the tolerance mechanism and for guidance for immunosuppressive management. To ensure ongoing graft stability, an assessment of creatinine, proteinuria, and Doppler ultrasonography should be performed monthly. Our case shows that combined transplantation is feasible, and this approach could be used but with high caution, and in a highly selected group of patients.

What Is Already Known on This Topic:

Combined kidney transplanttion is done in rare circumstances and in highly selected patients. These cases are rarely reported. Mortality is high, mainly in the first year post-transplant and due to infections.

What This Case Adds:

There have not been any reports to date of patients with combined kidney and alloHSCT in Bosnia and Herzegovina, and this is the first report of that type. Even with post-transplant complications that developed after the second transplantation, the patient had a normal-functioning renal allograft. This is an example of how tolerance was developed and how a renal allograft could be functional without maintenance immunosuppressive therapy.

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Case Report
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A Rare Case of Ruptured Heterotopic Pregnancy after Natural Conception Demanding Immediate Attention

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Abstract
Objective. We present the case of a ruptured heterotopic pregnancy after natural conception and its management during hospitalization. **Case Report.** The patient presented to the emergency department of our hospital with symptoms of lower abdominal pain and vaginal blood loss. She reported a confirmed intrauterine pregnancy at 8 weeks' gestation following natural conception. The patient was admitted to the hospital with the diagnosis of a ruptured ovarian cyst. Due to hemodynamic instability, an urgent exploratory laparotomy was performed. The histological review showed an ectopic fallopian pregnancy, confirming the final diagnosis of an heterotopic pregnancy. **Conclusion.** Heterotopic pregnancy is a very rare condition, even more so if it happens spontaneously. As a result, it has been insufficiently studied in relation to proper management and timely diagnosis.

Key Words: Eterotopic Pregnancy ▪ Ruptured Adnexa ▪ Pregnancy ▪ Ruptured Cyst ▪ Abdominal Pain.

Introduction

A globally acknowledged complication of Assisted Reproduction Techniques is the phenomenon of ectopic pregnancy. Heterotopic pregnancy is a category of ectopic pregnancy that is present at the same time as an intrauterine pregnancy. Its rate of occurrence in a natural pregnancy is very low, at about 1/10,000 – 1/50,000. Assisted Reproductive Technology elevates this percentage to 1/100 – 1/3600. 1% is not unheard of (1). Generally, well-known risk factors for the existence of an ectopic pregnancy are congenital uterine anomalies (1). Specifically, the embryo transfer of four or more embryos carries a great risk of a heterotopic pregnancy occurring. Distorted anatomy also raises the risk of development (2). The sooner the diagnosis is established, the easier it is to prevent lethal complications. Seventy percent of heterotopic pregnancies are diagnosed during the first 8 weeks' gestation, and the rest during the subsequent 3 weeks (1).

Ectopic pregnancies have the highest chance of presenting in the fallopian tubes, with the cornu coming second. That is also true for heterotopic pregnancies as a result of employing Assisted Reproduction Techniques. The most commonly found risk factors include assisted reproduction, ovarian hyperstimulation, abortion, pelvic inflammatory disease, or previous ectopic gestation with salpingectomy (3). Patients afflicted by an heterotopic pregnancy usually present with nondescript symptoms, such as abdominal pain or pain in the pelvic area. The painful feeling may radiate to the diaphragm or the shoulder, as a result of bleeding occurring in the abdomen. The expulsion of pieces of the decidua may cause spotting (4).

Detecting an heterotopic pregnancy poses several difficulties. Imaging techniques employing contrast agents are usually avoided due to insufficient literature data on the effects on the fetus

(5). An ultrasound scan (either transvaginal or abdominal) used to monitor a normal pregnancy will visualize an intrauterine pregnancy and may fail to show an ectopic one, misrepresenting it as a corpus luteal cyst. Beta human chorionic gonadotropin (B-hcG) levels will rise as in a normal pregnancy, thus causing this diagnostic option to be unreliable (6).

We present the case of a ruptured heterotopic pregnancy after natural conception, and its management during hospitalization.

Case Report

The patient, at 29 years old, presented to the emergency department of our hospital with symptoms of lower abdominal pain and spontaneous vaginal blood loss with blood clots. She reported a confirmed intrauterine pregnancy at 8 weeks' gestation following natural conception. Her obstetric history included a vaginal birth and an abortion for personal reasons. There were no post-abortal complications. Her medical history included hypothyroidism and appendectomy 15 years previously.

The patient was examined for basic clinical signs and they were found to be normal (blood pressure 132/68, heart rate 76 bpm). Vaginal inspection with a speculum showed blood clots with no apparent active hemorrhage. The patient's abdomen was tender on palpation. Blood tests were performed as per standard practice and showed the patient's hematocrit to be 23.9%, prothrombin time/international normalized ratio (PT/INR) 0.94 and she was blood type 0 positive. An ultrasound scan showed an intrauterine pregnancy of Crown-Rump Length: 19,7 mm, gestational age: 9w 2d, FHR: 136 (Figure 1) as well as a cystic formation, 27 × 21 mm, on the left adnexa (Figure 2), and significant free fluid in the pouch of Douglas (Figure 3).

The patient was admitted to the hospital with the diagnosis of ruptured ovarian cyst. As the patient presented with hemodynamic instability while hospitalized (blood pressure 84/46, heart rate 116 bpm), an urgent exploratory laparotomy was performed. During the operation, a significant amount of hemoperitoneum and a ruptured gestation sac on the right fallopian tube were detected. The peritoneal cavity was washed with sterile

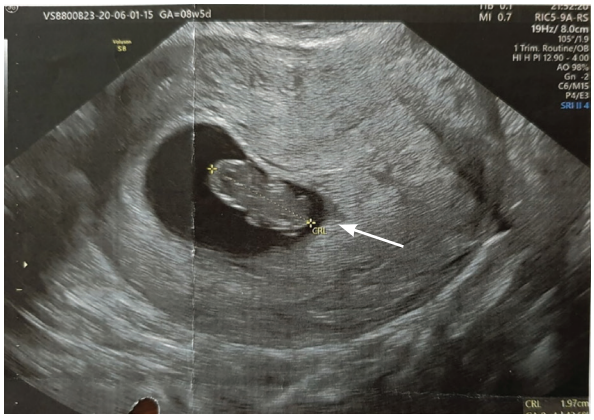

Figure 1. Intrauterine pregnancy Crown-Rump Length: 19.7 mm.

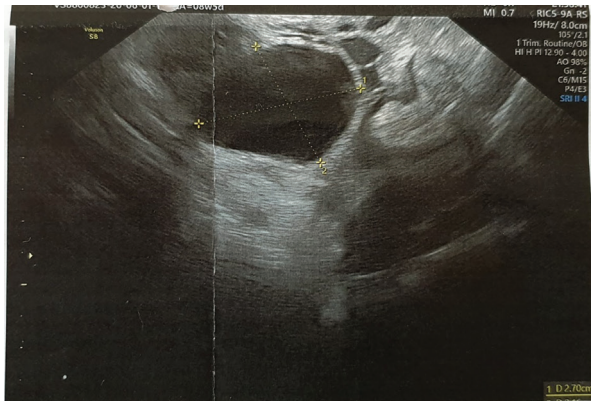

Figure 2. Left adnexa cyst 27 × 21 mm.

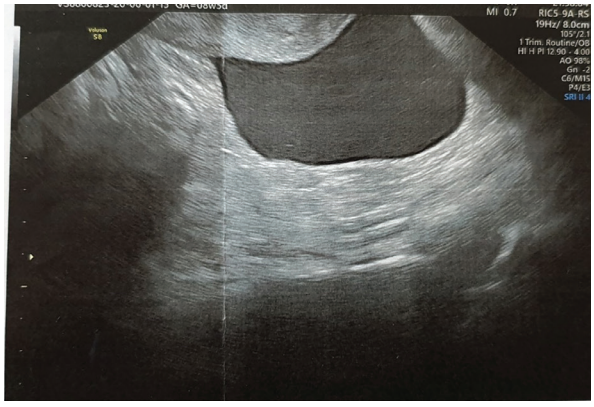

Figure 3. Free fluid in the pouch of Douglas.

saline solution. The right fallopian tube was arrested, ligated and removed alongside the ruptured sac. The uterus and the other adnexa were examined and no abnormalities were found. Complete hemostasis was achieved. A penrose catheter was installed. Intraoperatively, the patient received two blood bags of transfusion. At the end of the operation, a transvaginal ultrasound was performed, and the well-being of the intrauterine pregnancy was ensured.

The post-operative observation was uneventful. The patient was administered IV fluids and broad spectrum antibiotics. Stabilization of the hematoctrit was seen in blood tests. No post-operative complications were detected. No additional transfusion was deemed necessary. Penrose drainage was less than 100mL. The patient was discharged 3 days later in good condition. Prophylactic progesterone support was given, 100 mg × 2 for a week.

A month later, the patient attended for a follow-up examination. An ultrasound scan was performed and no pathological signs or free fluid were found in the peritoneal cavity. The pregnancy continued normally and the patient attended the hospital for a nuchal translucency scan at 12 weeks' gestation. The histological review showed that the sample was an ectopic fallopian pregnancy, confirming the final diagnosis of an heterotopic pregnancy.

Discussion

A natural heterotopic pregnancy is an extremely rare phenomenon. It is common for patients with a ruptured heterotopic pregnancy to be diagnosed during laparotomy, if it is not seen on an ultrasound scan. A gestation in the uterine appendages may be misdiagnosed as an hemorrhagic corpus luteum or an ovarian cyst, if it is noticed at all (7). Spontaneous abortions are a common occurrence to patients with heterotopic pregnancy. The chance for a live birth is 30% lower than in women carrying a normal intrauterine pregnancy. Due to the increased difficulty of the proper diagnosis, there is a great risk of maternal or fetal death (8).

