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Total Ferric Reducing Ability of Plasma as an independent caries risk marker in pregnant women with diabetes

Joanna Waligóra[1], Beata Kuśnierz-Cabala[2], Dorota Pawlica-Gosiewska[3], Katarzyna Gawlik[3], Jolanta Pytko-Polończyk[4]

[1] Department of Integrated Dentistry, Institute of Dentistry, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[2] Chair of Medical Biochemistry, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[3] Department of Clinical Biochemistry, Jagiellonian University Medical College, Kraków, Poland
[4] Chair of Department of Integrated Dentistry, Institute of Dentistry, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Joanna Waligóra, Ph.D.
Department of Integrated Dentistry, Jagiellonian University Medical College
ul. Montelupich 4, 31-155 Kraków, Poland
Phone: +48 12 424 54 65; E-mail: joanna.waligora@uj.edu.pl

Abstract: Pregnancy and diabetes increase the risk of developing pathologies in the oral cavity. Saliva biomarkers can be used as diagnostic markers in the monitoring of dental diseases.
The study aimed to evaluate hard dental tissue health in pregnant women with two forms of diabetes and salivary antioxidant activity to identify optimal non-invasive caries risk factors.
The study involved 104 pregnant women: 35 patients with gestational diabetes mellitus (GDM), 30 patients with type 1 diabetes (T1D), and 39 pregnant patients without diabetes mellitus (control group). Physical examination included caries assessment using the dental DMFT index, caries frequency, SiC index, and dental treatment index. Oral hygiene was also assessed using the Approximal Plaque Index (API). The study of unstimulated saliva measured the total Ferric Reducing Ability of Plasma (FRAP), as well as the activity of superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPX).
In the analyzed patient population, caries frequency totalled 100%, with active tooth decay observed in 72.12% of patients, whereas caries intensity was similar in the studied groups. In all groups, oral hygiene (API) was average. In T1D group, higher values of the API index ($p = 0.001$) were observed vs. the control group. Antioxidant activity was similar in the study groups. The independent determinants of caries occurrence included API ($p = 0.002$) and FRAP concentration ($p = 0.02$).
The health of dental hard tissues in the examined patients was unsatisfactory and similar in the studied groups. FRAP concentration and oral hygiene (API) functioned as determinants of caries.

Keywords: pregnancy, diabetes, caries, saliva, antioxidants.

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Introduction

Tooth decay is the most common chronic oral disease. It is a multifactorial, dynamic, biofilm-dependent disease, modulated by diet, leading to the loss of minerals from hard dental tissues. It is conditioned by biological, behavioral, psychosocial, and environmental factors and is manifested with demineralization of inorganic substances followed by proteolysis of organic substances in dental hard tissues due to the impact of acids produced by bacteria in the dental plaque as a result of the metabolism of exogenous and endogenous sugars. This process leads to development of carious lesions. [1, 2]

Caries in pregnant women and pregnant women with diabetes is an important healthcare problem, as both pregnancy and diabetes can increase the risk of developing oral disorders. A change of eating habits during pregnancy can affect hard tissues of the teeth, leading to their decay. Usually, carbohydrate intake increases, as well as the frequency of meals and snacking between main meals. This is also accompanied by indigestion and vomiting often occurring in the first trimester, as well as gastroesophageal reflux later in pregnancy. Reduced buffering capacity and pH of the saliva, as well as reduced concentration of mineral compounds also contribute to lesions in dental hard tissues. [3] Sex hormones directly affect the hard tissues of teeth, as it has been shown that odontoblasts and endothelial cells in the pulp feature estrogen receptors. [4] Increase in salivary estrogen levels leads to enhanced proliferation and exfoliation of epithelial cells. This creates better nutritional conditions for bacteria in the oral cavity with an increase in the cariogenic bacteria volumes. Estradiol is metabolized by caries-causing bacteria such as *Streptococcus mutans* and *Streptococcus sanguis.* Higher levels of salivary mucin contribute to the agglutination of these bacteria and enhanced plaque formation. [5]

In type 1 diabetes, higher incidence of caries may be related to such factors as reduced salivary flow rate, increase in salivary glucose, and increase in salivary alpha-amylase associated with both glucose production and adhesion of cariogenic bacteria to the tooth surface. [6, 7] Some authors point to low incidence of caries in patients with type 1 diabetes due to the application of a diabetic diet. It involves absence of sucrose and thus results in reduced glucose production in the oral cavity. [8, 9] No significant progress in carious disease was observed in pregnant patients with gestational diabetes mellitus. [10] Some studies, however, indicate that gestational diabetes mellitus is associated with reduced levels of beneficial oral bacteria, such as Veillonella, which plays an important role in maintaining oral health and preventing tooth decay. Imbalance in the oral microbiome increases the risk of dental diseases, including dental caries, because pathogenic bacteria become more dominant. [11]

Saliva is secreted by the larger salivary glands such as the parotid, submandibular, sublingual, and minor salivary glands. Under physiological conditions in adults, saliva is produced in volumes of about 500–600 ml per day, about half of which is resting saliva, while the rest is stimulated saliva produced in response to various stimuli. Saliva determines correct functioning of the bite apparatus, protects the surface of teeth and the oral mucosa, participates in the perception of taste, temperature and touch, facilitates speech, as well as bite formation and swallowing. The main component of saliva is water, which accounts for about 95–99% of its volume, with the remaining part formed of organic and inorganic components. [12] Saliva is a reservoir of calcium and phosphates, and is saturated with hydroxyapatite, which minimizes demineralization processes on the tooth surface and promotes remineralization. The anti-caries effect of saliva also involves dilution and neutralization of food acids and biofilm bacteria

metabolic products. It also has antioxidative properties, which is of significance in protection against tooth decay. [13]

In the healthy body, oxidative and reducing processes remain in balance. Predominance of oxidation processes leads to an uncontrolled increase in reactive oxygen species due to their overproduction or failure of the body's defense mechanisms. Such a situation is referred to as oxidative stress. This causes damage to many biological structures as well as disruption of processes taking place in the body. Oxidative stress accompanies inflammation and affects its development, also playing a major role in the pathogenesis of many diseases. Oxidative processes are prevented, among others, by antioxidants forming part of the body's immunological mechanisms. The antioxidant properties of saliva ensure balance among free radicals, thus significantly contributing to protecting the oral cavity. Salivary antioxidants include enzymatic and non-enzymatic systems. Glutathione peroxidase, catalase, and superoxide dismutase (SOD) are examples of enzymatic systems, whereas the non-enzymatic antioxidant system includes uric acid and glutathione, together creating the Total Antioxidant Capacity (TAC). [14]

In patients with caries, some biomarkers of oxidative damage were found, as well as changes in the parameters of the antioxidant defense system. [13] Tooth decay is not only a bacterial disease, but it is also host-dependent with factors including the oxidative–reductive balance in the oral cavity. Biomarkers of oxidative damage are elevated in individuals with tooth decay, reflecting an imbalance between free radicals and the antioxidant defense system in the saliva. This can affect the progression of tooth decay by promoting tissue damage and altering local immune responses. [15] There are many salivary molecules changes in concentration or activity of which may prove useful for detecting oral diseases or monitoring response to treatment. Measuring the activity of oxidative stress biomarkers and antioxidants in saliva can potentially serve as a diagnostic or prognostic tool to assess the risk or activity of dental caries. [16]

The present study was aimed to assess the health of dental hard tissues in pregnant women in the context of two different forms of diabetes. Furthermore, the study was to assess the antioxidant activity in saliva in order to determine the optimal, non-invasive factors conditioning the development of caries.

Materials and Methods

The patients were recruited among pregnant women diagnosed with diabetes and treated at the Metabolic Diseases Department of the Jagiellonian University Medical College in Krakow. During the same period, control group sample were recruited among pregnant women without diabetes at the Department of Gynecology and Obstetrics of the Jagiellonian University Medical College. Patients were eligible if they: (1) gave the informed consent; (2) were over 18 years of age; and (3) were in the 3rd trimester of pregnancy. Diagnosis of gestational diabetes mellitus (GDM) or type 1 diabetes (T1D) was made by the attending physician in accordance with the guidelines of the Polish Diabetes Association (Diabetes Poland). The patients were excluded if: (1) they did not give informed consent; (2) they had other types of diabetes mellitus; (3) they were diagnosed with other metabolic diseases affecting carbohydrate metabolism; The study protocol was in compliance with the ethical guidelines of the Helsinki Declaration of 1975 and was approved by the Ethics Committee of the Jagiellonian University Medical College (KBET/270/B/2013). Informed consent was obtained from each patient prior to enrolment in the study.

Medical history was collected from each patient on the basis of their medical records, an oral examination was performed, and saliva samples were collected at the same time. The medical history data collected included information on comorbidities, history of diabetes, current glycemia, family history of diabetes, the presence and type of oral symptoms, and the frequency of visits to the dentist.

A dentist with long experience conducted an oral examination on each patient. The examination was performed using a dental mirror (Pol-Intech, Łódź, Poland) and a single-sided Mifam type dental probe (ZG-1; Pol-Intech, Łódź, Poland). The oral examination included caries and oral hygiene assessment.

Tooth decay was assessed using the DMFT index. It is an indicator of the intensity of the carious process and consists in assessing the components: teeth with decay or temporary filling (D), teeth missing or extracted due to decay (M), teeth filled due to decay (F), and then summing up the individual components.

Caries frequency, i.e., the percentage of people with the DMFT index >0, was also assayed.

Next, SiC index (*Significant Caries Index*) was determined, namely the average DMFT value calculated for 1/3 of patients with the highest DMFT values in the study group.

The dental treatment index was established, expressed as the ratio of the number of filled teeth to the sum of filled teeth and teeth with decay. [17]

Oral hygiene was also evaluated using the Approximal Plaque Index (API). This index was expressed as a percentage and defined as the number of interdental spaces with plaque divided by the number of all assessed spaces. In quadrants I and III, the study was conducted from the lingual side, while in quadrants II and IV, the study was conducted from the buccal side. [18]

Two 1 ml samples of unstimulated saliva were collected from each patient. Sampling was performed between 8:00 a.m. and 11:00 a.m., at least 1 hour after the last meal and before the dental examination. Calibrated tubes were filled with saliva flowing freely from the mouth. The samples were immediately centrifuged at 10,000 g for 5 minutes at 4°C, then frozen and stored at −80°C until the end of the sampling period; they were thawed immediately prior to testing.

The analysis involved an assessment of Total Antioxidant Capacity (TAC), extracellular SOD activity, GR activity, and GPX activity. SOD activity was measured using the Misra and Fridovich method. [19] Total Antioxidant Capacity was measured using the Benzie and Strain Ferric Reduction Ability of Plasma (FRAP) method. [20] A modified Goldberg method was used to measure GR activity. [21] GPX activity was measured using the method described by Paglia and Valentine. [22] Antioxidant analysis was performed using the ELx808 Absorbance Microplate Reader (BioTek Instruments). The measurements were conducted at the Department of Diagnostics, Department of Clinical Biochemistry, Jagiellonian University Medical College in Krakow.

Statistical analysis was conducted using the STATISTICA PL data analysis software, version 9.1 (StatSoft Polska, Kraków, Poland), and the MedCalc® software, version 8.1.1.0 (https://www.medcalc.org/). Continuous variables have been presented as median and interquartile range (IQR), while categorical variables as number and percentage (n,%). The parameters were compared between patients with gestational diabetes mellitus, type 1 diabetes, and the control group. The Kruskal-Wallis test and post-hoc analysis were applied to compare continuous variables. For dichotomous variables, the χ^2 test was used. The stepwise logistic regression model was applied to assess the factors determining tooth decay. The accounted variables featured p-value <0.20. The results have been presented with odds ratios (OR) and confidence intervals (CI). The significance level was set at $p < 0.05$.

Results

Group description

The study involved 104 pregnant patients, including 35 patients with gestational diabetes mellitus (GDM), 30 patients with type 1 diabetes (T1D), and 39 pregnant patients without diabetes mellitus (control group). Pregnant women in each group did not differ in terms of age, height, pre-pregnancy body weight, pre-pregnancy body mass index (BMI), body weight during pregnancy, incidence of GDM in a previous pregnancy, and diabetes in the family. Hypothyroidism and Hashimoto's thyroiditis were more frequent in patients with T1D vs. patients with GDM and the control group (11 (36.7%) vs. 4 (11.4%), respectively, p = 0.0200; 11 (36.7%) vs. 6 (15.4%), p = 0.0001; and 10 (33.3%) vs. 1 (2.9%), p = 0.0010; 10 (33.3%) vs. 0 (0%), p = 0.0400). No differences were recorded among the groups regarding incidence of other diseases. Fasting plasma glucose (FPG, mmol/L) was the highest in T1D patients, lower in GDM patients, and lowest in the control group (6.22 (5.16–7.55) vs. 4.77 (4.50–5.16) vs. 4.34 (4.14–4.82), respectively; p <0.0001). HbA1c hemoglobin level in T1D patients amounted to 5.75% (5.10–6.10%). Detailed data have been presented in Table 1.

Table 1. Group data upon enrolment in the study.

Variable	Total	Control group	GDM	T1D	p
Number [n]	104	39	35	30	
Anthropometric data					
Age [years]	31.0 (28.0–33.0)	30.0 (26.0–33.0)	32.0 (29.0–34.0)	31.0 (28.0–33.0)	0.39
Height [cm]	165.0 (161.0–170.0)	165.0 (162.0–169.0)	164,0 (160.0–168.0)	167.5 (163.0–170.0)	0.12
Body weight before pregnancy [kg]	63.0 (56.5–69.0)	62.0 (57.0–65.0)	65.0 (56.0–74.0)	61.5 (54.5–73.0)	0.49
BMI before pregnancy [kg/m^2]	22.7 (21.6–25.2)	22.8 (21.8–23.6)	23.8 (21.6–27.5)	22.2 (20.8–25.9)	0.17
Body weight during pregnancy [kg]	73.6 (68.0–82.2)	73.6 (69.6–80.5)	74.5 (65.5–85.5)	73.5 (68.0–85.8)	0.90
Examination					
Prior gestational diabetes mellitus n (%)	8 (7.7)	2 (5.1)	5 (14.3)	1 (3.3)	0.19
Prevalence of diabetes in family n (%)	50 (48.2)	16 (41.0)	18 (51.4)	16 (53.3)	0.53
Hypothyroidism n (%)	21 (20.2)	6 (15.4)*	4 (11.4)†	11 (36.7)*	**0.03**
Hashimoto' disease n (%)	11 (10.6)	0 (0)*	1 (2.9)†	10 (33.3)*	**0.0001**

Table 1. Cont.