Although difficult, as mentioned, it is possible to detect an heterotopic pregnancy. A mass in the adnexa at the same time as an intrauterine pregnancy gives rise to suspicion of corpus luteum cysts or a heterotopic pregnancy, among other possibilities. The detection of a positive fetal heart rate in the adnexal formation is a sure sign of an ectopic pregnancy. Combining these signs with free fluid in the peritoneal area indicates the diagnosis of a ruptured heterotopic pregnancy (7).

Treatment of heterotopic pregnancy is controversial in the scientific community. The uncommonness of the condition increases the difficulty of shared guidelines in management. Treatment methods that have been used are surgical management by laparotomy or laparoscopy, expectant management, or aspiration of the ectopic sac under sonographic guidance. Aspiration can use drugs that euthanize the embryo in order to reaffirm the corrective course of the ectopic pregnancy (9).

All possible treatments have their positive and negative aspects. Expectant management can avoid the pitfalls of more invasive treatment, however, it is not an option for patients who are hemodynamically unstable or those that present with symptoms, such as abdominal pain or blood loss. For patients in whom expectant management is not an option, surgical treatment is preferred, where physicians have to balance the gain of complete removal of the heterotopic pregnancy with the increased chance of abortion of the intrauterine pregnancy. Another option is aspiration of the ectopic gestation sac under sonographic guidance, possibly with the use of drugs that euthanize the embryo. This is a minimally invasive technique, but highly dependent on the location of the heterotopic embryo, and should be offered as an option only if the the sac is distinctly visualized (10).

Conclusion

Heterotopic pregnancy is a very rare condition, even more so if it happens spontaneously. As a result, it has been insufficiently studied, regarding proper management and timely diagnosis. It is the

authors' belief that the recording of more cases of this phenomenon, as well as further research on preventing its occurrence, will contribute to better understanding and management.

What Is Already Known on This Topic:

A globally acknowledged complication of Assisted Reproduction Techniques is the phenomenon of ectopic pregnancy. Heterotopic pregnancy is a category of ectopic pregnancy that is present at the same time as an intrauterine pregnancy. Its rate of occurrence in a natural pregnancy is very low, at about 1/10,000 – 1/50,000.

What This Study Adds:

We present this case of a ruptured heterotopic pregnancy after natural conception and its management during hospitalization. Heterotopic pregnancy is a very rare condition, even more so if it happens spontaneously. As a result, it has been insufficiently studied, in relation to proper management and timely diagnosis.

Authors' Contributions: Conception and design: DG and CB; Acquisition, analysis and interpretation of data: KK and P-KK; Drafting the article: DG and IC; Revising it critically for important intellectual content: ID and FA; Approved final version of the manuscript: VB and NK.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report
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Case Report: Agranulocytosis in a Child Following Metamizole Use – A Diagnostic Challenge

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Abstract

Objective. This case report describes a young female who developed agranulocytosis with blast cells in peripheral blood following prolonged metamizole use after ankle surgery. **Case Report.** A 17-year-old female patient was admitted to the Department of Infectious Diseases due to high fever and sore throat. Initial diagnostics revealed agranulocytosis, followed by occurrence of blast cells and left shifted neutrophils in the peripheral blood, in subsequent days. Extensive further diagnostics were performed due to suspicion of leukaemia, which was excluded after flow cytometry and cytogenetic analysis of bone marrow aspirate. After all tests were completed, the patient disclosed that she had been using metamizole for four months following ankle surgery. **Conclusion.** In cases of agranulocytosis, involving a prolonged history of metamizole use accompanied by the presence of blast cells and granulocyte precursors in peripheral blood, we would recommend an initial diagnostic approach that includes a complete blood count with differential and flow cytometry of peripheral blood. Bone marrow aspiration may be postponed or deemed unnecessary if peripheral blood flow cytometry shows no aberrant populations and there are no other signs of leukaemia.

Key Words: Metamizole ▪ Agranulocytosis ▪ Leukaemia ▪ Flow Cytometry ▪ Blast Cell.

Introduction

Metamizole, or dipyrone, is a non-opioid drug commonly used for its antipyretic, antispasmodic and analgesic properties. It exhibits favourable gastrointestinal, cardiovascular and cerebrovascular tolerability in comparison to non-steroidal anti-inflammatory drugs (NSAIDs) (1, 2). Metamizole is used widely across numerous countries, and in Slovenia it is listed among the most prescribed medications. Furthermore, the prescription of metamizole has been increasing in recent years (1-3). Its administration is also included in the guidelines for postoperative pain management in children provided by the Pain Committee of the European Society for Paediatric Anaesthesiology (4). Despite its widespread use, metamizole has been banned in some countries due to its potential for severe adverse effects, such as agranulocytosis (1). Agranulocytosis is a severe form of neutropenia, characterized by an absolute neutrophil count less than 0.2×10^9/L, without any affect on other two cell lineages (5). This life-threatening condition poses a significant risk for severe infections, and commonly manifests within two months of initiating metamizole treatment, with one third to half of the cases occurring within the first week of treatment (1, 6). The mechanism of metamizole-induced agranulocytosis is not yet fully understood. Likely contributing factors include an immune mediated mechanism, and the direct toxic effects of metamizole and its metabolites on granulocyte precursors (5). The estimated incidence of metamizole-induced agranulocytosis varies significantly across studies, with some reports indicating a rate as high as one case per 1,439 prescriptions (6).

This report presents a case of agranulocytosis, most likely induced by metamizole use, with the unusual presence of blast cells in the peripheral blood, as a probable result of bone marrow regeneration and acute infection.

Case Report

A 17-year-old female patient presented at the Outpatient Clinic of the Infectious Diseases Department, complaining of high fever and a sore throat. These symptoms had persisted for the previous week and failed to improve despite phenoxymethylpenicillin therapy. Physical examination revealed pale skin and typical signs of upper respiratory tract infection. She had no history of significant illnesses, but had undergone ankle surgery five months earlier. She denied taking any medication regularly. The laboratory tests showed elevated C-reactive protein (CRP), leukopenia and moderate anaemia (Table 1). She was prescribed amoxicillin and sent home.

Two days later, on follow-up, left-shifted neutrophilic maturation with the presence of promyelocytes, myelocytes, metamyelocytes and blast cells, was reported in a peripheral blood smear (Figure 1).

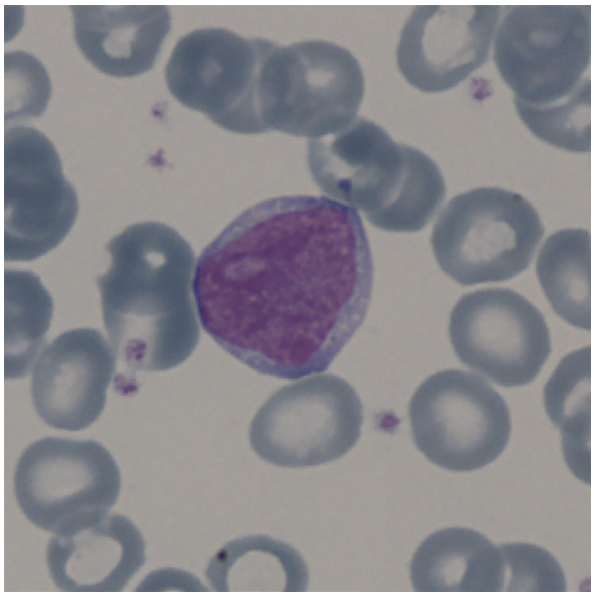

Figure 1. Blast cell in peripheral blood smear.

Table 1. Laboratory Results

Laboratory test [unit]	Outpatient clinic visit	Follow-up visit*	Discharge†	Follow-up visit‡	Reference range
Leukocytes (10^9/l)	0.8	1.6	12.6	5.5	4.2 – 10.8
Erythrocytes (10^{12}/l)	4.05	3.85	4.33	4.32	3.90 – 5.15
Haemoglobin (g/l)	97	95	108	1.7	120 – 154
Thrombocytes (10^9/l)	502	585	612	396	150 – 410
Blast cells (%)	NA§	5	0	0	-
Promyelocyte (%)	NA	2	10	0	-
Myelocyte (%)	NA	6	20	0	-
Metamyelocyte (%)	NA	2	12	0	-
Band neutrophil (%)	NA	4	4	0	-
Segmented neutrophil (%)	NA	10	22	55	36 – 77
Eosinophils (%)	NA	0	0	0	0.5 – 5.5
Basophils (%)	NA	0	0	0	0.00 – 1.75
Lymphocytes (%)	NA	61	26	40	20 – 44
Monocytes (%)	NA	9	5	5	1.5 – 9.0
Plasma cells (%)	NA	1	1	0	-
Band neutrophil (10^9/l)	NA	0,1	0,5	0	-
Segmented neutrophil (10^9/l)	NA	0.2	2.8	3.0	1.2 – 7.9
CRP (mg/l)	226	NA	NA	NA	< 8

*Two days after outpatient clinic visit; †Four days after hospitalization; ‡Two months after discharge; §Not assessed.

Due to the suspicion of leukaemia, she was admitted and underwent detailed diagnostic evaluation. A bone marrow aspiration was performed. Morphological examination showed hypercellular bone marrow with decreased erythropoiesis, decreased megakaryopoiesis and increased granulopoiesis. The myelopoiesis-to-erythropoiesis ratio was 11:1 (normal ratio 3:1). The blast cell count was within the reference range (<5%), and Auer rods were absent. One third of all the mononuclear cells were hypergranular promyelocytes, however maturation of the myelocytes beyond the promyelocyte stage was present. Flow cytometry revealed less than 1% of progenitor (CD34+) cells, with no aberrant lymphoid or myeloid populations detected in the bone marrow. Karyotyping and molecular genetics were negative for rearrangements typical for lymphoid and myeloid proliferation, such as BCR::ABL1 ((t(9;22)) and PML::RARA (t(15;17)).