Variable	Total	Control group	GDM	T1D	p
Hypothyroidism diagnosed during pregnancy n (%)	6 (5.8)	0 (0)	2 (5.7)	4 (13.3)	0.06
Hypertension n (%)	3 (2.9)	1 (2.6)	2 (5.7)	0 (0)	0.39
Hypertension diagnosed during pregnancy n (%)	7 (6.7)	3 (7.7)	3 (8.6)	1 (3.3)	0.67
Intrahepatic cholestasis of pregnancy n (%)	3 (2.9)	3 (7.7)	0 (0)	0 (0)	0.09
Migraine n (%)	1 (1)	0 (0)	1 (2.9)	0 (0)	0.37
Laboratory tests					
Fasting plasma glucose level [mmol/l]	4.82 (4.32–5.39)	4.34 (4.14–4.82)	4.77 (4.5–5.16)*†	6.22 (5.16–7.55)*	**<0.0001**

* p <0.05 vs. control group.
† p <0.05 between the groups of patients with type 1 diabetes and gestational diabetes mellitus.
Abbreviations used in the table: BMI — body mass index, T1D — type 1 diabetes, GDM — gestational diabetes mellitus, p — test probability.

Dental history

The most common oral ailment reported by patients during pregnancy was increased gingival bleeding, both spontaneous and provoked. It occurred with equal frequency in the study groups (p = 0.31) and concerned a total of 50 patients (48.1%), including 19 (54.3%) with GDM, 16 (53.3%) with T1D, and 15 (38.5%) from the control group. Other complaints included tooth and gum hypersensitivity, which affected a total of 14 (13.5%) patients, and bad breath reported by 2 (1.9%) pregnant women. The incidence of reported complaints was similar in all groups. The results have been presented in Table 2.

The frequency of visits to the dental office was similar in all groups (p = 0.44). Most pregnant women reported that they visit regularly, more than once per year. There were 23 such patients in the GDM group (65.71%), 13 in the T1D group (43.33%), and 21 such patients in the control group (53.85%). Also, a large number of pregnant women visited dentist once per year.

Assessment of tooth decay and oral hygiene

When assessing tooth decay, it was observed that the occurrence of caries, teeth extracted due to tooth decay, or the presence or absence of fillings were similar in all the study groups. The active tooth decay process occurred in 75 patients (72.12%), whereas 29 (27.88%) of them were free of

Table 2. Dental history data.

Variable	Total	Control group	GDM	T1D	p
Increased gingival bleeding n (%)	50 (48.1)	15 (38.5)	19 (54.3)	16 (53.3)	0.31
Tooth and gum hypersensitivity n (%)	14 (13.5)	6 (15.4)	3 (8.6)	5 (16.7)	0.58
Bad breath n (%)	2 (1.9)	1 (2.6)	0 (0.0)	1 (3.3)	0.58

Abbreviations used in the table: T1D — type 1 diabetes, GDM — gestational diabetes mellitus, p — test probability.

caries. In the control group, caries-related cavities were observed in 28 (71.79%) patients vs. 27 (77.14%) in the GDM group vs. 20 (66.66%) in the T1D group. Teeth extracted due to decay were recorded in exactly half of pregnant women: 52 (50%). There were 18 (46.15%) such patients in the control group, 19 (54.29%) in the GDM group, and 15 (50%) in the T1D group. Fillings were present in 100 (96.15%) patients, with only 4 women (3.85%) never treated conservatively for caries. In the control group, fillings were found in 38 (97.44%) patients vs. 34 (97.14%) the GDM group vs. 28 (93.33%) in the T1D group.

The average number of teeth with decay (D), missing/extracted due to decay (M) and filled (F) did not differ significantly among groups ($p = 0.11$; $p = 0.78$; $p = 0.06$, respectively). The minimum and maximum D values were 0 and 16; M values: 0 and 14, and F values: 0 and 20, respectively.

Caries frequency in the studied patient population totalled 100%, which indicates that all pregnant women examined were previously or currently affected by tooth decay. The mean DMFT score among the participants (caries intensity) was calculated as 15.1 (SD = 5.02) and ranged from 4 to 30. For the control group, mean DMFT value was 14.8 (SD = 4.0.9) vs. 14.1 (SD = 4.84) in the GDM group vs. 16.7 (SD = 6.0) in the T1D group. The median of DMFT values for the entire study group was set at 15 (11.5–18), whereas median DMFT values in individual groups were as follows: 14 (10–19) for GDM, 17 (12–22) for T1D, and 16 (12–18) for the control group. Statistical analysis did not reveal any significant differences among the groups ($p = 0.1$).

The SiC index (Significant Caries Index), being the mean DMFT value in 1/3 of patients with the highest values of the index, totalled 17 for the entire population. SiC values in individual groups were similar: 17 in the control group, 16 in the GDM group, and 18 in the T1D group. The mean caries treatment index amounted to 0.86 (range: 0.57–1.00).

In pregnant women with T1D, higher values of the API index ($p = 0.001$) were observed vs. the control group. In the group of patients with GDM, there were no statistically significant differences in the oral hygiene compared to the control group. In all groups, the mean API index value pointed to average oral hygiene.

Index values in individual study groups have been presented in Table 3.

When analyzing the prevalence of caries in individual groups of patients, a positive correlation was found between the advancement of caries measured with the D value and the HbA1c level ($R = 0.41$, $p = 0.02$). Because the HbA1c assay was available for patients with T1D, the correlation was found only in this group (Fig. 1). In the case of M, F, and DMFT values, no such correlation has been evidenced.

Table 3. Tooth decay and oral hygiene assessment in the studied population of pregnant patients.

Variable	Total	Control group	GDM	T1D	p
D	2 (0-5)	2 (0-5)	3 (1-6)	2 (0-4)	0.11
M	0.5 (0-2.5)	0 (0-2)	1 (0-3)	0.5 (0-3)	0.78
F	11.5 (7-14)	12 (7-15)	8 (6-12)	12.5 (7-16)	0.06
DMFT	15 (11.5-18)	16 (12-18)	14 (10-19)	17 (12-22)	0.1
Caries intensity	15.1	14.8	14.1	16.7	0.1
Caries dental treatment index	0.86 (0.57-1)	0.88 (0.58-1)	0.75 (0.46-0.93)	0.87 (0.71-1)	0.64
API (%)	50 (38-79)	46 (31–64)	50 (39-66)	69 (44-97)*	**0.03**

*p <0.05 vs. control group (values given in the text)
Abbreviations used in the table: T1D — type 1 diabetes, GDM — gestational diabetes mellitus, D — number of teeth with decay, p — test probability, M — number of teeth extracted due to decay, F — number of teeth filled due to decay, DMFT — caries intensity index.

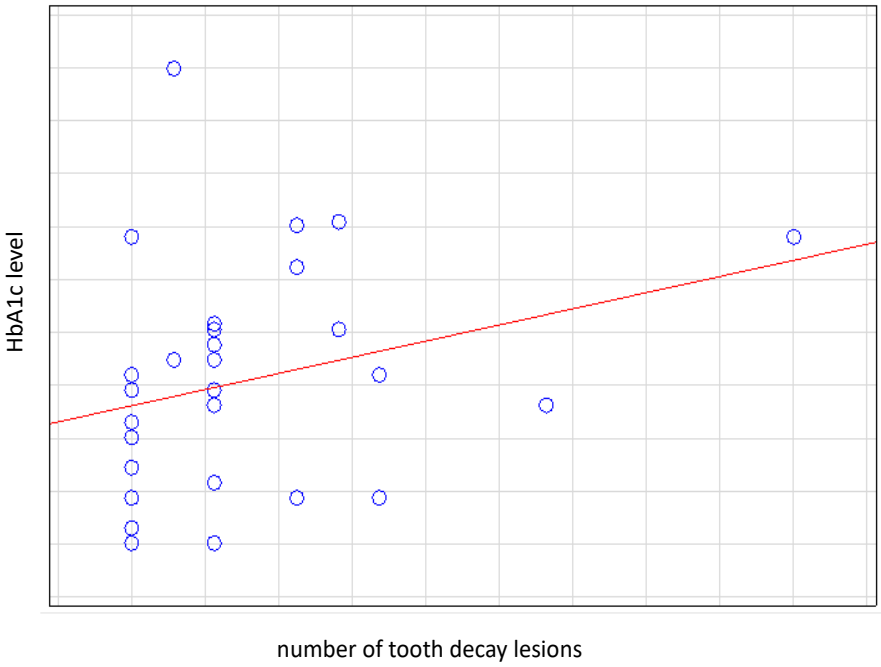

Fig. 1. Correlation between the number of carious cavities D (X axis) and the HbA1c level (Y axis) in patients with Type 1 DM.
X axis — number of tooth decay lesions
Y axis — HbA1c level
Line — correlation curve

Selected antioxidant levels in non-stimulated saliva

In all patients, total ferric reducing ability of plasma (FRAP) and the activity of extracellular superoxide dismutase (SOD), glutathione peroxidase (GP), and glutathione reductase (GR) were assayed.

In the groups studied, the above-mentioned parameters remained at a similar level. Detailed concentrations of salivary antioxidants assayed have been presented in Table 4.

Table 4. Selected salivary antioxidants levels in the study groups.

Variable	Total	Control group	GDM	T1D	p
FRAP [mmol/l]	0.42 (0.32–0.53)	0.41 (0.33–0.55)	0.42 (0.3–0.51)	0.43 (0.28–0.56)	0.97
Glutathione reductase [U/l]	9.85 (5.4–14.2)	11.5 (5.7–16.4)	10.3 (6.2–15.8)	7.9 (4.7–10.7)	0.22
Glutathione peroxidase [U/ml]	29.25 (14.5–71.35)	37.2 (15.1–67)	25.8 (11.7–71.0)	27.1 (19.1–96.3)	0.78
Superoxide dismutase [U/ml]	12.8 (10.65–15.7)	14.1 (10.9–17.7)	12.5 (9.5–14.2)	12.55 (10.6–15.3)	0.38

Abbreviations used in the table: T1D — type 1 diabetes, FRAP — Ferric Reducing Ability of Plasma, GDM — gestational diabetes mellitus, p — test probability.

The assessment of factors determining the occurrence of tooth decay was performed using the (stepwise) logistic regression models. When selecting determinants, variables accounted for included oral hygiene (measured with API), selected salivary antioxidant levels (FRAP, dismutase, peroxidase, reductase), diagnosed diabetes, hypothyroidism, and the patient's age. In the regression model, variables with $p < 0.2$ were taken into account in the subsequent steps. Variables with $p \geq 0.2$ were excluded from the model. Variables with $p < 0.05$ were considered significant determinants.

In the logistic regression model ($p < 0.0001$), the determinants of caries included API (OR = 1.03; 95% CI: 1.01–1.06; p = 0.002) and FRAP concentration (OR = 33.2; 95% CI: 1.74– 637.0; p = 0.02). Each increase in FRAP concentration by 1 mmol/L increases the odds of developing caries by 33 times, regardless of oral hygiene (an increase in API by 1% independently increases these odds by 3%). The results have been presented in Table 5.

Table 5. Logistic regression model.

Variable	OR	95% CI	p
API	1.03	1.01–1.06	**0.002**
FRAP	33.2	1.74–637.0	**0.02**

Abbreviations used in the table: API — Approximal Plaque Index, FRAP — Ferric Reducing Ability of Plasma, OR — odds ratio, p — test probability, CI — confidence interval.

Discussion

In the study, patients did not differ in the incidence of oral complaints or the frequency of visits to the dentist. The main reported complaints involved gingival bleeding and hypersensitivity of teeth and gums. An imbalance between the bacterial plaque activity and the body's defense reactions in pregnant women can lead to gestational gingivitis, manifested with redness, hyperplasia, and swelling of the papillae and marginal gingiva with associated bleeding while brushing. [23] Also, according to some authors, gingivitis may be more frequent and more severe in patients with type 1 diabetes. The intensity of periodontal lesions is associated with the duration of diabetes. [24] Most patients reported regular visits at the dental office once per year or more frequently. The patients declared that, since they became pregnant, the frequency of visits increased. This may be related to the growing patient awareness of the need to take care of their own health in such a special period as pregnancy, as well as the position of the Working Group on Dental Prevention in Pregnant Women of the Polish Chapter of the Alliance for a Cavity-Free Future, which recommends at least two dental consultations during pregnancy. [25, 26]

Active tooth decay process occurred in 72.12% of patients with caries frequency hitting 100%, which points to high prevalence of caries in the studied population. The values of the analyzed tooth decay indicators (D, M, F, DMFT, SiC, and treatment index) were comparable among the study groups, which indicates a similar level of tooth decay occurrence and severity regardless of GDM or T1D. Also López-Pérez *et al.*, when examining pregnant patients with type 2 diabetes, gestational diabetes mellitus, and those without diabetes, evidenced that they recorded a similar caries incidence with DMFT in all examined patients higher than 0. [10] In the conducted study, the highest values in all groups referred to the F numbers, which points to an intensive history of tooth decay, but also effective conservative treatment and control of caries lesions. Also, the values of the treatment index in the study groups point to a high level of benefiting from dental care and good availability of the services. Mean values of D — teeth with tooth decay, and especially M — teeth missing due to tooth decay achieved much lower values. The average D = 2 number indicates that the current disease activity is relatively high and requires dental intervention, particularly during pregnancy, or with additional complication of pregnancy with diabetes. In the studies by other authors, similar D-values were obtained, amounting to about 1.2–1.7 in pregnant women, which reflects a temporary increase in the risk of caries in the course of pregnancy, but at the same time suggests that, in most patients, the disease is controlled in a way owing to prior treatment and maintained access to dental care. [27, 28]

The study found that the incidence of tooth decay was higher in patients with higher HbA1c levels ($r = 0.41$, $p = 0.02$). Because the HbA1c assay was available for patients with T1D, the correlation was found exclusively in this group. HbA1c is not titrated in pregnant women without diabetes. There is also no evidence of HbA1c being useful as a metabolic control monitoring tool in GDM. [29]

Several studies have shown that a higher incidence of caries was observed in adolescents with diabetes and higher HbA1c values compared to those with good metabolic control. This is especially true for patients with persistently elevated values of this parameter, i.e., with poorly controlled diabetes. On the one hand, a decrease in the new tooth decay can be observed in the years following the diagnosis of the disease with appropriate metabolic control, but the long duration of the disease may contribute to an increase in caries intensity. [30, 31] According to a study by ElBshari *et al.*, diabetes and poor glycemic control have either no effect on the risk of caries, or the

impact is so low that it is masked by other known and more dominant risk factors, such as diet and obesity. [32] Also, in the studies by Tan *et al.*, no significant correlations were found between the risk of tooth decay and HbA1c levels. [33] However, the above-mentioned studies were conducted in children or adolescents, and were based on the overall DMFT index, not only on the number of teeth with caries.

In all groups, the mean API index value pointed to average oral hygiene. In pregnant women with T1D, higher API values were observed vs. the control group. Pregnant women may experience a temporary deterioration in oral hygiene. This is associated with increased frequency of eating, frequent fatigue, nausea, vomiting and, in the final stage of pregnancy, also reflux, as well as greater susceptibility to gingivitis. [3]

In the study, the salivary antioxidant levels were similar in all groups. In diabetes, oxidative stress is mainly observed due to poor glycemic control, so it is likely that patients in the gestational diabetes mellitus and type 1 diabetes groups had diabetes under proper control. The analysis of the literature indicates a lack of clarity in the previous reports, which is related to the evident discrepancy of the reported results. In studies comparing antioxidant levels in patients with gestational diabetes mellitus vs. pregnant women without diabetes, it was noted that the group with GDM did not show a significant difference in the total antioxidant capacity of saliva whereas all other antioxidant markers decreased compared to the healthy control group. [34] The literature also provides information that, in similar studies, the total antioxidant capacity of saliva increased [35] or decreased [36] in pregnant women with gestational diabetes mellitus compared to non-diabetic women. Discrepancies may result from differences in the methods of laboratory testing used and in the trimesters of pregnancy in which the tests were conducted.

Multifactorial analysis revealed that the variable determining the occurrence of tooth decay is the total Ferric Reducing Ability of Plasma (FRAP). Similar results were obtained by other authors. In their studies, Hegde *et al.* showed that, in adults, an increase in total antioxidant capacity in saliva and plasma correlated with the DMFT value increase. The same author in other studies also evidenced an increase in total antioxidant capacity in patients with tooth decay in relation to the caries-free control group. [37, 38] Uberos *et al.* showed in their study that, in patients with caries, total antioxidant capacity in saliva was 2.89 times greater than in patients without caries. [39] Similar results were also obtained in studies involving children and adolescents. [40, 41, 42] The authors used different methods to determine total antioxidant capacity, with Shaki, Silva and Mahjoub using the FRAP method, which has also been used in current research. Regardless of the method applied, the results were similar. Oxidative stress can initiate and participate in the progression of many inflammatory and infectious diseases, such as caries. The development of caries correlates with changes in salivary oxidative biochemistry, including an increase in lipid peroxidation and total antioxidant capacity in chronic carious lesions. After treatment of carious lesions, however, a decrease in lipid peroxidation is observed. Saliva may be the first line of defense against harmful effects of oxidative stress in oral diseases. The increase in total antioxidant capacity in saliva in people with tooth decay may point to a compensatory mechanism against free radicals. [40, 43] In the study conducted, the activity of other antioxidants did not affect caries occurrence. According to some authors, the activity of superoxide dismutase was increased in patients with caries compared to the control group without caries. [44, 45] The proposed multivariate regression model did not, however, confirm these reports.

Tooth decay was also affected by oral hygiene measured with API. This is not a surprising result, as the presence of plaque is a documented factor involved in the development of caries.

Dental biofilm determines the initiation and progression of demineralization lesions. In the oral cavity, especially in conditions of impaired hygiene, excessive growth of cariogenic bacteria occurs with predominance of *Streptococcus mutans*, *Streptococcus sobrinus*, as well as acidophilic and acid-forming bacteria such as *Lactobacillus spp*. The bacterial biofilm metabolizes carbohydrates, leading to the formation of organic acids which initiate the process of enamel demineralization through calcium ions and phosphates being washed out of its structure. Prolonged persistence of biofilm in an acidic environment shifts the balance towards more acid-forming bacteria and reduces the ability of saliva to neutralize acids. [46]

The effect of diabetes on the presence of caries has not been demonstrated. Gestational diabetes mellitus is one of the most common metabolic complications of pregnancy. [47] According to the data from the literature, no increased tooth decay intensity was observed in pregnant patients with gestational diabetes mellitus vs. healthy pregnant patients. [10] According to some reports regarding type 1 diabetes, high concentrations of glucose in the saliva and secretions of the gingival pockets, as well as reduced salivation, lower pH, and increased density, as well as frequent meals contribute to the development of tooth decay in patients. [48, 49] Other reports indicate that dietary restrictions excluding simple carbohydrates lead to lower incidence of caries in such patients. [50] Caries intensity may also depend on HbA1c levels. The regression model did not account for HbA1c level because the data were available exclusively for 30 patients with type 1 diabetes, which would limit the possibility of conducting the analysis.

All the examined patients were of a similar age, so it is not surprising that age was not decisive for the incidence of caries.

Hypothyroidism did not affect caries incidence in the study population either. Reports from the literature are unclear. Venkatesh *et al*. showed that, in children with hypothyroidism, slightly higher values of the DMFT index were observed but these differences were not statistically significant. [51] Some studies conducted on extracted teeth in patients with hypothyroidism point to structural changes in them within the enamel, dentin, and cementum, both healthy and decayed teeth. [52]

The conducted study has some limitations. One of these involves the inability to perform tooth decay assay on the basis of dental x-ray imaging. X-rays during pregnancy are subject to medical restrictions, except in the most urgent cases. Another limitation is that only a few saliva biomarkers were included in the study, which indicates the need for further research.

The health of hard dental tissues in the examined patients was unsatisfactory and similar in the studied groups. No correlation was found between FRAP and caries in pregnancy subgroups. The study showed that the total Ferric Reducing Ability of Plasma (FRAP) and oral hygiene (API) functioned as determinants of caries.

Authors' contributions

J.W. collected, interpreted the data and was a major contributor in writing the manuscript. B.K.-C., D.P.-G., K.G. were responsible for laboratory analyses. J.P.-P. was responsible for the final version of the manuscript and approved it for submission. All authors read and approved the final manuscript.

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Conflict of interest

None declared.

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Pregnancy and postpartum period as risk factors for cervical artery dissection

Janusz Skrzat, Anna Yevstifeieva, Grzegorz Goncerz

Department of Anatomy, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Janusz Skrzat, Ph.D., D.Sc.
Department of Anatomy, Jagiellonian University Medical College
ul. Kopernika 12, 31-034 Kraków, Poland
Phone/Fax: +48 12 422 95 11; E-mail: j.skrzat@uj.edu.pl

Abstract: Pregnancy and the postpartum period are associated with physiological, hormonal, and hemodynamic changes that may increase susceptibility to cervical artery dissection. Structural alterations of the arterial wall can result from a variety of factors, predisposing vessels to injury and subsequent neurovascular events. In the present study, we analysed the contribution of multiple factors to the development of cervical artery dissection in pregnant women, including advanced maternal age, hormonal status, hypertension, hyperlipidaemia, connective tissue disorders, arterial malformations, migraine, adverse or rapidly changing head and neck positions, neck trauma, chiropractic manipulation, the presence of an elongated styloid process, pressure-inducing activities, and physical exercise.

Keywords: carotid artery dissection, cervical artery dissection, pregnancy.

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Introduction

Pregnancy is associated with considerable cardiovascular and hemodynamic changes such as: increased blood volume, cardiac output, maternal heart rate, whereas blood pressure may either remain constant or be slightly decreased. [1] An initial increase of blood volume is observed about the 12th week of gestation, reaching a peak of 40–50% above non-pregnant levels at 32 to 34 weeks of gestation. The maternal heart rate begins to rise as early as 7 weeks of gestation. By the time of delivery, the heart rate is approximately 20% higher than the rate prior to pregnancy.

Maternal cardiac output starts to increase as early as 10 weeks of gestation, peaking at 50% above the maternal prepregnant cardiac output by the end of the second trimester. During the first stage of labour, cardiac output can increase up to 30%, mainly due to an increase in stroke volume. At term, the cardiac output for a pregnant woman at rest is between 6 and 7 L/min,

compared to the normal range of 4 to 5 L/min observed in non-pregnant women. [1] This elevated level of maternal cardiac output stabilizes and remains consistent until term. Physiological adaptations are essential to meet the increased metabolic demands of both the mother and the developing foetus, and directly influence maternal–foetal oxygen transport. Furthermore, maternal arterial blood pressure declines during the first trimester, reaches its lowest point in the second trimester, and then gradually rises, approaching prepregnant levels in the final two months of pregnancy. [2, 3] Women generally tolerate the physiological changes of pregnancy well; however, under certain conditions, impaired adaptation to hemodynamic and hormonal shifts can weaken vessel walls, leading to vascular complications such as arterial dissection. [4] Thereby, pregnancy may be regarded as a risk factor for artery wall dissection primarily due to altered interplay between hormonal, hemodynamic, and mechanical changes that affect the vascular system.

With regard to the aforementioned issues, this review summarizes the evidence on pathophysiological and mechanical factors associated with cervical artery dissection during pregnancy and the postpartum period, with particular emphasis on factors that compromise arterial wall integrity. A targeted literature search was conducted in PubMed, Scopus, and Google Scholar, considering articles published in English up to December 2025. Search terms included combinations of "cervical artery dissection," "carotid dissection," "vertebral artery dissection," "pregnancy," and "postpartum." Original studies, systematic reviews, meta-analyses, and relevant case series were included, while case reports were selectively used to illustrate specific associations.

Pathophysiology and epidemiology of arterial wall dissection

An artery dissection occurs when there is a tear in the inner layer of the artery wall, leading to a separation of the intima and media layers of the artery wall. When the inner layer of the artery is torn, blood enters the vessel wall, creating a clot or a "false lumen" (an abnormal space between layers of the arterial wall). This condition can disrupt or reduce blood flow to the brain which may lead to ischemic stroke and various neurological impairments. [5, 6] In case of the cervical arteries (the carotid and vertebral arteries) there are two types of dissections: spontaneous and traumatic. Spontaneous dissection does not result from trauma or injury to the neck but usually is a consequence of connective tissue disorders, high blood pressure, migraines, respiratory infections, contraceptive use, pregnancy, autoimmune disease (systemic lupus erythematosus) and smoking, as well. [7] In turn, traumatic dissection of the cervical arteries is caused by external forces that may act during traffic accidents, sports, or even chiropractic manipulations. [8]

Arterial dissections were noted in 5.5 per 100,000 hospitalized pregnant or postpartum women, with the highest occurrence observed during the postpartum period. The most common locations for dissections were coronary (38.2%), vertebral (22.9%), aortic (19.8%), and carotid (19.5%). [9] The average annual incidence of cervical artery dissection has been estimated at 2.6 per 100,000 individuals. [10] Furthermore, Schievink and Roiter noted the incidence of spontaneous cervical artery dissection is highest in autumn (approximately 5 per 100,000). [11] Trager *et al.* found that women during pregnancy and the postpartum period have a twofold increased risk of cervical artery dissection compared to non-pregnant women. [12]

Cervical artery dissection that occurs spontaneously may cause ischaemic stroke or transient ischaemic attack in both young and middle-aged persons, regardless of their sex, although the risk of stroke is higher in women than in men. Additionally, the stroke outcomes for women are

more severe compared to men. [13] Recent studies indicate that cervical artery dissection may occur in both males and females at nearly the same rate. [14] Conversely, Metanis *et al.*, found that cervical artery dissection occurs more frequently in males, who are more likely to have associated risk factors and traumatic neck injuries. [15] As reported by Griffin *et al.*, the incidence rate of spontaneous cervical artery dissection has increased nearly four times from 2002 to 2020, with a pronounced increase of over twelvefold in women. [16]

Contribution of pathophysiological and traumatic factors increasing risk of cervical artery dissection during pregnancy

According to Omran *et al.* pregnancy roughly doubles the risk of cervical artery dissection compared to non-pregnant women, but this risk escalates in postpartum phase, reaching a rate that is 5.5 times higher. [17] This condition provides a possible explanation for why pregnancy is a risk factor for stroke, and why neurologic symptoms such as headache or neck pain occur. [18, 19] Borelli *et al.* noted that postpartum headaches are relatively common and can sometimes be a symptom of serious cerebrovascular events resulting from spontaneous cervical artery dissection. [20] However, not all pregnant women face the same risks of cervical artery dissection, as specific pregnancy-related factors elevate susceptibility in certain cases. The risk is highest in the late third trimester and early postpartum period, when hormonal, hemodynamic, and mechanical stresses overlap. According to Kärkkäinen *et al.* the carotid artery elasticity decreases progressively during pregnancy, particularly in the third trimester. [21] The stiffening of the carotid artery results primarily form advanced gestational age, and does not have a direct correlation with maternal hyperlipidaemia, the diameter of the carotid artery, or alterations in endothelial function. Visontai *et al.* found that during pregnancy, carotid artery stiffness increases, leading to reduced baroreflex sensitivity, despite a concurrent increase in systemic arterial distensibility. [22] This reduction in carotid elasticity is primarily associated with advancing gestational age and is independent of maternal hyperlipidaemia, carotid diameter, or endothelial function. These findings suggest that decreased carotid elasticity represents an adaptive response to pregnancy-related hemodynamic changes, including increased cardiac output and blood volume.

Analysis of risk factors of cervical artery dissection

Various factors, including both pathological and non-pathological, can influence the likelihood of arterial wall dissection by either weakening or damaging the structural composition of the wall. A thorough retrospective cohort study or meta-analysis of these conditions were performed by: Abdelnour *et al.*, [23] Beyer *et al.*, [9] Biller *et al.*, [24] Jacobs *et al.*, [6] Rist *et al.*, [25] Sun *et al.*, [26] Trager *et al.* [12]

From the whole spectrum of risk factors of cervical artery dissection we selected and analysed the conditions that increase vulnerability of the cervical arteries to injury, either by weakening the arterial wall or by exposing it to mechanical stress (Table 1). Thereby, selected factors were classified into two categories:

1. Intrinsic factors / pathogenic conditions — predisposing factors represent underlying conditions that weaken arterial wall, increasing susceptibility to dissection.
2. Mechanical factors / environmental factors — triggering factors that directly cause dissection of arterial wall through mechanical stress.

Table 1. Potential factors predisposing development of the cervical artery dissection during pregnancy and postpartum period.