After the bone marrow aspiration test, the patient revealed a history of regular metamizole use, three times daily for the first two months following her ankle surgery. Her complete and differential blood count (CBC DIFF) prior to ankle surgery was reviewed, showing no abnormalities. Following two months of regular metamizole use, she continued with occasional use for following two months. The metamizole treatment was stopped one month before the admission to our hospital. After consideration of additional anamnestic data and previous negative testing, she was diagnosed with agranulocytosis and increased bone marrow production, which could potentially be attributed to prolonged metamizole use and recent infection. At discharge, her condition was stable with a slightly elevated blood leukocyte count, and the mild anaemia persisted with an evident left shift, although no blast cells were detected. At the two-month follow-up appointment, complete resolution was observed, and her laboratory tests were unremarkable. No additional work up or follow-up was necessary.

Discussion

The patient was prescribed metamizole for postoperative pain after her ankle surgery. However, the patient did not have regular blood checks during her metamizole use, despite the fact that this is recommended when it is administered for extended periods (7). She did not inform her primary care physician nor the physician at the outpatient clinic about her prior metamizole use. As previously emphasized by Hoffmann et al., patients and healthcare workers must be aware of the risks associated with metamizole use. It is essential to seek medical attention promptly when a patient is using metamizole and symptoms of infections occur. The patient should be evaluated and treated by specialists experienced in managing febrile neutropenia. Furthermore, if a patient is still taking metamizole, it must be discontinued immediately (2, 5). The lack of awareness in this case endangered the patient's safety, and contributed to delayed diagnosis of agranulocytosis. Since drug-induced agranulocytosis may manifest after a prolonged interval from the last dose of metamizole, it is crucial to obtain detailed anamnestic data and provide preventable action (2). If it is clinically assessed that the patient can be discharged to home care, they must be specifically advised upon discharge to seek immediate medical attention if any signs of new infection appear (5).

The detection of blast cells in the peripheral blood during a follow-up visit was a particularly critical finding, suggesting a possible malignant disease such as acute leukaemia (5). In our experience, almost all children with the presence of blasts in their peripheral blood, are diagnosed with acute lymphoblastic leukaemia or have preleukemia. However, it is important to note that blast cells can also be present in newborns with low birth weight, in individuals with Down syndrome, and in severe cases of sepsis. More important than the proportion of blasts in the peripheral blood is their interpretation in accordance with the clinical presentation. However, any presence of blasts should be evaluated by a haematologist (5, 8). When interpreting blast cells in a peripheral blood smear, the concomitant presence of other cells is important to consider in the differential diagnosis. If blast cells are accompanied by granulocyte precursors, this primarily suggests

regenerative proliferation (9). This case scenario was seen in our patient, as an evident left shift was present in the peripheral blood smear. A useful parameter for differentiating agranulocytosis from leukaemia and other haematological disorders is also haemoglobin concentration. In agranulocytosis, anaemia is generally absent or, when present, only mild. In contrast, other haematological disorders accompanied by neutropenia typically exhibit more pronounced anaemia (5). In our case, moderate anaemia was observed. Consequently, since abnormalities in multiple blood cell lineages increase the suspicion of leukaemia, bone marrow aspiration was performed to exclude the diagnosis. On the basis of the findings of the bone marrow examination, the diagnosis aligned more closely with recovering bone marrow, a condition that might be related to the patient's prolonged metamizole use and concurrent infection.

Conclusion

While the use of metamizole has been on the rise, an inadequate understanding remains among both patients and physicians regarding its potential severe adverse effects, such as agranulocytosis. Given the potentially fatal outcomes of agranulocytosis, it is crucial for patients who develop clinical signs of infection during or after metamizole administration to seek immediate medical assistance from specialists experienced in managing febrile neutropenia. Healthcare professionals should promptly initiate suitable diagnostics and treatment. Additionally, discontinuation of metamizole is essential to prevent further complications. However, accurately diagnosing this condition can sometimes be particularly challenging. As evidenced by our case, the presence of blast cells, which are not typically seen in drug induced agranulocytosis, can mimic the manifestations of leukaemia. In the case of a history of metamizole treatment, we would recommend a more rational initial approach that involves CBC DIFF and flow cytometry of peripheral blood. If the flow cytometry does not reveal any aberrant lymphoid or myeloid populations, an invasive bone marrow analysis may be postponed or considered unnecessary. Subsequent patient management should include careful monitoring, with additional diagnostic interventions undertaken only if there are indications of disease progression, particularly if any signs suggestive of evolving leukaemia occur.

What Is Already Known on This Topic:

Metamizole is a widely used non-opioid analgesic and antipyretic. Despite its widespread use and inclusion in guidelines for postoperative pain management in children, it has been banned in some countries due to the risk of severe adverse effects, most notably agranulocytosis. Agranulocytosis is a potentially life-threatening condition due to a significant risk for infections and is known to occur within two months of initiating metamizole therapy. Although the incidence of metamizole-induced agranulocytosis varies, its potential severity underscores the need for careful monitoring of patients on prolonged treatment.

What This Study Adds:

This study emphasizes the diagnostic challenges of metamizole-induced agranulocytosis, particularly when atypical findings, such as peripheral blast cells, mimic leukaemia. It highlights the importance of thorough patient history-taking, regular blood monitoring during prolonged metamizole use, and awareness among healthcare providers of delayed manifestations of agranulocytosis. The study proposes a rational diagnostic approach involving CBC DIFF and flow cytometry to minimize invasive procedures, while ensuring timely and accurate diagnosis, thereby improving patient safety and management.

Authors' Contributions: Conception and design: AP, TP, JJ and ATB; Acquisition, analysis and interpretation of data: AP, TP, JJ and ATB; Drafting the article: AP; Revising it critically for important intellectual content: AP, TP, JJ and ATB; Approved final version of the manuscript: AP, TP, JJ and ATB.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report
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Genetic Analysis of Osteosarcoma Cells in a 9-year-old Boy: Genes Involved in Cell Cycle Control

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Abstract

Objective. This study describes mutations of genes that stimulate and regulate cell growth, programmed cell death, DNA repair, and cell growth suppression in a boy with osteosarcoma. **Case Report.** We report a case of bone sarcoma in a 9-year-old boy with possible familial predisposition. In our patient, only a subset of tumor cells expressed the ATRX protein, which is known to control the expression of several genome regions. The function of the p53 protein, which acts as a transcription factor that regulates the DNA damage repair response, cell cycle progression, and apoptosis pathways, is lost in 40-50% of malignant cells. Retinoblastoma was positive in the predominant subset of tumor cells. Deletion is found on chromosome 9, cytoband 9p21.3, where the genes for CDKN2A and CDKN2B are located. Neoplastic cells were SATB2-positive in a substantial subset, with nuclear staining. The SATB2 protein is a DNA-binding protein involved in transcriptional regulation and chromatin remodeling. Chromosomal losses of 8p and 19q11-q13.43 were also found. These regions contain several tumor suppressor genes, including *NKX3.1*, whose reduced expression correlates with 8p loss in high-grade tumors. Although there was no known cancer syndrome in the family, the maternal grandfather had a similar tumor requiring amputation. **Conclusion.** Chromosomal instability is a hallmark of osteosarcoma and is characterized by heterogeneous and extensive genetic complexity. Various numerical and structural genomic rearrangements have been described in cancer cells. However, there is little consistent genetic change to understand the etiopathogenesis of this aggressive tumor.

Key Words: Osteosarcoma ▪ Regulation ▪ Genes ▪ Mutation ▪ Tumorigenesis.

Introduction

Osteosarcoma (OS) is one of the most common primary bone tumor in children and adolescents, although its incidence is very low (1, 2). The timing of tumor diagnosis in patients coincides with the developmental growth spurt that occurs in this patient population (3), indicating a possible role for the growth hormone-insulin-like growth factor axis in the development and progression of osteosarcoma (4). On average, 4.4 cases of osteosarcoma are diagnosed per million children per year (5, 6). Statistical data show that this number has remained unchanged over the past few decades, while the introduction of multi-agent chemotherapy in the 1980s significantly reduced mortality (7). About 80% of patients present with grossly localized disease (8). Surgical resection following induction chemotherapy is the standard for local control of osteosarcoma. Systemic therapy with high-dose methotrexate, including adriamycin, cisplatin, and ifosfamide (MAP), demonstrated a five-year survival rate of about 60% (9). If the tumor is resectable, radiation therapy is not applied as a first-line definitive treatment approach because osteosarcoma is not a radiosensitive disease. The primary localization is in the metaphysis of the long bone, most often in the femur, tibia, and humerus, although cases with multifocal lesions have also been described (10). The tumor is

derived from bone-forming mesenchymal cells. Histologically, the presence of different malignant mesenchymal cells that produce the bone stroma characterizes this mesenchymal tumor. Several histological subtypes of osteosarcoma have been defined, including osteoblastic, chondroblastic, fibroblastic, and telangiectatic (11). Although the origin of the tumor cells is uncertain, scholars believe that the malignant transformation occurs in osteoblasts or preosteoblasts (12, 13). The results of recent research suggest that malignant transformation occurs at the level of multipotent mesenchymal stem cells that differentiate into bone-differentiation lineages (14, 15).