Intrinsic factors / Pathogenic conditions	Mechanical factors / Environmental factors
Advanced age	Adverse head position and rapid movements
Hormonal level	Neck injuries
Hypertension and hyperlipidaemia	Chiropractic manipulation
Connective tissue disorders	Elongated styloid process
Arterial malformations	Pressure-inducing activities
Migraine	Physical exercises

The influence of intrinsic factors or pathogenic conditions on the arterial wall

Advanced maternal age

Advanced maternal age is associated with three-fold increased risk of cervical artery dissection during pregnancy. In a large case-control and cohort study, pregnant women aged ≥35 years had a higher relative incidence of cervical artery dissection (IRR 3.0) compared with younger women (IRR 1.9) during pregnancy. [17] According to Beyer *et al.*, older maternal age (mean 33 years in women with dissection vs. 28 years in those without) was associated with a higher risk of arterial dissection. Postponed pregnancy may increase the risk of negative pregnancy outcomes, including hypertensive disorders, possibly because of changes in arterial structure and function or inadequate vascular adaptations throughout pregnancy. [9] Lee *et al.* evaluated the impact of advanced maternal age on both vascular and autonomic functions during all three trimesters. [27] Compared with younger pregnant women, those of advanced maternal age demonstrated increased arterial stiffness and elevated carotid intima-media thickness, along with impaired autonomic regulation throughout pregnancy, even though there was no significant variation in endothelial function. This could imply that older pregnant women might have early latent changes affected vascular function. However, no large studies have shown a strong age-specific increase in the risk of cervical artery dissection during pregnancy or the postpartum period.

Although vascular changes associated with advanced maternal age offer a plausible biological explanation for increased susceptibility, this association has not been confirmed by clinical outcome studies.

Hormonal level

Hormonal changes during pregnancy may influence arterial wall structure and function and potentially modify susceptibility to dissection. Hormones, especially: estradiol**,** progesterone, and relaxin play a significant role for maintaining the integrity of the arterial wall.

During pregnancy, elevated estradiol levels have complex effects on arteries: they may weaken the arterial wall structurally, but also improve vascular compliance and reduce hemodynamic stress. As a result, the net effect on arterial dissection risk depends on individual susceptibility. Arterial wall mechanics and endothelial function critically influence both dissection behaviour and repair capacity. [28, 29]

Progesterone exerts protective effects on the cardiovascular system through its anti-inflammatory actions on blood vessels and modulation of vascular function. [30] It influences collagen and elastin synthesis, thereby contributing to the maintenance of arterial wall stability. Furthermore, progesterone affects placental arteries and veins by promoting endothelium-independent relaxation, which supports adequate uteroplacental blood circulation. [31]

In turn, relaxin is a hormone that, during pregnancy, confers cardiovascular benefits. [32] It modulates arterial wall structure by influencing collagen synthesis and maintaining elastin integrity, thereby preventing excessive stiffening while promoting vascular flexibility. It also relaxes vascular smooth muscle to enhance blood flow, dilate vessels, lower arterial pressure, and reduce the risk of arterial wall dissection. [33]

Overall, hormonal influences should be regarded as modifiers of vascular vulnerability rather than independent causal factors for cervical artery dissection.

Hypertension

Arterial hypertension is regarded as a major risk factor for arterial dissection. Pregnancy combined with hypertension elevates the risk of cervical artery dissection encompassing both carotid and vertebral artery dissection. [34] Pezzini *et al.* identified a correlation between arterial hypertension and increased risk of spontaneous cervical artery dissection when compared to the control group. [35] The association was particularly pronounced in patients who also experienced cerebral infarction, especially in cases of vertebral artery dissections. In turn, Arnold *et al.* found that the overall frequency of hypertension did not differ significantly between patients affected with spontaneous cervical artery dissection and controls. [36]

Hypertension, particularly in the form of hypertensive disorders of pregnancy such as preeclampsia or eclampsia and gestational hypertension, appears to be a recurrent and clinically relevant comorbidity in reported cases of pregnancy-related and postpartum cervical artery dissection. Although the available evidence is largely derived from case reports and small case series, the systematic review suggests that elevated blood pressure may contribute to arterial wall vulnerability through hemodynamic stress and endothelial dysfunction during pregnancy and the postpartum period. [23] Roberts *et al.*, in their analysis of population-based trends in hypertensive disorders of pregnancy, reported considerable variation in absolute rates across populations; [37] however, overall prevalence estimates indicate that gestational hypertension affects approximately 3–9% of pregnancies, preeclampsia occurs in 1.4–4%, and eclampsia is comparatively rare.

According to Beyer *et al.* gestational hypertension and preeclampsia/eclampsia were significantly more prevalent among pregnant women with arterial dissection compared with pregnant women without arterial dissection (6.0% vs. 0.6% and 2.7% vs. 0.4%, respectively). [9] Moreover, Omran *et al.* observed that 45% of women diagnosed with postpartum cervical artery dissection had a documented history of hypertensive disorders of pregnancy. [17]

Consequently, precise role of hypertension in dissecting cervical artery remains intricate and needs further studies due to conflicting findings from clinical research. Therefore, the aim of future research should be to establish what the most critical combined effect of hypertension and additional factors is in raising the risk of cervical artery dissection during pregnancy.

Hyperlipidaemia

Several studies have explored hyperlipidaemia as a potential risk factor for cervical artery dissection. However, only a limited number have directly compared serum lipid levels between patients with spontaneous cervical artery dissection and control groups, and these investigations used heterogeneous lipid profiles and assessment methods.

Arnold *et al.* reported that major vascular risk factors including hypercholesterolemia, hypertension, diabetes, and smoking did not differ significantly between patients that experienced cervical artery dissection comparing to the control group. [36] In turn, Abdelnour *et al.* reported in a meta-analysis an inverse association between spontaneous cervical artery dissection and hyperlipidaemia in the general population. [38] However, the authors noted that this association lost statistical significance in sensitivity analyses and that definitions of hyperlipidaemia varied across studies. Although hyperlipidaemia is a well-established risk factor for atherosclerotic cardiovascular disease, it has not, to date, been regarded as a significant direct risk factor for cervical artery dissection during pregnancy or the postpartum period. Instead, hyperlipidaemia appears as potential contributing factor that may interact with physiological factors related to pregnancy which in combination may amplify the risk of vascular changes. The relationship between spontaneous cervical artery dissection and hyperlipidaemia remains unclear, emphasizing the need for well-designed studies and meta-analyses to draw more definitive conclusions. Thereby, further prospective studies are warranted to better define the strength and nature of this association, particularly in the context of cervical artery dissection occurring during pregnancy.

Connective tissue disorders

Connective tissue disorders have been suggested as a potential underlying condition contributing to the aetiology of spontaneous cervical artery dissection. [39] The Ehlers-Danlos syndrome, Marfan syndrome or fibromuscular dysplasia may increase the risk of arterial dissection. Patients with Ehlers-Danlos syndrome have a significantly increased likelihood of hospitalization for carotid and vertebral artery dissection compared with the general population, despite low absolute event rates. [40] Nevertheless, associations with spontaneous cervical artery dissection were also reported as very rare, accounting for less than 2% of all cervical artery dissections in vascular Ehlers-Danlos syndrome, whereas in Marfan syndrome it was less than 1%. [41, 42] Only, fibromuscular dysplasia is more likely to lead to the occurrence of spontaneous cervical arterial dissections. [43, 44] These conditions are all relatively rare genetic disorders, therefore the number of pregnant women who have any of these diseases is relatively low.

Arterial malformations

Cervical arterial malformations, including coils, loops, and elongated segments, significantly alter hemodynamics and increase mechanical strain on the arterial wall making it susceptible to dissection. Kim *et al.* identified a clear link between internal carotid artery tortuosity (reported as loops, kinks or coils) and spontaneous dissection. [45] As reported Giossi *et al.* patients exhibited cervical artery dissection had increased arterial tortuosity. Greater tortuosity of the cervical artery may indicate an increased risk of arterial dissection. [46] The prevalence of any tortuosities of the extracranial portion of the internal carotid artery was higher in the patients with spontaneous

cervicocerebral artery dissection. [47] Furthermore, Barbour *et al.* found a significant correlation between internal carotid artery redundancy and dissection, particularly if redundancy is present bilaterally. [48]

In pregnant women, the risk posed by the arterial malformations can be exacerbated by the combined influence of hemodynamic and hormonal changes. The increased blood volume and blood pressure fluctuations characteristic of pregnancy, particularly in the context of preeclampsia, put additional strain on weakened arterial wall from malformation. Under these conditions, even routine neck motion can act as a mechanical trigger, precipitating a dissection of the compromised arterial wall.

Migraine

Migraine is a common disorder affecting approximately 18% of the female population. The prevalence of migraine increases until approximately the age of 40, after which it begins to decrease. [49] Multiple studies have shown that individuals with a history of migraine have increased (approximately a two-fold) risk of cerebral ischemic events, particularly among young individuals compared to those without migraine, regardless of gender, though few studies presented gender-specific data. [25, 26, 50] Pregnancy usually reduces the frequency and severity of migraine attacks. Rising estrogen and endorphin levels suppress migraines, and post-pregnancy breastfeeding can extend this benefit. Sex hormones affect both migraine types, but in slightly different ways. [51, 52] Approximately 60–70% of women suffering from migraine experience improvement during pregnancy, especially in the second and third trimesters. [53] However, a small percentage of women may experience their first migraine during pregnancy or postpartum period.

Because the incidence of migraine coincides with advanced maternal age (30–40 years), it should be considered a potential contributing factor to cervical artery dissection, particularly in in conjunction with other pathophysiological conditions.

Traumatic factors that can trigger cervical artery dissection

Adverse head position and rapid movements

Prolonged adverse head positioning, rapid head and neck movements have been proposed as potential risk factors for cervical artery dissection. [6] However, such activities during pregnancy can be rather incidental so their role in triggering cervical carotid artery dissection seem to be marginal. For a healthy pregnant woman, avoiding extreme neck movements is more about general comfort, rather than being a direct precaution against cervical artery dissection.

Neck injuries

Neck injuries in pregnant women may result from postural changes. Pregnancy may elevate the risk of certain injuries because of altered body mechanics and the increased weight of the enlarging uterus. As pregnancy advances, the centre of gravity in a woman's body changes position due to the abdominal growth, thereby increasing strain on the cervical musculature and making pregnant women more vulnerable to neck injuries. Studies have shown that pregnancy significantly increases the spine curvatures, especially between the second and third trimesters. [54]

Automobile accidents represent an additional source of neck injuries; therefore, this should be considered for pregnant women as a potential situation that may result in arterial dissection. Injury severity, and fatality rate were significantly lower in pregnant women than in non-pregnant women as found by Koh *et al.* [55] However, this observation should not be regarded as a universal standard in all countries.

Chiropractic manipulation

Chiropractic manipulation (cervical spine manipulation), during pregnancy can be used to help alleviate back pain, pelvic discomfort. There is a potential risk of cervical artery dissection from chiropractic manipulation, especially the vertebral artery. [24, 56] The number of cases is extremely low, and many of these cases involved people with pre-existing vascular risks, such as a history of arterial disease or trauma. According to Curch *et al.* there is no convincing evidence to support a causal link between chiropractic manipulation and cervical artery dissection. [57] Therefore, it's not considered a significant factor for most pregnant women, especially those without pre-existing health conditions like arterial disease or trauma. To minimize risks, pregnant women should consult their healthcare provider before seeing a chiropractor.

Elongated styloid process

An elongated styloid process, and ossified stylohyoid ligament can increase likelihood of dissection in extracranial portion of the internal carotid artery compared with people having normal styloid process. [58] Due to close proximity between these structures, an elongated styloid process or ossified stylohyoid ligament can lead to irritation or compression of the carotid artery, and such condition may trigger dissection of its wall. The prevalence of a styloid process that is elongated (exceeding 30 mm in length) may fluctuate from 1.4% to 30%, based on the criteria for length evaluation and population study. [59] However, only a small proportion (4%) of individuals with an elongated styloid process develop clinical manifestations of Eagle's syndrome. [60] Therefore, its role as a causative factor in internal carotid artery dissection during pregnancy appears to be highly unlikely.

Pressure-inducing activities and physical exercises

Physical activities such as: carrying heavy objects, breath-holding (Valsalva manoeuvre), forceful coughing, vomiting, have been recorded as conditions that probably triggered arterial dissection Nevertheless, there is no strong evidence that everyday activities or physical exercises meaningfully increase the risk of cervical artery dissection. [61] During pregnancy, hormonal and hemodynamic changes may predispose to sudden increases in blood pressure; thus, intense physical exertion or lifting heavy objects may be risky, particularly in women with additional factors predisposing to cervical artery dissection.

Limitations of the study

The present study did not address the following issues:

- Risk of recurrent cervical artery dissection during pregnancy, childbirth and postpartum.
- The risk of cervical artery dissection recurrence in future pregnancies.

- Long-term risk of repeated cervical artery dissection and stroke following pregnancy.
- Spontaneous multiple cervical artery dissection in the pregnancy and postpartum period.
- Cervical artery dissection in women after caesarean delivery during the postpartum period.

Conclusions

Cervical artery dissection is a multi-factorial disorder, particularly in pregnancy and the postpartum period because of physiological changes, which include elevated blood volume, and hormonal imbalance that contribute to stiffening of arterial walls. Additionally, the risk of cervical artery dissection may be amplified by advanced maternal age, arteriopathies and physical traumatic events, as well. Multiple reports suggest that cervical artery dissection may contribute to pregnancy-related or postpartum stroke. The early identification of cervical artery dissection in pregnant women will enable prompt intervention and minimize related neurological complications.

Conflict of interest

The authors declare no conflict of interest, nor any financial interest associated with the current study.