Although osteosarcoma is a sporadic disease, in a small number of cases, it occurs as a component of a hereditary cancer syndrome, which includes Li-Fraumeni syndrome, retinoblastoma, Rothmund-Thomson syndrome, and Bloom's and Werner's syndromes, with individuals inheriting germline inactivating mutations of the respective genes (16, 17). Genetic analysis of osteosarcoma cells in the 9-year-old boy presented here discusses a possible mechanism of tumorigenesis and a possible familial predisposition.

Materials and Methods

Neoplastic tissue was obtained from the right tibial mass by a fluoroscopy-guided core biopsy and a core needle biopsy. Tissues were formalin-fixed and paraffin-embedded. Cytospins of the right distal tibia mass cyst fluid were stained with hematoxylin and eosin (H&E) and Wright-Giemsa. The cell block was stained with H&E. The histologic preparations were reviewed by the responsible pathologist and found to be adequate in terms of the quality of fixation, processing, microtomy, and H&E staining. Appropriate positive and negative controls for special stains, immunohistochemistry, and/or in situ hybridization showed the expected reactivity. Immunohistochemical testing and special stains, as applicable, were performed, and the performance characteristics were developed by the Anatomic Pathology Laboratory at St. Jude Children's Research Hospital (SJCRH) in Memphis, United States of America. Immunostaining for SATB2 was performed at Mayo Clinic Laboratories and reviewed at SJCRH. All tumor specimens were evaluated histopathologically for diagnostic purposes and to assess specimen adequacy.

The Archer VariantPlex Custom Panel assay is used to identify variants within the selected target region(s) only. A negative Archer VariantPlex targeted sequencing test result does not rule out the presence of variants at a level below the sensitivity of detection. This test will not detect variants in areas outside the targeted genomic regions, nor will it detect copy number alterations and translocations. The test is not intended for minimal residual disease testing. This test evaluates variants in both tumor and germline tissue(s) and may not be able to distinguish between somatic and germline variants. Only pathogenic or likely pathogenic variants are reported, and only canonical splice sites (exon +/- 2 bp neighboring intronic regions) are evaluated. The test does not report variants categorized as being of uncertain clinical significance, benign, or likely benign.

Analyzed Genes

Gene symbol	Reference sequence	Target exons
RB	NM_000321	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27
TP3	NM_000546	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11

Tech: LK.

Analyte Specific Reagent Notification

The Pathology Laboratory at SJCRH developed this test and determined its performance characteristics. The test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The use of Analyte Specific Reagents does not require FDA approval [21CFR809.30].

Fluorescence in Situ Hybridization (FISH)

Target Probe CDKN2A: Locus 9p21 (laboratory developed). Control Probe: Locus 9q31.2

(laboratory developed). Target Probe CDK4: Locus 12q14 (Empire Genomics) orange. Control Probe: Locus CEP 12 (Empire Genomics) green. Scoring Method: Manual. Images captured and processed by GenASIs software, ASI (Applied Spectral Imaging).

History of Present Illness

A 9-year-old boy presented for evaluation of a 5.5 cm × 3 cm × 3 cm bone mass in the distal right tibia that had grown over the preceding month. The patient and his family provided the history. The boy had developed right ankle pain and swelling three months prior. His primary care physician referred him to orthopedics. The boy was found to have a lytic lesion on XR that was thought to be a bone cyst (Figure 1).

An MRI was obtained (Figure 2), and a biopsy was recommended. A routine pre- and post-contrast MRI of the right tibia and fibula was performed. No definite diffusion restriction was identified, although diffusion images were limited due to signal loss in the presence of calcified/ossified tissue. Out-of-phase imaging showed no definite signal loss within the tumor, suggesting the absence of microscopic fat.

The heterogeneously enhancing, expansile lesion in the distal metadiaphysis increased in size over the interval. It measured 2.9 cm × 2.8 cm axially. The cortex showed thinning, and marrow edema extended to the adjacent tibial diaphysis. The lesion appeared to have multiple cystic components without appreciable fluid-fluid levels. There was no appreciable soft tissue component. The lesion was highly suspicious for telangiectatic osteosarcoma.

According to outside hospital records, a lack of insurance prevented the scheduling of a biopsy. The patient was placed in a boot, but pain and swelling continued to worsen. Due to severe pain, the family called 911, and the emergency room evaluated the patient. The MRI and XR were repeated and showed interval growth of the right distal tibial mass. Orthopedics was consulted and referred the boy for further evaluation due to the concerning findings on imaging. The patient was using Motrin and Tylenol, but the pain sometimes

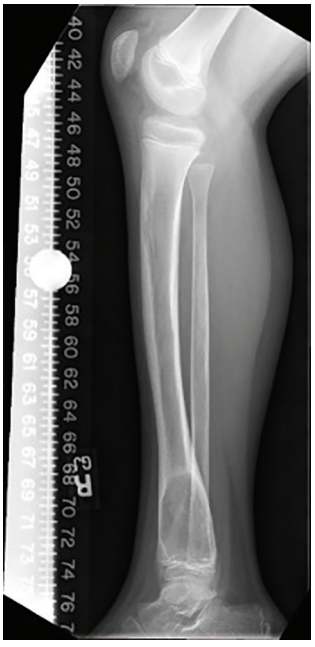

Figure 1. XR of the tibia/fibula right. A lytic lesion within the distal tibia primary, involving the metaphysis and abutting the physis.

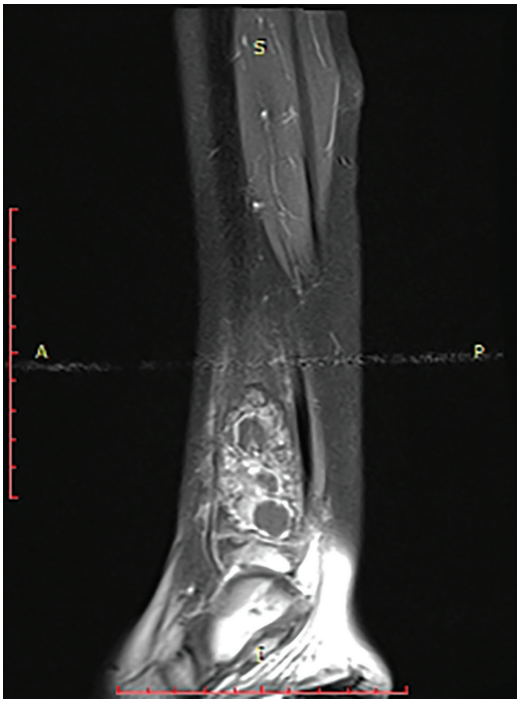

Figure 2. MRI of the right tibia/fibula.

awakened him from sleep. He was given a morphine prescription, which helped him rest better. He denied any trauma (prior to or since the onset of pain). He had no fevers, night sweats, weight loss, bony pain, fatigue, or malaise, nor did he experience changes in the color of the foot, numbness, or tingling. Imaging demonstrating an enlarging cystic lesion raised concern for telangiectatic osteosarcoma, while aneurysmal bone cyst (ABC) was also possible. Giant cell tumor was also considered, as air-fluid levels were not visualized on the MRI. He was started on a nurse practitioner training program (NPTP) with MAP chemotherapy. Following the first 5 weeks of MAP, imaging revealed no obvious evidence of disease progression, and the patient exhibited clinical evidence of response with reduced pain and localized soft tissue swelling. He developed significant hearing loss after the first cycle, and cisplatin was held; he received week 6 doxorubicin alone and was due for week 9 high-dose methotrexate (HDMTX). A month later, the right distal tibial mass underwent a biopsy. After surgery, due to hearing loss, the therapy was changed to an OS99 regimen with carboplatin, ifosfamide, and doxorubicin. The patient reported less pain and was using crutches or a wheelchair and avoiding bearing weight.

Perinatal, Social, and Family History

The boy was born after a full-term, uncomplicated pregnancy. He was breastfed, had normal early childhood development, and lived with his mother and father, two brothers, and two sisters. To date, he had received early childhood vaccines, but the family was not certain whether he was "up to date". He had not received COVID-19 or flu vaccines. The boy did not suffer from varicella or herpes simplex (fever blisters). There were no medical conditions in the family. His four siblings were healthy. His mother and father, who are cousins, were also healthy and without any medical problems. There were no genetic disorders or cancers that ran in the family. The patient's maternal grandfather had "this tumor" in his leg in childhood, which resulted in the amputation of his ankle and foot.

Results

A CT of the chest (Figure 3) showed clear lungs and no pleural or pericardial effusions. There was no adenopathy in the chest. The visualized portions of the thyroid and upper abdomen were unremarkable. There was no evidence of pulmonary metastatic disease.

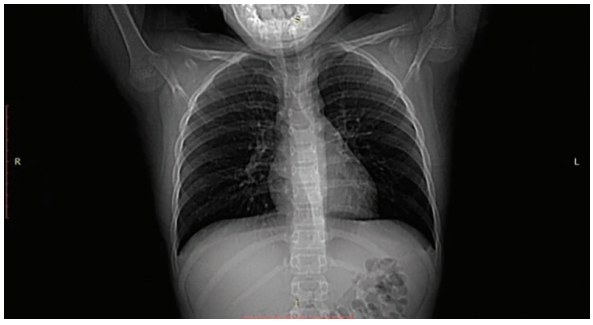

Figure 3. A chest CT revealed a hazy ground glass opacity along the minor fissure in the lateral segment of the right middle lobe.

Positron Emission Tomography (PET) imaging (Figures 4A and 4B) showed an expansile lesion of the distal right tibia, demonstrating significant hypermetabolic activity. This is nonspecific as hypermetabolic activity may be seen with giant cell tumors, aneurysmal bone cysts, and sarcoma. Hypermetabolic right inguinal and iliofemoral lymph nodes were noted. These were indeterminate and may have been reactive/inflammatory, especially considering the recent biopsy. However, metastatic disease could not be excluded.

The pathology of the biopsy sections revealed a hypercellular neoplasm composed of large cells with pleomorphic nuclei, abundant eosinophilic cytoplasm, and indistinct cell borders (Figure 5). Many tumor cells contained multiple large, hyperchromatic nuclei. Other tumor cells had smaller nuclei with granular chromatin and one to several small nucleoli. Frequent mitoses were present, including markedly enlarged, atypical mitoses. Occasional apoptotic cells and small areas of necrosis were seen, comprising less than 5% of the tumor. Frequent admixed multinucleated osteoclast-type giant cells were seen. Small amounts of loose fibromyxoid tissue were noted in some

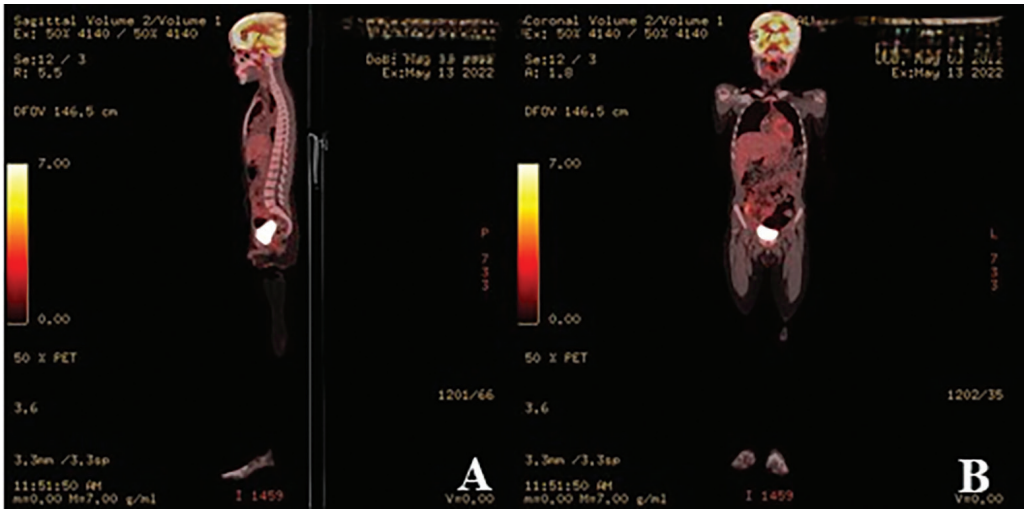

Figure 4. Body PET (Figures 4A and 4B) showed significant hypermetabolic activity of the distal right tibia and right inguinal and iliofemoral lymph nodes.

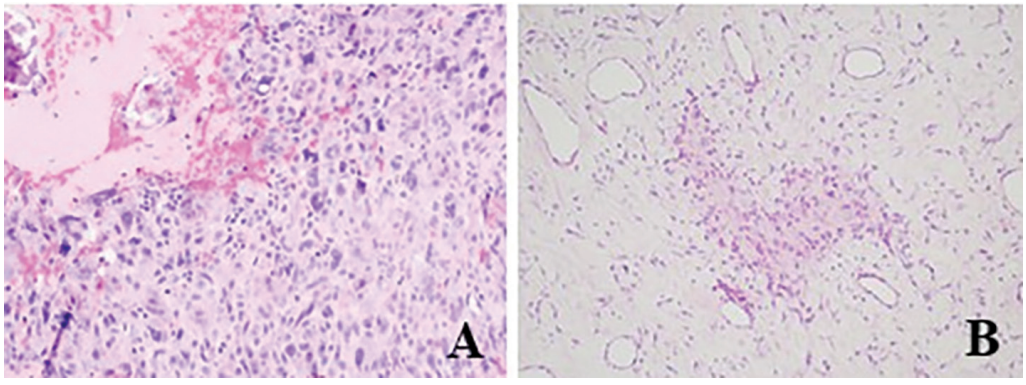

Figure 5. Malignant neoplasm, most consistent with high-grade osteosarcoma with cystic features and frequent multinucleated cells (A). Histologic response to induction chemotherapy (B).

sections. The osteoid matrix was not widely seen, but a partially mineralized matrix with cellular atypia consistent with neoplastic bone was noted focally. The performed Trichrome stain was weakly positive in the fibromyxoid tissue and positive in thin strands and small amounts of collagenous tissue admixed with tumor cells. Cytospins showed occasional large, atypical cells, including mononuclear and multinucleated forms, against a background of numerous red blood cells, scattered acute and chronic inflammatory cells, occasional macrophages, and occasional osteoclast-type giant cells (not shown).

Immunostaining

- CD68: Negative in tumor cells; positive in frequent mononuclear histiocytes; positive in frequent multinucleated osteoclast-type giant cells.
- ATRX: Positive in a predominant subset of tumor cells (retained). Although the specific function of the ATRX protein is unknown, studies suggest that it helps regulate the activity (expression) of other genes through a process known as chromatin remodeling. ATRX controls the expression of several genomic regions.
- p53: Positive in a subset - approximately 40 to 50% - of tumor cells.

- Retinoblastoma: Positive in a predominant subset of tumor cells (retained).
- SATB2 was positive in a substantial subset of lesional cells, with nuclear staining.

RNA transcriptome sequencing (RNA-Seq) was performed on sections and did not detect recurrent fusion transcripts. Sequencing of the whole exome was attempted on the tissue but could not be performed due to insufficient nucleic acid yield.

Fluorescence in Situ Hybridization (FISH)

CDKN2A – Positive for homozygous deletion of *CDKN2A* in the 200 evaluable nuclei. The following patterns of deletion were observed [TC (21%) (cutoff = 48%); C (20.5%); CC (22.5%) (cutoff = 8%); 3C (6%); TCC (6%) (cutoff = 25%)]. The following additional signal pattern was observed [TTC (2%)]. *CDK4* – Negative for amplification of *CDK4* in the 200 evaluable nuclei. Approximately 26% of the nuclei showed 3 to 8 signals of both target and control [3×(13%) (cutoff = 16%); 4×(7%); 5-8× (6%)]. A gain of one *CDK4* signal was observed in 7% of the nuclei [3T2C (5%) (cutoff = 9%); 4T3C (2%)] along with a relative loss of one *CDK4* signal being observed in 2.5% of the nuclei [2T3C (1.5%); 3T4C (1%)]. The following additional signal patterns were observed [TC (16%); TCC (4.5%); TTC (5.5%)].

Methylation Array-Based Copy Number Analysis

Copy number analysis was performed on recut sections using a methylation array, yielding the following results (Table 1).

Table 1. Large-Scale DNA Copy Number Variations

Gene	Alteration	Cytoband
CDKN2A	Deletion	9p21.3
CDKN2B	Deletion	9p21.3

Chromosomal losses: 8p, Chromosome 9, 17p13.1 (segment includes the *TP53* gene), 19q11-q13.43; *Chromosomal gains*: No large-scale chromosomal gains. Targeted sequencing analysis was positive for the following likely pathogenic variant of the *TP53* gene (Table 2).

Table 2. Targeted Sequencing Analysis of the *TP53* Gene

Alteration *TP53* P278S	HGVS Nomenclature	Allele Frequency Approximately 40%
NM_000546: c.832C>T; p.Pro278Ser	-	-

Molecular Pathology Final Report

Targeted sequencing analysis performed on this patient's sample was positive for the following likely pathogenic variant: *Alteration HGVS Nomenclature Allele Frequency.* TP53 P278S NM_000546: c.832C>T; p.Pro278Ser ~40%. *Comments*. Since tumor-only tests cannot reliably distinguish between somatic and germline alterations, correlation with clinical data, genetic counseling, and germline testing may be recommended if clinically indicated.

Discussion

Our patient was a 9-year-old boy. Differential diagnosis for primary bone lesions in this age group is wide and includes osteosarcoma, Ewing sarcoma, giant cell tumor, osteoblastoma, eosinophilic granuloma, aneurysmal bone cyst, chondromyxoid fibroma, osteomyelitis, simple bone cyst, osteoid osteoma, and fibrous dysplasia. Based on location, progression, and multicystic appearance on MRI, the leading differentials were aneurysmal bone cyst and telangiectatic osteosarcoma. Giant cell tumor was ruled out because CD68 staining was negative in tumor cells and positive in frequent mononuclear histiocytes and frequent multinucleated osteoclast-type giant cells. Very rarely, lung metastases are detected in patients concurrently with a diagnosis of the primary tumor (18, 19), a condition known as early metachronous osteosarcoma (MOS), for which there appears to be a male prevalence. In our patient, no evidence of pulmonary metastatic disease was found. The lungs were otherwise clear, with no nodules to

suggest pulmonary metastatic disease. There were no pleural or pericardial effusions and no adenopathy in the chest. Hypermetabolic lesion activity of the distal right tibia is nonspecific, as this may be seen with giant cell tumors, aneurysmal bone cysts, and sarcoma. Pathohistological examination described a poorly differentiated, high-grade (grade 3) osteosarcoma with 5% necrosis. The morphologic features and the suggestion of blood and fibrin-filled cystic spaces raised the possibility of telangiectatic osteosarcoma. Telangiectatic osteosarcoma is a rare subtype of the disease, accounting for 2-12% of cases (20).