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Management of two osteomas in the frontoethmoidal region using an osteoplastic flap, endoscopic approach and 3D CT imaging

Julia Hypnar[1], Marcin Rudziński[2], Mateusz Magdziarz[1], Jakub Pośpiech[1], Kamil Możdżeń[1], Michał Błażejowski[1], Maria Przeklasa[2], Patryk Hartwich[2], Marcin Konior[2], Jerzy Tomik[2]

[1] Student Scientific Group at the Department of Otolaryngology, Jagiellonian University Medical College, Kraków, Poland
[2] Department of Otolaryngology, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Prof. Jerzy Tomik, M.D., Ph.D.
Department of Otolaryngology, Jagiellonian University Medical College
ul. Jakubowskiego 2, 30-688 Kraków, Poland
Phone: + 48 12 400 27 50; E-mail: j.tomik@uj.edu.pl

Abstract: The aim of this study was to demonstrate the application of preoperative three-dimensional (3D) computed tomography (CT) reconstruction in the surgical management of paranasal sinus (PNS) osteomas. Osteomas are the most common benign PNS tumors and can lead to significant symptoms, including chronic rhinosinusitis, frontal pressure, and orbital or intracranial complications. Surgical excision is recommended for symptomatic cases or those with potential complications, with endoscopic and external approaches being the primary techniques. A 74-year-old patient presented with symptoms of chronic rhinosinusitis, including nasal obstruction and anosmia. CT revealed osteomas in the left ethmoid and frontal sinuses, measuring 27 × 21 × 28 mm and 15 × 17 mm, respectively. The patient was qualified for surgical excision of lesions. Preoperative planning included 3D CT reconstruction to assess the osteomas' size, location, and surgical access, particularly for a planned osteoplastic flap (OPF). During surgery, an endoscopic approach was initially utilized, resulting in frontosphenoethmoidectomy and removal of ethmoid osteoma. The other osteoma extended from the anterior to the posterior wall of the frontal sinus and required conversion to an external approach using the preplanned OPF. The surgery was successfully completed, with intraoperative findings being consistent with imaging. Postoperative complications were limited to frontal sinus outflow stenosis, managed endoscopically during follow-up. PNS osteomas may require complex surgical planning, especially when endoscopic and external approaches are combined. Preoperative 3D CT reconstruction provides valuable insights into tumor characteristics and aids in precise surgical planning. This case highlights the utility of 3D imaging in safe and effective management of challenging PNS osteomas.

Keywords: paranasal sinus osteoma, 3D computed tomography, osteoplastic flap, endoscopic sinus surgery, frontal sinus surgery, surgical planning, sinus tumor.

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Introduction

Osteomas are benign bone tumors and with prevalence rate of 3% in patients presenting with chronic rhinosinusitis, they constitute the most common benign tumors of the paranasal sinuses. The frontal and ethmoid sinuses are, respectively, the first and the second most frequent location of osteomas of the paranasal sinuses. [1]

The first symptoms of paranasal sinus (PNS) osteoma usually appear in the third to sixth decade [2] and vary depending on size and localization. Osteomas of the frontoethmoidal region are usually associated with earlier occurrence of symptoms. Possible manifestations involve frontal pressure, headache, chronic rhinosinusitis, mucocele, facial deformities, proptosis, periorbital pain and epiphora. Most osteomas are, however, an incidental asymptomatic finding.

Surgical excision is recommended for symptomatic osteoma and in certain cases also for asymptomatic osteoma. [1] Possible surgical methods of excision comprise endoscopic approach, external approach and combination of both. [3, 4] The decision concerning the approach is made based on characteristics of the osteoma, its location and direction of extension, as well as experience of the surgeon. [1, 3] Osteoplastic flap is a well-established technique belonging to external approaches. However, due to significant morbidity its use is nowadays limited and endoscopic procedures are preferred method of management of PNS osteoma in most patients with favorable anatomy. [5] The imaging methods, including three-dimensional 3D reconstruction, provide a more precise characterization of osteoma's size, location and relation to the surrounding tissue, playing an important role in planning appropriate surgical approach**.** [4, 6]

Multiple osteomas might be associated with Gardner's syndrome, an inherited autosomal dominant condition, a variant of familial adenomatous polyposis (FAP) [6] with the incidence estimated to be 1 in 8300 to 16000 in American population. [2] The manifestations of Gardner's syndrome include cutaneous and soft tissue tumors, osteomas, supernumerary or unerupted permanent teeth and multiple intestinal polyps with propensity for malignant transformation. [7]

Development of colon polyps begins in the second decade, with the average age at diagnosis being 22 years. Without treatment, the risk of malignant transformation reaches almost 100% in affected patients and is usually observed in individuals aged 30 to 50 years. [8] Development of clinically significant osteomas precedes the diagnosis of FAP by an average of 17 years. As an early diagnosis and surgical treatment prevent the malignant transformation of the polyps, it is beneficial for patients with osteomas to undergo assessment for exclusion of the Gardner's syndrome. [2]

The aim of the study is to demonstrate the application of preoperative 3D planning with the use of 3D computed tomography reconstruction in removal of ethmoid and frontal sinus osteomas via endoscopic and osteoplastic flap approach.

Case presentation

A 74-year-old man presented with symptoms suggestive of chronic sinusitis, including nasal congestion and anosmia. Patient reported no gastrointestinal symptoms. Findings from CT imaging performed 1 year previously were indicative of ethmoid and frontal sinus osteomas. Nasal examination was significant for obstruction and polyps in the left nasal cavity. No pathological discharge was found. CT scan of the head was performed and revealed osteomas in the left ethmoid and frontal sinus, measuring 27 × 21 × 28 mm and 15 × 17 mm, respectively (Fig. 1, 2). The CT scan description suggested osteomas' probable association with Gardner's syndrome. Patient's

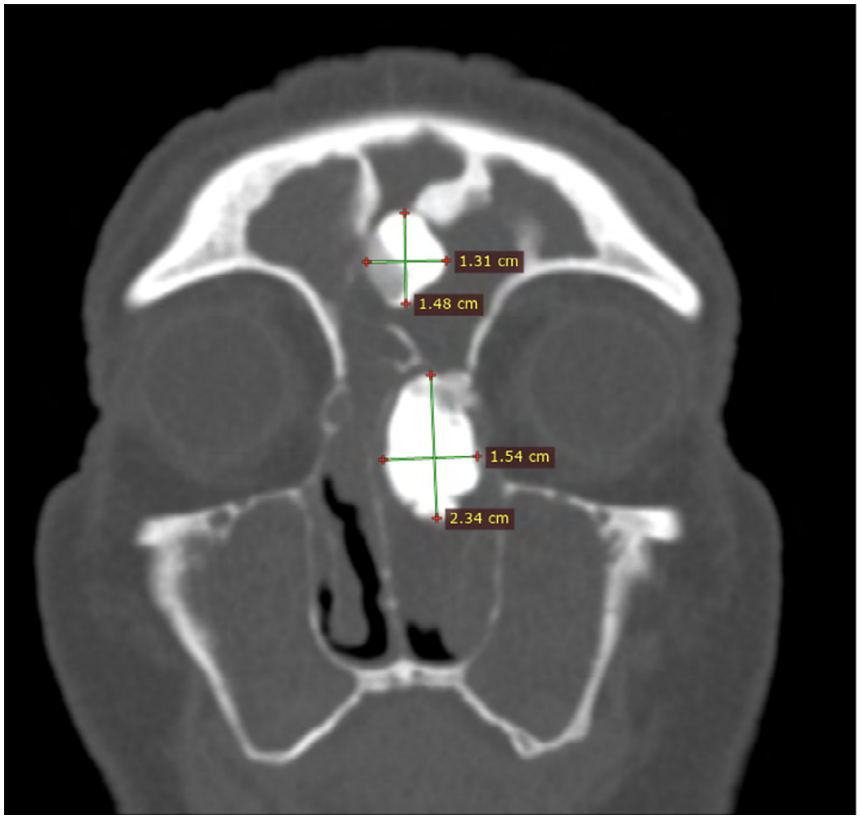

Fig. 1. Computed tomography image of osteomas in ethmoid and frontal sinus with diameter of the osteomas measured in coronal projection.

history was remarkable for bilateral polypectomy and bilateral ethmoidectomy performed, respectively, 26 and 17 years previously.

The patient was qualified for surgical excision of lesions in ethmoid and frontal sinus. The presurgical operation planning first involved generating a three-dimensional computed tomography (3D CT) reconstruction, using dedicated software (RadiAnt). Based on it, the team of operators determined and visualized the image of the planned anterior osteoplastic flap with its exact measurements (Fig. 3, 4). Operators also used 3D CT reconstruction images to plan the optimal external access to the frontal sinus osteoma in case the lesion was not amenable to an endoscopic approach.

Using the endoscopic approach, polypectomy was performed in the left nasal cavity, enabling the identification and dissection of oval-shaped osteoma, with dimensions consistent with those found in the CT. Considering the diameter of the lesion, it proved necessary to cleave it into 2 fragments for safe extraction through the left nostril. Frontosphenoethmoidectomy was performed. Examination of the frontal sinus revealed the other osteoma, consistent with the CT scan description. The nasal septum was dislocated. The endoscopic examination of the right nasal cavity and the sinuses included polypectomy, followed by frontoethmoidectomy. The endoscopic approach

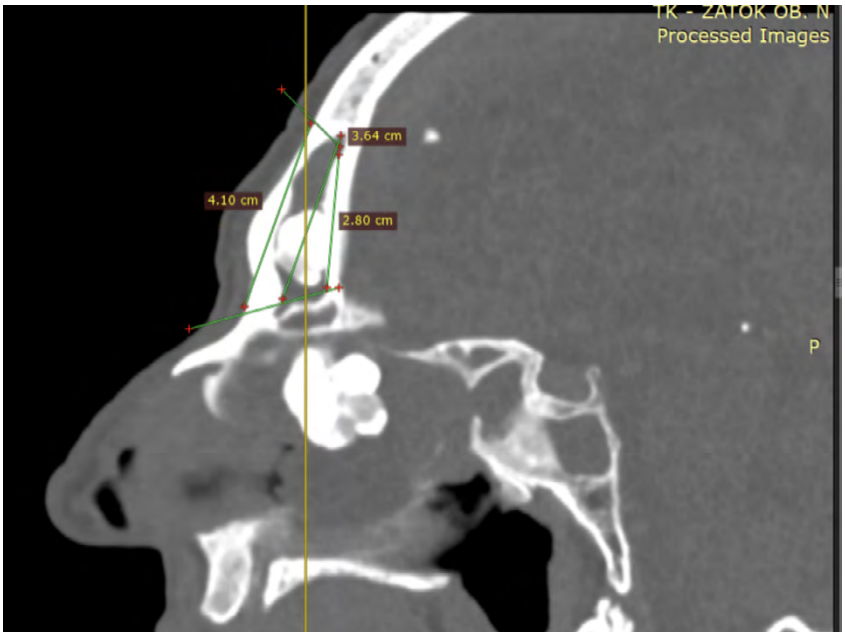

Fig. 2. Computed tomography image of osteomas in ethmoid and frontal sinus with diameter of the frontal sinus measured in sagittal projection.

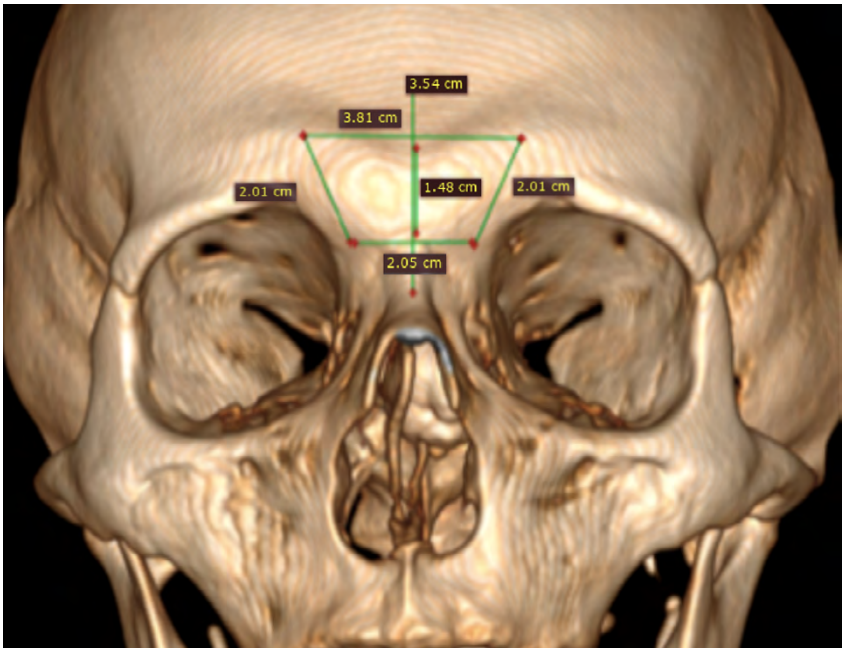

Fig. 3. The three-dimensional computed tomography reconstruction showing the frontal osteoplastic flap which was planned to be removed during surgery, including its exact measured diameter.

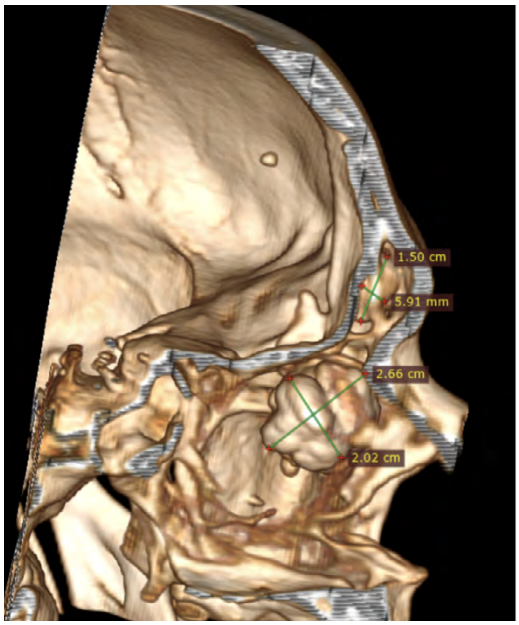

Fig. 4. The three-dimensional computed tomography reconstruction showing the ethmoid and frontal sinus osteomas with measured diameters in sagittal projection.

provided bilateral assessment of the frontal ostium leading to the decision to extract the frontal sinus osteoma using the external approach.

T-shaped incision above the frontal sinuses was made and followed by the removal of the trapezoidal bone flap in accordance with the preoperative plan (Fig. 5, 6), which provided access to the frontal sinuses. Examination of the frontal sinuses revealed the osteoma located in the median line, measuring approximately 30 × 20 mm, connected to both anterior and posterior wall. Using the

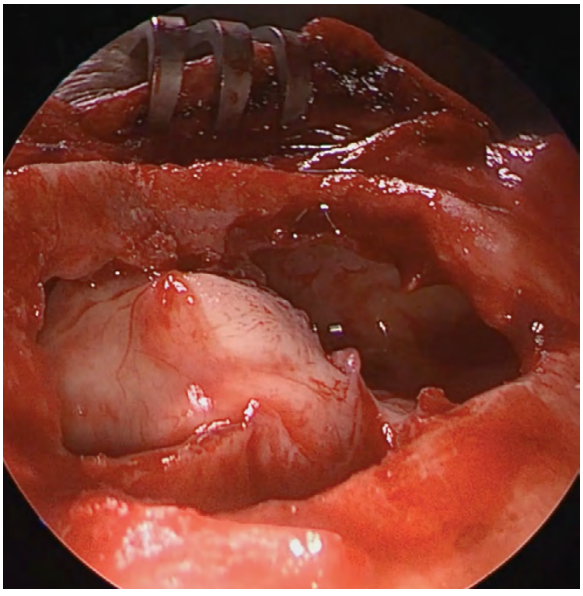

Fig. 5. Intraoperative image of the frontal bone including exposed frontal sinuses and osteoma after the removal of the frontal bone flap.

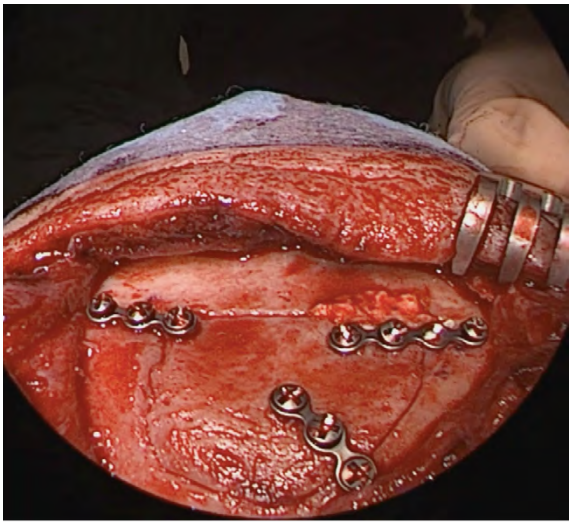

Fig 6. Intraoperative image of the frontal bone after securing the frontal bone flap in place with plates and screws.

cutting burr, the lesion was separated from the anterior wall, allowing it to be extracted. Polyps in the frontal sinus were exposed and removed. The openings of frontal sinus connecting frontal sinuses to the nasal cavity were widened as in the endoscopic modified Lothrop procedure, providing appropriate drainage. The bone flap was replaced and secured in place with plates and screws. The wound drainage and the anterior nasal packing with steroid were kept for 72 and 16 hours, respectively.

Pathological examination confirmed the lesion removed from the frontal sinus was an osteoma. At the follow-up visits 2 weeks and 4 months after the surgery endoscopic examination revealed obstruction on the left side of the frontal sinuses opening. The opening was widened, no pathological discharge was detected. The follow-up examination was otherwise insignificant. The patient was referred to a gastroenterologist for further examination regarding the suspicion of Gardner's syndrome.

Discussion

Osteoma is the most common benign neoplasm of the nose and paranasal sinuses [3] and the most common primary bone tumor in the craniofacial region. Apart from the paranasal sinuses, osteomas develop in maxilla, mandible, mastoid sinus, external auditory canal, the cranial vault, [9] and rarely outside the head and neck region. [3] Aksakal *et al.* [10] calculated the prevalence rate of paranasal sinus (PNS) osteoma in patients who underwent paranasal sinus, maxillofacial computed tomography (CT) and brain CT angiography to be nearly 1%. The frontal and ethmoid sinuses were the most frequent locations. The study conducted by Erdogan *et al.* [9] determined the prevalence rate PNS osteoma in patients undergoing CT scans for sinus symptoms to be 3% with frontal and ethmoid being, respectively, the first and second most frequent location. Semerci *et al.* [11] in a recent study estimated the prevalence of PNS osteomas in patients undergoing cone beam CT tomography for various dental indications to be 7%, with ethmoid sinus constituting the most frequent location, followed by the sphenoid and frontal sinus in decreasing order. In the forementioned studies the size of osteomas varied from 1.15 mm to 50 mm. [9, 10, 11]

The spectrum of clinical manifestations of PNS osteomas is wide and depends on size, location and direction of extension. As most PNS osteomas are asymptomatic, they are frequently identified incidentally in imaging studies or other clinical procedures, [1] similarly to bony defects of the posterior and middle cranial fossa [12] and other lesions in the sinonasal and orbital region. [13] The literature states that only 4 to 10% of all osteomas produce clinical symptoms, with frontal pressure, headache and chronic rhinosinusitis being the most common. Other manifestations include mucocele, external deformities and symptoms resulting from orbital or intracranial extension. Slow growth of osteomas, however, usually precludes development of manifestations other than headache and rhinosinusitis. [1] The peak incidence of PNS osteoma varies in literature and is stated to be in the third [9] and seventh [14] decade.

Multiple osteomas are rare. Aksakal *et al.* [10] reported that multiple osteomas were found in 24 out of 176 patients with at least one osteoma. Erdogan *et al.* [9] stated that in 4 out of 57 cases osteomas were found in more than one sinus. Presence of multiple osteomas might be associated with Gardner's syndrome, an autosomal dominant disease genetically indistinct from familial adenomatous polyposis (FAP), characterized by a triad of symptoms comprised of osteomas, mostly of the mandible and PNS, cutaneous and soft tissue tumors, and intestinal polyposis with propensity for malignant transformation. The literature suggests that development of clinically significant osteomas precedes the diagnosis of FAP by 17 years. Thus, it is crucial for patients diagnosed with PNS osteomas to undergo further examination for Gardner's syndrome. [2]

In this case, 71-year-old patient presented with two PNS osteomas, one located in the left ethmoid sinus and the other in the left frontal sinus, in the median line. The sizes were stated in the description of the CT scan as $27 \times 21 \times 28$ mm and 15×17 mm, respectively. The symptoms present on admission involved nasal congestion, more pronounced on the left side, and anosmia. The symptoms were suggestive of chronic sinusitis and therefore consistent with the most common symptoms of osteomas described in the literature. [1] The patient did not, however, report headache or frontal pressure, which are considered the most common symptoms. The literature states that in individuals affected with Gardner's syndrome the risk of developing colon cancer is nearly 100% by the age of 40. [3] In this case, the patient was 74 at the time of admission to the hospital and presented with no symptoms associated with colon cancer. Nonetheless, the patient underwent further gastroenterological evaluation, and the diagnosis of Gardner's syndrome patient was ruled out.

Indications for surgical excision of an osteoma include symptomatic osteoma (following the exclusion of other explanations for the symptoms), osteoma associated with current complication, and under certain conditions asymptomatic osteoma. [1, 3] As far as asymptomatic osteoma is concerned, Georgalas *et al.* [1] recommends excision of a growing osteoma, an osteoma extending more than 50% of the frontal sinus and an osteoma associated with imminent complications, involving complete obstruction of the frontal recess and intraorbital or intracranial extension. In a more recent study, Sofokleous *et al.* [3] extended those indications to include all osteomas located in the ethmoid sinus, in proximity to frontal recess or in the sphenoid sinus, regardless of their size and lack of symptoms. In this case, the patient presented with symptoms suggestive of chronic rhinosinusitis, associated with two osteomas located in frontal and ethmoid sinus and was therefore qualified for surgical resection of both osteomas.

Surgical resection of an osteoma of the frontoethmoidal region can be performed via an external (open) approach, endoscopic approach, and combined external and endoscopic approach. Since endoscopic approach provides an excellent visualization of the operating field, including the posterior wall of the frontal sinus, anterior ethmoid sinus and orbital walls, [15] and open approach

exposes the patient to longer recovery course and higher complication rates, the endoscopic approach is advisable whenever applicable. [3] Thus, throughout the years attempts have been made to determine the criteria for identification of frontal sinus osteomas amenable to exclusively endoscopic approach. [1] A grading system proposed by Chiu *et al.* [16] is widely used in literature. The authors recommend endoscopic approach for management of only grade I and II osteomas, which include osteomas medial to a virtual sagittal plane through the lamina papyracea with base of attachment posterior-inferior along the frontal recess, and external or combined approach for management of grade III and IV osteomas. Grade III osteomas are defined as those with base of attachment located anteriorly or superiorly within the frontal sinus, or as tumor extending lateral to a virtual sagittal plane through the lamina papyracea. Grade IV osteomas are referred to as tumors filling the entire frontal sinus. According to the literature grading system by Chiu *et al.* [16] has now been surpassed by the current practice, and the limitations of exclusively endoscopic approach are still being explored. [1, 3] In a recent study Sofokleous *et al.* [3] proposed a grading system that classifies grade I and II tumors as manageable through an endoscopic approach and leaves the feasibility of endoscopic resection of grade III tumors at the discretion of the surgeon, as grade III tumors require appropriate training and experience. Grade I tumors according to Sofokleous *et al.* [3] involve former grade I and II tumors, grade II corresponds to former grade III, grade III involves former grade IV tumors and those with a point of attachment in far-lateral aspect of a well-pneumatized frontal sinus. Grade IV tumors are referred to as osteomas with certain characteristics, which makes them currently unamenable to exclusively endoscopic approach. As far as ethmoid osteoma is concerned, anterior extension is considered a limitation of the endoscopic approach. Additionally, extension anteriorly to the nasolacrimal duct and under the skin requires a combined approach. [1]

Endoscopic management of frontal sinus diseases, including osteoma, can be achieved through procedures first described by Draf, [17] which allow opening the frontal sinus outflow more widely. The procedures vary in extensiveness, with Draf III being the most extensive and involving expansion of the frontal recess, superior septectomy and establishing common frontal sinus cavity. Draf III can be a method of choice in management of severe polyposis, benign or malignant tumors and conditions related to trauma or in patients with specific anatomic variations. [18] On the other hand, osteoplastic flap (OPF) technique, belonging to external approaches, for decades was considered the gold standard in the management of frontal sinus diseases. Since the popularization of endoscopic sinus surgery, the role of OPF is mostly reduced to managing frontal sinus diseases in patients with unfavorable anatomy or complications due to previous surgeries. Frontal OPF can be performed with or without obliteration of the frontal sinus. The latter usually includes not only abstaining from occluding the frontal recess, but also widening it to provide drainage to the nasal cavity, restoring physiological function of the sinus. [5]

In this case, three-dimensional (3D) computed tomography (CT) reconstruction was used during preoperative planning for assessment of the frontal sinus osteoma and the anatomy of the frontal sinus. Considering osteoma's anterior attachment within the frontal sinus, the frontal sinus osteoma could be characterized as at least grade III according to Chiu *et al.* [16] or grade II according to Sofokleous *et al.*, [3] classifying it as amenable to endoscopic approach, provided the surgeon had appropriate experience and training. Given osteoma's both anterior and posterior attachment to the frontal sinus and patient's anatomy, a decision was made to perform left frontosphenoethmoidectomy and resection of the left ethmoid osteoma, followed by intraoperative assessment of the frontal ostium to determine if conversion to osteoplastic flap was needed.

3D CT reconstruction was used to determine appropriate size, location and precise measurements of planned frontal OPF. Intraoperative bilateral assessment of the frontal ostium resulted in conversion to the external approach, which was performed according to preoperative 3D reconstruction. The frontal OPF was performed without obliteration of the frontal sinus and appropriate drainage from the sinus to the nasal cavity was provided during the endoscopic part of the surgery.

Despite being a well-established procedure, which is in use for more than 40 years, OPF technique is associated with substantial morbidity. The literature lists dural injury, bone flap fracture and orbital fat exposure as the most common intraoperative complications of OPF. [5] The most significant postoperative complications involve frontal sinus outflow stenosis, mucocele, frontal pain, paresthesia, anesthesia and impaired cosmesis. [1, 5] In a study conducted by Pagella *et al.* [5] frontal sinus outflow stenosis was present in about 36% of patients. Both frontal outflow stenosis and mucocele may be managed with endoscopic endonasal salvage procedure. Similarly, the most common complication related to Draf III procedure is frontal sinus outflow stenosis. Its likelihood might be reduced by regular debridement and intranasal steroid use. [18] In this case, no intraoperative adverse events occurred. Endonasal examination during follow-up visits 2 weeks and 4 months after the surgery revealed left frontal sinus outflow stenosis, which was managed endoscopically.

Conclusions

Paranasal sinus osteoma is the most common tumor of the paranasal sinuses and might produce significant symptoms and complications, including chronic rhinosinusitis and symptoms related to orbital or intracranial extension. Whenever applicable, surgical management of paranasal sinus osteomas might require meticulous preoperative planning, which in certain cases might result in external approach being safer for the patient than endoscopic approach. In this case, successful excision of ethmoid and frontal osteomas was performed. The latter was managed with osteoplastic flap technique, preceded by thorough preoperative planning with the application of 3D CT reconstruction for determining the location and measurements of the bone flap. It resulted in successful excision with no intraoperative adverse events and postoperative complications limited to frontal sinus outflow stenosis.

Author contributions

Conceptualization: M.R., M.P., P.H., M.K. and J.T.; methodology: M.R., M.P. and J.T.; validation M.R., M.K. and J.T.; resources: J.H., M.M. and M.B.; writing—original draft preparation: M.R., J.H., M.M., J.P., K.M. and M.B.; writing—review and editing: M.R., M.K., P.H. and J.T.; visualization: J.H., M.M., K.M. and M.B.; supervision: M.R., M.P., P.H., M.K. and J.T.; project administration: J.H., M.M., M.B., J.P. and K.M.; funding acquisition: J.P., K.M. and J.T. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

None declared.

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CAR-Tography of modeling approaches and opportunities in cellular therapy of cancer

Monika Jesionek

Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Monika Jesionek
Faculty of Pharmacy, Jagiellonian University Medical College
ul. Medyczna 9, 30-688 Kraków, Poland
Phone: +48 620 56 00; E-mail: monika.jesionek@student.uj.edu.pl

Abstract: Chimeric Antigen Receptor CAR-T therapy represents a potent adoptive cell immunotherapy for cancer, yet its clinical application remains constrained by pronounced interindividual variability, severe adverse events, and restricted efficacy in solid tumors. Computational modeling has emerged as a critical framework for analyzing and characterizing CAR-T cell behavior to mitigate these clinical limitations. This systematic review synthesizes 20 computational models identified through targeted bibliographic search that were developed using human clinical datasets.