ATRX staining was positive in the predominant subset of tumor cells (retained). The ATRX protein controls the expression of several genomic regions through a process known as chromatin remodeling. ATRX mutations have been shown to cause diverse changes in the pattern of DNA methylation, which may provide a link between chromatin remodeling, DNA methylation, and gene expression in developmental processes. Inherited mutations of the *ATRX* gene are associated with X-linked mental retardation (XLMR) syndrome, most often accompanied by alpha-thalassemia (ATR-X) syndrome (21). Acquired mutations in *ATRX* have been reported in several human cancers, including osteosarcomas (22). A subset of tumor cells (approximately 40-50%) were positive for TP53. Our results showed that most tumor cells had lost the segment of chromosome 17 (17p13.1), a segment that includes the tumor suppressor *TP53* gene. Loss-of-function *TP53* mutations occur in 75% of osteosarcoma cases (23). The p53 protein acts as a transcription factor that regulates DNA damage repair response, cell cycle progression, and apoptosis pathways (24). Mutation of p53 was found in essentially all tumor types. In Li-Fraumeni syndrome (LFS), characterized by a germline mutation of the *TP53* gene, the risk of osteosarcoma is much higher (25). Retinoblastoma (Rb) was positive in the predominant subset of tumor cells (retained) of our patient. Rb plays a regulatory role in the G_1-to-S cell cycle transition by binding to E2F family transcription factors in the absence of mitogenic stimuli. When the function of this protein is lost, this cell checkpoint is lost (26). pRb is one component in a cell-cycle control pathway that includes the p16 (encoded by the *CDKN2A* gene) and cyclin-dependent kinase 4 (cdk4, encoded by the *CDK4* gene) proteins. *CDKN2A*, also known as cyclin-dependent kinase inhibitor 2A, is a gene located in humans at chromosome 9, band p21.3. It is ubiquitously expressed in many tissues and cell types. The gene codes for two proteins, including the INK4 family member p16 (or p16INK4a) and p14arf.

Analysis was negative for amplification of *CDK4*; a gain of one *CDK4* signal was observed in 7% of the nuclei, and a relative loss of one *CDK4* signal was observed in 2.5% of the nuclei. However, on chromosome 9, cytoband 9p21.3, where the genes for *CDKN2A* and *CDKN2B* are located, deletion was found. This agrees with the findings that tumor suppressor *CDKN2A* was inactivated in osteosarcomas that lack *RB* mutations and that the p16-pRb cell-cycle control pathway was deregulated in a large number of high-grade osteosarcomas (27). One study has reported a constitutional inversion at chromosome 9p11-9q12 in a patient, along with non-clonal balanced translocations in the tumor (28), and a familial occurrence of telangiectatic osteosarcoma in cousins, but without any apparent hereditary components (29). Special AT-rich sequence-binding protein 2 (SATB2) is also known as DNA-binding protein. SATB2 is a protein encoded by the *SATB2* gene in humans. It is a DNA-binding protein that specifically binds nuclear matrix attachment regions and is involved in transcriptional regulation and chromatin remodeling. Although not specific to osteosarcoma, SATB2 is a marker of osteoblastic differentiation in benign and malignant mesenchymal tumors (30). Lesional cells in our patient were SATB2 positive in a substantial subset, with nuclear staining. Chromosomal losses 8p and 19q11-q13.43 were also found in our patient. Several tumor suppressor genes are located on chromosome 8p. One of these genes is *NKX3.1*, whose reduced expression correlates with 8p loss in high-grade tumors (31). Osteosarcomas exhibit karyotypes with an unusually high degree of aneuploidy and structural rearrangements. Frequent structural alterations at

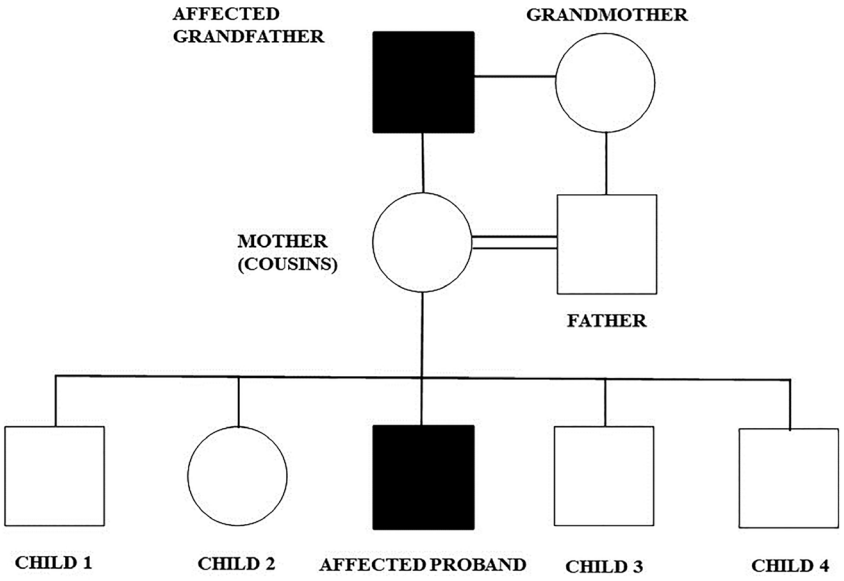

Figure 6. Possible hereditary predisposition.

chromosome bands or regions 19q11-13 were reported (32). In prostate cancer, research indicates that 19 regions contain susceptibility loci that regulate tumor aggressiveness (33).

Although there was no known cancer syndrome in the family, the maternal grandfather had a similar tumor requiring amputation. The parents were cousins, raising the possibility of inherited mutations by 40% (Figure 6). The family was advised to undergo a genetic analysis in order to detect a possible predisposition to malignant diseases.

Conclusion

Cancer is a multistage process characterized by the accumulation of epigenetic alterations and the mutation of genes that regulate cell growth, apoptosis, DNA repair, and tumor-suppressor genes. Genetic changes can be inherited, making a person predisposed to developing neoplasia. The accumulation is associated with neoplasia risk and can be utilized for cancer risk diagnosis. Chromosomal instability is a hallmark of osteosarcoma and is characterized by heterogeneous and extensive genetic complexity. The field of mutations is highly complex and differs significantly between tumors. Unlike other sarcomas, osteosarcomas do not exhibit genetic translocations; instead, we find widespread and heterogeneous abnormalities in the number and structure of chromosomes, illustrating the numerous DNA alterations that can occur in cancer. In sarcoma cells, DNA-related/chromatin remodeling is not evidence for transformation into a cancerous phenotype. In order for a tumor to spread and metastasize, tumor cells acquire six hallmarks of cancer during their development (typically by mutations in the relevant genes): limitless replicative potential, tissue invasion and metastasis, insensitivity to anti-growth signals, self-sufficiency in growth signals, evading apoptosis, and sustained angiogenesis. Therefore, this study examined only a subset of genes that frequently mutate in patients with osteosarcoma. The authors hope that discovering cancer biomarkers will lead to the development of targeted therapies.

What Is Already Known on This Topic:

Cancer of the bones and joints is a rare genetic disease accounting for approximately 20% of all benign and malignant bone neoplasia and 2% of pediatric cancers. The majority of osteosarcoma (OS) cases are sporadic but occur at increased rates in individuals with Paget's disease of bone, after therapeutic radiation, and in certain cancer predisposition syndromes. Although some subtypes exhibit characteristic genetic features and biological behaviors, the molecular basis for each subtype remains poorly understood. The etiological factors and pathogenetic mechanisms underlying OS development are complex, but significant progress has been made toward understanding its causes. The efforts made over the past few decades have focused on identifying so-called 'driver' mutations present in cases of inherited predisposition, as well as in sporadic OS. Cancer-causing genes (often called driver genes or drivers) contain driver mutations, which confer a proliferative advantage to cancer cells, leading to tumor clone outgrowth (34).

What This Study Adds:

So-called 'driver' mutations, including tumor suppressor genes p53, Rb, RECOL4, BLM, and WRN, play a critical role in developing OS. The molecular basis of OS is not well understood, so studying driver genes and their interaction in OS development will eventually advance preclinical investigations into new therapeutic strategies and drugs.

Acknowledgments: We thank the patient, the parents, and the physicians for their participation in this study. The parents provided written consent for the use of the medical documentation and the publication of a paper. In our institution, the Institutional Review Board (IRB) also functions as the Ethical Committee. The IRB number for this work is 012025-001.

Authors' Contributions: Acquisition of data: AT and DJ; Conception, interpretation and analysis of data: DJ, AT and SD; Drafting the article: DJ; Approved final version of the manuscript: DJ, AT and SD.

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Narrative Review
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Theodoros Aretaios (1829-1893) and the Foundation of Greek Orthopedic Surgery

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Abstract

Objective. This historical vignette aims to elucidate the contributions of Professor Theodoros Aretaios (1829-1893) to orthopedic surgery in nineteenth-century Greece. **Methods.** Documentary research was conducted of Aretaios' personal patient archives, preserved in the National Library of Greece, to validate and evaluate his surgical practices. **Results.** The archival records revealed a total of 22 orthopedic cases. Aretaios primarily performed upper and lower limb amputations and dislocation reductions. Reconstructive bone and joint operations were less frequent in his practice. **Conclusion.** The role which may be played by the archival records is demonstrated within this historical vignette. The work of Theodoros Aretaios highlights the procedures performed in 19th century orthopedics in the new Greek state. Aretaios contributed to the establishment of the Greek school of surgical practice and many of the surgeons of his era followed his steps.

Key Words: Hellenic Orthopedics ▪ Amputation ▪ Dislocation ▪ Blast Injuries ▪ Reconstructive Surgery.

Introduction

The practice of orthopedic surgery in the nineteenth century was still in the hands of physicians who practiced general surgery. It was in the early twentieth century that orthopedics became an independent surgical branch in Greece (1). The leading figure of the era in Greek surgery was Theodoros Aretaios (1829-1893) (Figure 1).