The reviewed studies employ a range of mathematical frameworks, including cellular kinetics and population pharmacometrics models, tumor-immune interaction models, quantitative systems pharmacology approaches, and multiscale mechanistic models. Across these frameworks, CAR-T cells are represented as dynamic populations undergoing expansion, contraction, persistence, and functional exhaustion. Many models further incorporate phenotypic stratification into functional subsets, most commonly effector and memory cells, to capture the multiphasic kinetics observed in clinical settings. Additional variables frequently include tumor burden, antigen expression, host immune cells, cytokines, and CAR-target complexes, reflecting different levels of biological detail and modeling objectives.

Based on this analysis, I propose a unified set of core variables that captures the key biological processes represented across existing models while providing a consistent structure for future modeling efforts. Together, these studies demonstrate that the choice and structure of variables used to describe CAR-T cell populations and their interactions are key determinants of model interpretability and translational relevance. Improved access to longitudinal clinical datasets and phenotype-resolved measurements will be essential for developing more predictive and clinically applicable computational models of CAR-T therapy.

Keywords: CAR-T therapies, cancer models, computational models, pharmacokinetics, immunotherapy.

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Introduction

Cancer is characterized by the uncontrolled proliferation of transformed cells that undergo evolutionary processes driven by natural selection. Its development can be initiated by a wide range of factors, including genetic alterations, pathogenic agents, environmental exposures, and lifestyle-related influences. [1] Despite significant advances in cancer research, the disease remains a major global health challenge, and the development of effective and durable therapies continues to be a critical unmet need.

Chimeric Antigen Receptor (CAR)-T cell therapy is an adoptive cell immunotherapy that involves the genetic modification of patients or healthy donor T cells to express chimeric antigen receptors targeting cancer-associated antigens. These engineered cells are capable of specifically recognizing and eliminating malignant cells, offering a powerful therapeutic option. [2] CAR-T cell therapy has emerged as one of the most transformative approaches in cancer immunotherapy, with several products approved by the U.S. Food and Drug Administration (FDA) for the treatment of hematological malignancies. [3] Some approved therapies include Axicabtagene ciloleucel (Yescarta), Tisangileucel (Kymriah), Brexucabtagene autoleucel (Tecarus), Lisocabtagene maraleucel (Breyanzi), Idecabtagene vicleucel (Abecma) and Ciltacabtagene autoleucel (Carvykti). [4]

Despite these advances, CAR-T cell therapy is still associated with significant challenges. These include substantial interindividual variability in treatment response, difficulties in achieving durable or complete remissions, the occurrence of severe adverse events, and limited efficacy in patients with solid tumors and even those suffering from other hematological malignancies. [5] To address these challenges, computational modeling has emerged as valuable quantitative tool for analyzing and predicting CAR-T cell behavior, and therapy outcomes. [6]

Over the past few years, numerous computational models have been developed to investigate diverse aspects of CAR-T therapy, including the effects of lymphodepleting preconditioning, the role of inflammatory cytokines, exposure-response relationships, and the impact of intrinsic and extrinsic factors on treatment efficacy. Alongside the development of these models, several review articles have been published that comprehensively summarize modeling strategies and provide valuable insights into the current state of computational approaches in CAR-T research.

Chaudhury *et al.* proposed a taxonomy of cellular kinetic models describing the *in vivo* dynamics of CAR-T cells and their interactions with cancer cells. [7] Nukala *et al.* systematically reviewed models focusing on T-cell activation, malignant and host T cells population dynamics, and overall patient survival, with an emphasis on cost-effective therapeutic strategies. [8] Qi *et al.* reviewed models addressing the cellular phases and complex mechanisms of CAR-T therapy, with particular attention to immunological factors influencing treatment efficacy. [9] In 2024, Kirouac, Zmurchok and Morris utilized available CAR-T clinical data to demonstrate how mathematical models can quantitatively address key challenges in CAR-T therapy and support the development of effective clinical strategies. [10] More recently, in 2025, two additional reviews were published. Putignano *et al.* examined how computational approaches can enhance CAR-T therapy design and predict therapeutic outcomes, with a focus on antigen binding and CAR-T cell dynamics. [3] In parallel, Murias-Closas *et al.* emphasized the practical utility of existing models, highlighting that their effectiveness depends less on model complexity and more on the appropriate choice of mathematical framework and the availability of high-quality data. [11]

In this review, I provide a comprehensive and systematic overview of existing computational models of CAR-T cell therapies whose development has been informed by cancer clinical data.

I focus specifically on how CAR-T cell behavior is represented across different modeling frameworks, with particular attention paid to the key variables and assumptions used to describe CAR-T cell dynamics, interactions with cancer, tumor and host cells, and measures of treatment effectiveness. By comparing a range of various modeling approaches, I aim to highlight both common strategies and important differences in how CAR-T therapies are conceptualized and simulated. Finally, I outline future directions for computational modeling, emphasizing the potential role of integrative, data-driven approaches supporting the optimization and personalization of CAR-T cell therapies.

Methods

A systematic literature review was conducted using the PubMed database to identify existing models of CAR-T therapies. An advanced search was employed using the keywords "CAR-T" AND "model" and "CAR-T" AND "computational model." Additionally, selected review articles addressing CAR-T modeling were reviewed to identify further relevant publications. [3, 7, 8, 9, 10, 11] The screening was conducted based on the full text of the identified articles. In total, 49 publications describing CAR-T therapy models were initially identified. These publications were subsequently evaluated for the use of human clinical data from cancer studies, while studies using data for other diseases, as well as those based exclusively on preclinical or other non-clinical data were excluded. As a result, 20 models met the inclusion criteria and were included in the final analysis.

Results

Sources and characteristics of clinical data

Clinical data form the foundation of computational models of CAR-T therapies, enabling quantitative descriptions of cell kinetics, treatment response, and interindividual variability to calibrate, validate, and assess the clinical relevance of these models. The reviewed studies relied on diverse clinical datasets covering multiple cancer indications and CAR-T cell constructs.

The majority of analyzed models were informed by hematological malignancies, including B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), large B-cell lymphoma (LBCL), non-Hodgkin lymphoma (NHL), mantle cell lymphoma (MCL), and multiple myeloma (MM) study data. [5, 6, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22] Several studies focused on chemotherapy-resistant or relapsed/refractory disease, reflecting clinical challenges associated with cancer treatments. [23, 24, 25, 26, 27]

Across hematological malignancies, CD19-targeting CAR-T cells were the most frequently modelled therapies. [13, 14, 15, 16, 17, 18, 19, 21, 24, 25, 26] These investigations focused predominantly on the Axicabtagene ciloleucel (Yescarta) [13, 14, 17, 18, 24] and Tisagenlecleucel (Kymriah) [14, 16, 18, 24, 26] while other utilized Lisocabtagene maraleucel (Breyanzi), [19] the IM19 product [25] and variant constructs including FMC63 or CAT19. [19] Furthermore, some evaluate various anti-CD19 constructs, featuring for example CD28 or 4-1BB co-stimulatory domains. [15, 21]

In multiple myeloma, computational models incorporated clinical data from trials evaluating anti-B-cell maturation antigen (BCMA) CAR-T cells, such as Ciltacabtagene autoleucel (Carvykti), Idecabtagene vicleucel (Abecma). [5, 14, 22, 27] Some studies also included multiple types of therapies, in cases when clinical data were from patients suffering from various malignancies. [5, 6, 12, 14, 20, 23]

Two studies utilized clinical data from allogeneic CAR-T therapies, with one incorporating information on lymphodepleting regimens with fludarabine or alemtuzumab to investigate their effects on CAR-T dynamics. [6, 28] Other models accounted for the management of treatment-related toxicities, including the use of corticosteroids or tocilizumab. [26]

Although less frequent, several models extended to solid tumors. These data were generally more heterogenous and limited in scope, and, in some cases, complemented by imaging-derived measurements, such as MRI data, or by patient-derived cellular data. [29] Models used data from patients suffering from non-small cell lung cancer, glioblastoma, melanoma, ovarian cancer, pancreatic cancer, advanced hepatocellular carcinoma, and metastatic castration-resistant prostate cancer. They addressed a broader range of CAR-T targets, including EGFR, EGFRvIII, IL13Rα2, mesothelin, GPC3, and PSMA. [5, 12, 18, 23, 29]

Administered doses of CAR-T cells varies widely upon analyzed frameworks. Across modeling and clinical studies, total doses generally range from 10^7 to 10^9 cells per patient, with many analyses assuming representative flat doses around 10^8 cells or evaluating discrete levels between $1 \times 10^7$and 2.5×10^8 cells. [5, 6, 13, 14, 18] Several studies also examine specific clinical cohorts such as 6×10^6 to 2.4×10^8 cells or flat dose levels of 50, 150, 450, and 800 million cells. [14, 22, 28] Weight based doing is commonly used in hematological malignancies, typically within 3×10^5 to 3×10^6 cells/kg and broader ranges of $0.2 - 2 \times 10^6$ cells/kg, while other therapies are administered at approximately $0.5 - 2 \times 10^6$ cells/kg. [16, 17, 19, 25, 26, 27] Some models incorporate split infusion regimens of 10, 30, and 60 percent over three days, [6, 24] whereas others scale CAR-T cells dynamics to bone marrow levels. [15] Additional reported scenarios include patient specific doses such as 9.23×10^7 cells or 1.2×10^7 and 1.4×10^6 cells/kg in pediatric cases, [12, 20, 21] as well as body surface area-based regimens in solid tumors ranging from tens to hundreds of millions of cells/m^2 and total doses up to several billion cells. [23] Experimental analyses also evaluate effector to target ratios between 1:5 and 1:20 to study proliferation and exhaustion dynamics. [29]

Across all cancer types, clinical data were obtained directly from early-phase clinical trials, [16, 17, 22, 26, 27, 28] published clinical studies, [5, 6, 12, 15, 19, 20, 21, 22, 23, 24, 25] and patient-level datasets [5, 14, 15, 29] or supplemented with aggregated data from literature. [13, 18, 19] In one case, investigators constructed virtual patient populations based on existing clinical data to capture interindividual variability and the reliability of model predictions. [14] Additionally, some models combined clinical and preclinical data to compensate for limited data availability. [19, 22]

Parameters

The parametrization of the reviewed computational models generally relied on the combination of fixed values derived from existing literature and parameter estimates obtained from the characteristics of the analyzed datasets. [6, 17, 18, 19, 20, 22, 23, 24, 25] In addition to using fixed parameters, Antonini *et al.* applied Bayesian estimation methods including Monte Carlo Integration, Bayesian Inference, and Markov Chain Monte Carlo (MCMC) to estimate kinetic parameters and evaluate different algorithms to determine which provided the most accurate predictions. [12]

Another strategy involved estimating model parameters using both computational and experimental tools. Optimization methods included particle swarm optimization (PSO) based on minimizing the log mean squared error (MSE), the stochastic approximation expectation maximization algorithm for nonlinear mixed-effect modeling (NLME) with fixed and random parameters, and gradient-based optimization via Broyden–Fletcher–Goldfarb–Shanno (BFGS). [5, 13, 14, 15,

16, 20, 26, 27, 28] Moreover, real-time cell analyzer (RTCA) systems were used to obtain dynamic in vitro measurements that directly informed parameter estimation. [29]

Variables

The analyzed models represent diverse approaches to describing the dynamics of CAR-T cell therapy. The choice of modeling framework was primarily determined by the type of available data as well as by the study endpoints. While some models incorporated phenotypic differentiation of CAR-T cell subpopulations such as effector, memory, or exhausted cells, others treated CAR-T cells as a single aggregated variable and focused on their overall activity. A subset of models explicitly accounted for interactions with cancer cells, host immune cells, or both. Although the models varied considerably in complexity and scope, all could capture key mechanisms and clinically relevant outcomes of CAR-T cell therapy (Table 1).

Table 1. Overview of published mathematical models of CAR-T cell therapy across different cancer types, including the original model state variables and their unified categories.

Author, year	Cancer type(s)	Model state variables	Unified variables
Phenotype-structured CAR-T cell models			
Mueller-Schoell *et al.*, 2021 [17]	NHL (DLBCL, PMBCL, TFL)	Naïve CAR-T cells T_N [cells/μL], Terminally differentiated effector CAR-T cells T_{Eff} [cells/μL], Effector memory CAR-T cells T_{EM} [cells/μL], Central memory CAR-T cells T_{CM} [cells/μL], Metabolic tumor volume *CD*19+ [mL]	*I*, *E*, *M*, *T*
Liu *et al.*, 2021 [5]	B-ALL, CLL, DLBCL, MM, NSCLC, GBM	Effector CAR-T cell concentration *E* [copies/μg DNA], Memory-phenotypic CAR-T cell concentration *M* [copies/μg DNA]	*E*, *M*
Paixão *et al.*, 2022 [6]	B-ALL, CLL, DLBCL, MCL	Distributed CAR-T cells C_D [cells], Engrafted (Effector) CAR-T cells C_T [cells], Memory CAR-T cells C_M [cells], Exhausted CAR-T cells C_E [cells], Tumor cells *T* [cells]	*I*, *E*, *M*, *X*, *T*
Kirouac *et al.*, 2023 [14]	CLL, B-ALL, LBCL, MM	Effector CAR-T cells *TE* [cells or cells/ml], Memory CAR-T cells *TM* [cells or cells/ml], Exhausted CAR-T cells *TX* [cells or cells/ml], Tumor B cells *B* [cells or cells/ml], Tumor antigen levels B_A [amount]	*E*, *M*, *X*, *T*
Antonini *et al.*, 2024 [12]	Hematological malignancies	Distributed CAR-T cells C_D [cells], Effector CAR-T cells C_T [cells], Memory CAR-T cells C_M [cells], Exhausted CAR-T cells C_E [cells], Tumor cells *T* [cells]	*I*, *E*, *M*, *X*, *T*
Models incorporating immune interactions and toxicity			
Hardiansyah & Ng, 2019 [24]	CLL, B-ALL	Effector CAR-T cells *CARTE* [cells], Memory CAR-T cells *CARTM* [cells], B-cell tumor burden B_{PB} [cells], Pro-inflammatory cytokines *IL-6*, *IL-10*, *IFN-γ* [pg/ml]	*E*, *M*, *T*, *B*
Derippe *et al.*, 2022 [28]	B-ALL	Stem central memory CAR-T cells T_{SCM} [cells/L], Central memory CAR-T cells T_{CM} [cells/L], Effector memory CAR-T cells T_{EM} [cells/L], Host T cells or NK cells in the *Host*X1 [cells/L], Expanding host immune cells *Host*X2 [cells/L], Interleukin-7 concentrations *IL-7* [pg/mL], Lymphodepleting variables for Alemtuzumab *Alem* [μg/mL] and virtual chemotherapy *FC* [unitless]	*S*, *M*, *H*, *B*, *L*

Table 1. Cont.