Records are kept today in the Manuscript Department of the National Library of Greece, where documentary research was conducted during the 2023-2024 academic year. A series of surgical operations showed the framework and possibilities of treatment in the practice of orthopedic surgery in 19th century Greece. These surgical procedures consisted primarily of amputations, which were performed using Esmarch's technique. That is, an ischemic bandage was used, as massive bleeding during amputation was the fundamental issue that surgeons had to deal with. Amputation functioned as a means of treating serious injuries,

Figure 1. Theodoros Aretaios (1829-1893).

severe infections that caused caries, and bone necrosis or gangrene in the soft tissues that surrounded them. The same fate awaited limbs with tumorous diseases, especially those presenting signs of malignancy. Other procedures were: reduction of fractures and dislocations, bone branching, which meant the excision of all or part of a bone without the simultaneous excision of the surrounding soft tissues, operations to resolve pseudoarthrosis, or treatment of distortion of fractures that had healed. Osteosynthesis, osteocoagulation, sculpting, arthroplasia, arthrodesis, and osteoplasia were procedures absent from the Greek surgery for most of the nineteenth century. However, they were performed gradually from the end of the century forward, as Greek surgeons acquired greater skills by studying abroad, or through practice. Soon, surgeons demonstrated better perception of the creative regeneration of bones and joints. This progress became evident in the surgical textbooks and atlases that were published at the end of the nineteenth and the beginning of the twentieth century, such as the relevant works of Evangelos Kalliontzis (1862-1922) (2), Gerasimos Phokas (1861-1937) (3), and Konstantinos Mermigkas (1874-1942) (4).

The aim of this historical vignette is to elucidate Aretaios' pivotal role in advancing nineteenth-century Hellenic orthopedic surgery, by surveying his extensive patient archives.

Methods

We conducted research in the Theodoros Aretaios Archives kept today in the Manuscript Department of The National Library of Greece. The information in these archives gave us information about Aretaios' contribution to the foundation of orthopedic surgery in Greece at the beginning of the modern scientific era. Within Theodoros Aretaios' archives, a small number of orthopedic cases treated in his clinic were recorded and saved. These series of cases aroused interest due to the variety of issues described. A total of 22 cases were encountered, consisting of eleven amputations, six dislocations, and five reconstructive operations. These procedures were performed in both sexes and various age groups. Reconstructive operations were limited to pediatric and adolescent patients.

Results

Notable cases of upper limb amputations resulted after accidents with dynamite explosions involving civilians. These injuries occurred when patients were either handling the explosive directly, or during close proximity detonation, often self-initiated. None of the incidents were war-related, and they occurred during peacetime activities, primarily involving dynamite use in fishing or construction work. This historical data provides an insight into the etiology of traumatic upper limb amputations in nineteenth-century Greece, highlighting occupational hazards and the negligence of civilians in the use of explosives. Such traumas were classified as transradial or transhumeral.

The earliest case of amputation in Aretaios' archives was dated in 1875, when the amputation was recorded of the left hand of a fisherman from Methana from the level of the wrist (5). This patient was transferred to the clinic five days after his injury, when the destruction of his hands from an explosion was ascertained, as well as the presence of burns on his chest, neck and face. Sepsis was evident in the tissues of the stump, while the patient had a fever of 38.5 °C. The surgical technique employed involved circumferential amputation of the injured stump utilizing two semicircular incisions encompassing the zone of injury, which were subsequently approximated. The archives indicate that one of the amputations was carried out by Theodoros Aretaios' assistant, Mr. Vassiliadis, and the other by Mr. Doukas. The patient exhibited tetanus symptoms on the second postoperative day, characterized by dysphagia and contractions of the masseter and cervical muscles. Treatment was initiated with subcutaneous injections of 20 chlorinating acetaldehyde granules administered every two hours, along with the application of warm covers. Despite intervention, the patient's condition deteriorated, marked by progressive muscle contractions and somnolence. The patient

succumbed to the infection on the fourth postoperative day.

An amputation of both forearms was documented in 1879 on a 28-year-old painter who was fishing with dynamite in Faliro. Both his hands were detached from the level of the wrist due to the explosion (6). A similar operation was performed in 1892, when Aretaios decided to amputate the lower third of both forearms of a worker from Kythnos, when cartridges of dynamite exploded prematurely during sewer drilling work (7).

Death from tetanus had already been described as early as 1875, connected with a previous injury. This was the case of a 50-year-old female patient who was shot by a gun carrying spherical projectiles at close range (8). The projectiles caused a penetrating wound to the left breast, and ruptured the tissue of the corresponding arm, shattering the humerus in its lower third. The projectiles became embedded in the soft tissues of the arm. There was no palpable pulse in either the radial or the ulnar artery. Initially, a bandage was applied and ice was placed, as the limb was not cold. The next day the patient developed a fever of up to 38° C, although her fingers and forearm were cold. Aretaios therefore decided to perform upper limb amputation due to severe arm injury. However, although the fever subsided from the first postoperative day, the patient showed symptoms of crackling and signs of universal tetanus. Although she received high doses of chlorinating acetaldehyde, which helped reduce her symptoms, the patient died after 48 hours.

Amputations performed to confront severe infections were also recorded by Theodoros Aretaios. Post-surgery lesions, septic conditions, bacteria and viruses, all contributed to severe limb infections. In 1874, a 30-year-old patient underwent amputation at the level of the middle third of his left forearm (9). Although not mentioned, the execution of the amputation would have been the result of an infection after a firearm injury eight days previously. However, the patient developed osteomyelitis postoperatively. Theodoros Aretaios, therefore, excised an additional seven centimeters of the ulna, which was necrotized due to osteomyelitis. As early as 1873, the case of a 22-year-old patient with erysipelas throughout the body was recorded, resulting in the decision to amputate the arm (it is not mentioned which arm, or the level of the amputation) due to necrosis (10).

A subsequent case from the same year documented arm necrosis in a 26-year-old farmer. Treatment involved an ischemic bandage and bone preservation rather than amputation, although detailed outcomes were not reported. This approach is aligned with historical management of severe soft tissue infections, where conservative measures were often attempted before resorting to amputation.

Scrofula was an infectious disease that was common at that time, especially in populations living in meagre conditions. In 1875, the case was described of a 23-year-old patient who had been suffering from scrofula for five years, which caused periostitis of the right ulna (NLG 1873: leaf 105). The patient developed decay on the phalanges of the ring finger of the same hand, and this had caused distortion. The surgeon performed dislocation of the finger by creating two lateral flaps. Another case was that of a 17-year-old patient in 1876 (11). This patient had a history of scrofula that caused bone decay without being clearly identified. The cause of bone degeneration could not be determined because of preexisting concomitant osseous pathologies. Four years prior, the patient had presented with a neoplasia that had the characteristics of an oversized spindle formation, extending from the lower third of the arm (it is not specified which) to the middle third of the forearm. Theodoros Aretaios proceeded to amputate at arm level. The patient had a smooth postoperative course apart from postoperative fever. No biopsy report is recorded in the archives.

In 1892, a 37-year-old farmer presented with chronic right ankle pain and progressive swelling. Concurrently, a lymphatic tumor in the left parotid region was noted, which apparently resolved spontaneously. The ankle joint developed fungal growths, which Theodoros Aretaios initially attempted to debride. However, recurrence occurred, with evident cavernous lesions. A below-knee amputation at the distal third of the tibia was

performed. This case illustrates the management of chronic ankle infections in the late nineteenth century, highlighting the progression from conservative debridement to amputation when faced with persistent fungal infection. The presence of a concurrent parotid tumor suggests potential systemic involvement, though the relationship between the two conditions remains unclear from the available information (12).

In 1892, Theodoros Aretaios performed a Pirogoff amputation on a 24-year-old male patient presenting with chronic right metatarsal pain and swelling. The patient's history included unsuccessful drainage by a local folk healer, and concurrent sub genital lymphadenopathy. Upon examination, Aretaios observed significant foot edema, a crater-like ulceration on the dorsum with hyperplastic margins, and first toe swelling. Aretaios' differential diagnosis was firstly a malignant neoplasm and secondly a fungal infection. On the basis of the clinical presentation, he concluded it was a fungal infection. Noting the intact ankle joint and tarsal structures, Aretaios opted for a Pirogoff amputation, demonstrating his diagnostic acumen and familiarity with contemporary surgical techniques. This case highlights Aretaios' approach to salvaging a limb, prioritizing minimal functional impact. Postoperative follow-up at day 33 revealed no fever, with only a small, unhealed cavernous tract remaining. The Pirogoff technique, involving calcaneal rotation and tibio-calcaneal arthrodesis, can provide a full weight-bearing stump and allow short-distance ambulation without prosthesis in carefully selected patients (13).

Theodoros Aretaios used a pulley in 1875, having sedated his 55-year-old patient, to reduce an old dislocation of the left arm, as it had occurred more than three months before (14). However, the attempt was unsuccessful, as the head of the arm did not move at all. An incomplete reduction was described in a 17-year-old female patient in 1875, who, after a fall about which no further information is given, presented a dislocation of the right arm from two months earlier. At first, she had resorted to a general practitioner, to no avail, and could not move her arm. It is described how the arm was hanging downwards and her elbow was close to her chest, and in front of and below the anterior rim of the clavicle there was a spherical prolapse that was considered to be the displaced head of the humerus. It was emphasized that the patient received deep sedation with chloroform, but the attempt was not completely successful. It is stated that the patient left the clinic, without giving any additional information. In contrast, successful results were achieved in 1875 by using the pulley again, in the case of a 50-year-old patient from Psarra (15). Twenty days before this she had developed dislocation of the arm (the side was not noted). The file records that a physician was then called, who allegedly performed the reduction but in fact did not. The surgeon described how the head of the humerus could be seen under the armpit. Due to the age of the injury, he sedated the patient with chloroform to treat the pain during reduction and, using a pulley, he restored the bone to its place, placing a bandage, as is advised for fractures of the clavicle.

Once more, in 1875, the surgeon succeeded in restoring the left arm of an 80-year-old patient, which had been dislocated at shoulder height after a fall, eight days earlier (14). This time he used the Hippocratic Method by placing his own right forearm as a fulcrum under the patient's armpit, and by falling downwards and inwards he succeeded, as he stated "probably", in reducing the dislocation. The same happened to a 22-year-old patient in 1876, who already had a dislocation of his left arm 25 days before, when he fell during a failed attempt to climb onto a moving carriage (16). With the use of a pulley and chloroform sedation, the surgeon succeeded in reducing the arm, as stated, at the first attempt.

Reconstructive procedures were aimed at removing affected areas that had elements of stiffness, to resolve the obstacle to movement, but not to create a new functional connection of the bones. This was the case for most operations in the nineteenth century. In 1874, Theodoros Aretaios treated an ankylosis of the elbow (it is not mentioned whether it was the right or left elbow) of a 24-year-old patient, which was the result of

pseudoarthrosis after being shot in the lower part of the arm (17). The surgeon planned to perform pseudoarthrosis exfiltration. However, during the operation, he noticed that there was decay and part of the joint of the humerus was also fragile, leading to the clearance of both the affected part of this bone, the pseudoarthrosis, and also the ulnar and the radius head. The operation was performed with two incisions on either side of the elbow joint. Apart from the removal of these parts of the bones, there is no mention of attaching materials or other manipulations to the stumps.

Creative reconstructive surgical treatment, but again in the form of cutting small parts of the bones or soft tissue, may be seen in the case of a three-year-old infant female in 1875 (14). The infant, due to a burn in infancy at the age of six months, had a scar on the palmar surface of the ring finger of the left hand that had caused "contraction", as stated in the file. Theodoros Aretaios excised the scar, and the infant was then considered healed, as it was recorded that the finger was subsequently almost straight. The same core thinking was applied to the case of an 8-year-old child in 1876 who had a supernumerary finger on the outer side of his right thumb (18). It was an apparently incomplete finger, formed with only two phalanges, articulated by an exostosis of the metacarpus, clearly in an ectopic position (hexadactyly). By performing an oval incision, excision of the exostosis was performed, along with the supernumerary finger.

A fourteen-year-old teenager with bilateral congenital clubfoot underwent surgical correction by Theodoros Aretaios, in stages in 1891 and 1892. The right foot was treated first with a wedge-shaped metatarsal resection, involving the cuboid, posterior sphenoid, and partial scaphoid, combined with Achilles tendon division. Postoperatively, the foot was immobilized until healing reportedly occurred within 3-4 months. The patient achieved unassisted ambulation without orthotic devices. The following year, the left foot was addressed. Initially planned as simple ankle projection removal using Nelaton's technique, the severity of the deformity necessitated a more extensive procedure. This included resection of the lateral ankle apex, partial articular surface removal of the tibia and scaphoid, and Achilles tendon division. The leg was then immobilized. Postoperative follow-up was documented until day eleven, noting the absence of fever and "very moderate" blood flow without signs of ischemia (19). This case highlights the evolving surgical approaches to severe clubfoot deformities in the late nineteenth century, emphasizing the importance of staged correction and tailored surgical techniques based on intraoperative findings.

Discussion

The archival evidence from Theodoros Aretaios' practice reveals a discrepancy between his theoretical knowledge, as presented in his textbooks on surgical techniques (20) (Figure 2) and pathology (21), and his actual clinical practice in orthopedic surgery. While his textbooks describe a wide range of surgical approaches for bone diseases, with limited coverage of spinal surgery, the archived cases demonstrate a narrower scope of procedures. Aretaios' documented surgeries primarily focused on amputations and dislocation reductions. Reconstructive orthopedics, particularly for foot deformities, were limited in number and employed relatively simple techniques involving bone and tendon excisions to restore joint function. This disparity likely reflects the gap between the advanced orthopedic knowledge available in Europe at the time and the practical limitations of implementing these techniques in Greece in the late nineteenth century. The archives provide valuable insights into the actual state of orthopedic practice in Greece during this period, highlighting the challenges faced in translating theoretical knowledge into clinical application.

Aretaios used the old but well established Hippocratic method for dislocations, a method widely used in European orthopedics (22). Credit for the method of winding a strip of tensile material around the limb (Esmarch's bandage) is usually given to Johann T. Friederich August von Esmarch (1823-1908), Professor of Surgery at Kiel (23). Meanwhile, studies have revealed that Pirogoff's

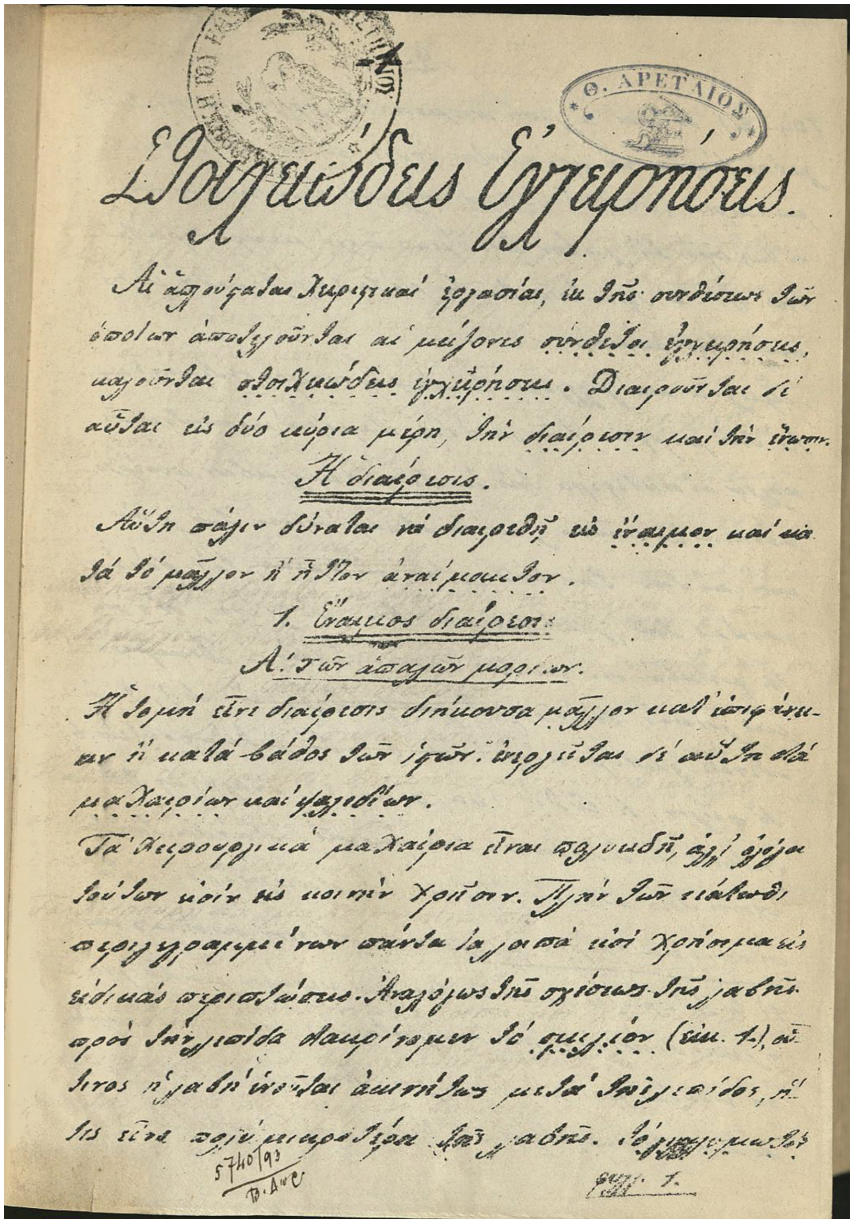

Figure 2. Front page of Theodoros Aretaios's Textbook "Surgery". National Library of Greece Manuscript no. IATP-2594-Q:Sheet 1. Under license of the National Library of Greece.

amputation was more successful, with less post-surgical implications (24). Aretaios was educated in Europe, attending universities in Berlin Vienna and Paris, and was fond of modern techniques and introduced them to Hellenic surgery (25, 26). Theodoros Aretaios' surgical practices were adopted by his successors and students, as evidenced in the medical reports of the clinic under Spyridon Magginas' directorship (27). Late nineteenth and early twentieth century Greek surgeons, including Professors Evangelos Kalliontzis, Gerasimos Phokas, and Konstantinos Mermigkas (1874-1942), further advanced these techniques by incorporating European training into their practice in Greece (28).

Conclusion

While Aretaios' published works on surgical techniques and pathology are well-known, this archival study provides novel insights into the actual scope of orthopedic procedures performed in Greece during his time. Despite the limited range of operations, due to contemporary constraints, Aretaios' work laid the foundation for the subsequent development of orthopedic surgery in Greece. It also highlights the discrepancy between theoretical knowledge and practical application in nineteenth-century Greek orthopedic surgery, and underscores Aretaios' pivotal role in advancing the field.

What Is Already Known on This Topic:
Previous research has primarily focused on Theodoros Aretaios' published textbooks on surgical techniques and pathology.

What This Study Adds:
This article provides detailed examination of Theodoros Aretaios' actual orthopedic surgical practices, based on analysis of his personal patient archives housed in the Manuscript Department of the National Library of Greece.

Authors' Contributions: Concept and design: KL and PM; Acquisition, analysis and interpretation of data: KL and PM; Drafting the article: KL, PM, IN and PL-S; Revising it critically for important intellectual content: PL-S, DZ and DM; Approved final version of the manuscript: PM, PL-S, IN, DZ, DM and KL.

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