Author, year	Cancer type(s)	Model state variables	Unified variables
Phenotype-structured CAR-T cell models			
Santurio *et al.*, 2025 [21]	B-ALL, CLL, MM	Injected CAR-T cells C_I [cells], Expander CAR-T cells C_E [cells], Persister CAR-T cells C_p [cells], Antigen-positive tumor cells T_P [cells], Antigen-negative tumor cells T_N [cells], Naïve macrophages M_i [cells], Activated macrophages M_a [cells], Interleukin-6 blood concentration *IL6* [pg/mL]	I, A, M, T, H, B
Models addressing antigen resistance and heterogeneity			
Liu *et al.*, 2022 [15]	B-ALL	Non-activated CAR-T cells n_{TN} [cells], Activated CAR-T cells n_{TA} [cells], CD19+ B-ALL cells n_P [cells], CD19- tumor cells n_N [cells]	*I, A, T*
Santurio *et al.*, 2024 [20]	B-ALL, ALL, CLL, MCL, DLBCL	Effector CAR-T cells C_T [cells], Memory CAR-T cells C_M [cells], Antigen-positive tumor cells T_P [cells/antigen], Antigen-negative tumor cells T_N [cells/antigen]	*E, M, T*
Predator-prey and tumor-immune interaction models			
Sahoo *et al.*, 2020 [29]	GBM	CAR-T cells *Y* [cells or cell index], Cancer cells *X* [cells or cell index]	*E, T*
Kimmel *et al.*, 2021 [13]	LBCL, *applicable to CLL, B-ALL, MCL	CAR-T cells *C* [cells/μL], CD19+ tumor cells *B* [cells], Normal T cells *N* [cells/μL]	E, T, H
Owens & Bozic, 2021 [18]	DLBCL, CLL, Metastatic Melanoma	CAR-T cells *C* [cells], Tumor cells *T* [cells], Endogenous effector cells *E* [cells], Chemotherapy concentration *M* [μM]	*E, T, H, L*
Multiscale mechanistic models			
Singh *et al.*, 2021 [22]	MM	Effector CAR-T cells $CAR - T_e$ [cells], Memory CAR-T cells $CAR - T_M$ [cells], CAR-target complexes C_{plx} [complexes/cell], Total tumor cells *Tumor* [cells], Serum M-protein *M – protein* [pg/mL], Soluble BCMA *sBCMA* [pg/mL]	*E, M, C, T, B*
Salem *et al.*, 2023 [19]	ALL, DLBCL	Effector CAR-T cells *E* [cells/L or cells/μL], Terminal effector CAR-T cells T_{Eff} [cells/L or cells/μL], Stem-cell memory CAR-T cells T_{SCM} [cells/L or cells/μL], Memory CAR-T cells *M* [cells/L or cells/μL], Central memory CAR-T cells T_{CM} [cells/L or cells/μL], Effector memory CAR-T cells T_{EM} [cells/L or cells/μL], CAR-target complexes C_{mplx} [complexes/cell], Tumor cells *Tumor* [cells/L or cells/μL]	*E, S, M, C, T*
Minnuci *et al.*, 2024 [25]	B-cell NHL	Infused CAR-T cells T_{inf} [nmol], Activated CAR-T cells T_{act} [nmol], Effector CAR-T T_{eff} [nmol], Memory CAR-T cells T_{mem} [nmol], Free CAR receptors *CAR* [nmol], Bound CAR-target complexes *CAR:CD19* [nmol], Antigen-expressing malignant cells *Tumor* [nmol], Endogenous lymphocytes *Endo* [nmol], Free CD19 antigen receptors m_{Ag} [nmol]	*I, A, E, M, C, T, H*

Table 1. Cont.

Author, year	Cancer type(s)	Model state variables	Unified variables
Phenotype-structured CAR-T cell models			
Parmar *et al.*, 2025 [23]	B-cell lymphoma, MPM, OVCA, PDAC, HCC, mCRPC	Effector CAR-T cells C_e [cells/L], Memory CAR-T cells C_m [cells/L], CAR-target complexes C_{TE} [number of complexes], Antigen-expressing tumor cells V^{TAA} [L], Antigen-negative tumor cells V^{NO-TAA} [L], Host T lymphocytes R [cells/L], Virtual lymphodepleting therapy A [virtual units]	E, M, C, T, H, L
Population and pharmacometric models of CAR-T kinetics			
Mueller *et al.*, 2018 [16]	B-ALL	Observed transgene levels of CAR-T cells in copies/μg of genomic DNA	
Stein *et al.*, 2019 [26]	B-ALL	Observed transgene levels of CAR-T cells in copies/μg of genomic DNA, as a sum of effector CAR-T cells E and memory CAR-T cells M	
Wu *et al.*, 2022 [27]	MM	Observed transgene levels of CAR-T cells in copies/μg of genomic DNA, as a sum of fast-eliminated CAR-T and sustained CAR-T subpopulations	

Legend: I — Infused/Initial CAR-T cells (Distributed, Naïve, Injected, Non-activated, Infused), A — Activated/Proliferating/Expander CAR-T cells, E — Effector/Engrafted CAR-T cells (active tumor-killing population) [in case of only CAR-T cells, assumed Effector], S — Stem-cell memory CAR-T cells, M — Memory CAR-T cells (Central, Effector, Persister), X — Exhausted CAR-T cells (Non-functional), C — Complexes CAR-Target, T — Tumor/Cancer cells and tumor-related variables (unified all malignant cells/solid tumors, antigen positive/negative cells), H — Host cells (T cells, NK cells, Macrophages), B — Biomarkers (Cytokines, Soluble proteins), L — Lymphodepletion/chemotherapy

Phenotype-structured CAR-T cell models

A major group of models represents CAR-T cells as multiple phenotypic compartments to capture multiphasic expansion–contraction profiles, functional maturation, and long-term persistence. Paixão *et al.* developed an ordinary differential equation (ODE)-based model aimed at establishing a biological foundation for multiphasic kinetics of CAR-T. CAR-T cells were represented as phenotypic subpopulations, including distributed (C_D), effector (C_T), memory (C_M) and exhausted (C_E). By incorporating tumor cells (T) variable, the authors described the CAR-T cell life cycle during therapy, in which distributed cells undergo activation, engraftment and differentiate into active effector cells. Tumor persistence stimulates CAR-T cell expansion but also promotes differentiation into a memory pool that supports long-term persistence or transition into an exhausted state due to chronic stimulation. [6]

Building upon this framework, Antonini *et al.* introduced Bayesian parameter estimation to optimize patient-specific model calibration. They evaluated several MCMC algorithms, including Metropolis-Hastings, DEMetropolis, and DEMetropolisZ, to address sparse clinical data and interindividual variability. While retaining the same mathematical description of CAR-T cell dynamics, their work emphasized the probabilistic nature of biological interactions, thereby providing an option for personalized treatment planning. [12]

Liu *et al.* focused on retrospective characterization of multiphasic CAR-T cell kinetics to identify factors influencing proliferation and persistence. Their immune-dynamic model included effector (*E*) and memory (*M*) CAR-T cell compartments. Effector cells were assumed to undergo exponential expansion upon antigen recognition. Following tumor clearance, a fraction of effector cells differentiated into long-lived memory cells, while the remaining population underwent activation-induced cell death. [5]

Phenotypic differentiation was also incorporated by Kirouac *et al.* in an antigen-driven toggle switch ODE model integrated with machine learning techniques. This study focused on deconvoluting clinical variability and predicting patient outcomes based on pre-infusion CAR-T product transcriptomes. CAR-T cells were classified as memory (*TM*), effector (*TE*) differentiated into non-cytotoxic effector (*TE*1) and cytotoxic effector cells (*TE*2), and exhausted cells (*TX*). The model additionally included variables describing B-cell tumor burden (*B*) and tumor antigen levels (B_A), which drive the transition of memory cells into expanding non-cytotoxic and subsequently cytotoxic effector populations. Persistent tumor burden leads to chronic antigen stimulation and CAR-T cells exhaustion, whereas effective tumor lysis by effector cells reduces antigen levels and enables regeneration of the memory compartment. [14]

A distinct approach was proposed by Mueller-Schoel *et al.* who developed a quantitative systems pharmacology (QSP) framework for early survival prediction. CAR-T cells were represented as naïve (T_N), central memory (T_{CM}), effector memory (T_{EM}), and terminal effector (T_{Eff}) cells, alongside metabolic tumor volume variable (*CD*19+). The model demonstrated maturation of CAR-T cells from naïve to central and effector states, with proliferation and differentiation driven by metabolic tumor volume. All phenotypes except terminal effector cells contributed to tumor reduction through nonlinear cytotoxic killing mechanisms. [17]

Models incorporating immune interactions and toxicity

Several mechanistic frameworks explicitly incorporated host immune compartments and inflammatory mediators to explain variability in CAR-T expansion and the emergence of clinically relevant toxicities. Derippe *et al.* applied a mechanistic modeling approach to quantify the interplay between lymphodepletion, homeostatic cytokines, and the host immune system in the context of allogeneic CAR-T cells kinetics. CAR-T cells were represented as stem central memory (T_{SCM}), central memory (T_{CM}), and effector memory (T_{EM}) subpopulations in blood and tissue compartments. Additional variables included host T or NK cells (*HostX*1), expanding host immune cells (*HostX*2), interleukin-7 (*IL* – 7) concentration, lymphodepleting agent alemtuzumab (*Alem*), and virtual chemotherapy (*FC*). The model demonstrated that host immune cells recognize and eliminate allogeneic CAR-T cells. However, lymphodepletion reduces host cell abundance, thereby increasing the availability of homeostatic cytokines such as IL-7, which in turn enhances CAR-T cell expansion and persistence. [28]

Cytokine dynamics were also incorporated in the model developed by Santurio *et al.*, who used a multilayer mechanistic approach to characterize the temporal development of macrophage-mediated cytokine release syndrome (CRS), a common toxicity associated with CAR-T therapy. CAR-T cells were divided into injected (C_I), expanding (C_E), and persisting (C_P) subpopulations. The inclusion of antigen-positive and antigen-negative tumour cells (T_P, T_N), naïve and activated macrophages (M_i, M_a), and IL-6 concentrations allowed the authors to demonstrate that tumour cell killing releases damage-associated molecular patterns (DAMPs), which activate naïve macrophages.

Furthermore, interactions between CAR-T cells and macrophages establish a positive feedback loop that drives excessive IL-6 secretion by activated macrophages, potentially leading to CRS. [21]

Hardiansyah and Ng developed a QSP model to quantify the relationships between CAR-T cell dose, disease burden, and systemic inflammatory responses. CAR-T cells were represented as effector (*CART E*) and memory (*CART M*) populations in blood and tissue compartments. By incorporating malignant B cells (B_{PB}) and cytokines such as *IL-6, IL-10*, and *IFN-γ*, the model demonstrated that effector CAR-T cells expand upon interaction with malignant B cells, triggering localized secretion of pro-inflammatory cytokines. Importantly, the results indicated that cytokine peak levels correlate more strongly with baseline tumour burden than with the administered CAR-T cell dose. [24]

Models addressing antigen resistance and heterogeneity

A smaller but conceptually important set of models extended tumor dynamics to include antigen-negative or low-antigen phenotypes, enabling the study of immune escape under therapeutic pressure.

Several studies focused on antigen resistance and heterogeneity. Liu *et al.* developed a nonlinear mixed-effects ODE model to predict late responses to CAR-T therapy. CAR-T cells were described as activated (n_{TA}) and non-activated (n_{TN}), while tumor cells were divided into antigen-positive (n_P) and antigen-negative (n_N) populations. Activated CAR-T cells proliferated while eliminating antigen-positive tumor cells. Therapeutic pressure shifted the tumor population toward antigen-negative cells, which evade direct CAR recognition but may be partially eliminated via bystander killing mediated by lysis of neighboring antigen-positive cells. [15]

In complementary study, Santurio *et al.* investigated how a continuous distribution of antigen density on the tumor surface influences resistance development and treatment outcomes. CAR-T cells were described as effector (C_T) and memory (C_M) cells. Tumor cells were distributed along a continuous antigen expression variable (x), with state variables representing densities of antigen-positive and antigen-negative tumor cells at antigen level x (T_P, T_N), as well as the total tumor population (T). CAR-T-mediated therapeutic pressure induced a reversible phenotypic drift, whereby tumor cells temporarily downregulated antigen expression to evade immune attack and returned to homeostatic levels as CAR-T cell pressure declined. [20]

Predator-prey and tumor-immune interaction models

To reduce model dimensionality while retaining interpretability, several studies adopted predator-prey or tumor-immune interaction structures that focus on mutual stimulation, cytotoxicity, and exhaustion.

A subset of studies employed predator–prey frameworks to describe CAR-T cell behavior. Kimmel *et al.* developed a tumor extinction model incorporating CAR-T cells as a cumulative variable (C), normal T cells (N) and antigen-presenting tumor cells (B). CAR-T cells and endogenous T cells competed for a limited homeostatic carrying capacity, with CAR-T cells acting as predators. Therapeutic success depended on CAR-T cells driving tumor cells to stochastic extinction before being outcompeted by the recovering host immune system. [13]

In the modified Lotka–Volterra CARRGO model, Sahoo *et al.* focused on deconvoluting the proliferation and exhaustion rates of CAR-T cells to better understand treatment efficacy in solid

tumors. The model described interactions between CAR-T cells (Y) and cancer cells (X) as the only state variables. Cancer cells acted as a stimulus for CAR-T cell expansion while simultaneously serving as a driver of functional exhaustion. Using this framework, the authors demonstrated that lower CAR-T cell doses were associated with higher per-cell tumor killing efficiency but led to more rapid exhaustion of the CAR-T cell population. [29]

Owens and Bozic adapted the tumor–immune interaction model originally proposed by Kuznetsov *et al.*, [30] to assess the impact of preconditioning on CAR-T therapeutic success. In this ODE system, CAR-T cells (C) expanded and eliminated tumor cells (T), while chemotherapy (M) reduced tumor burden and endogenous effector cells (E), thereby decreasing competition for resources. [18]

Multiscale mechanistic models

Multiscale models link CAR-T pharmacology and trafficking with tumor burden and biomarkers, allowing CAR-target binding and tissue distribution to be reflected in systemic kinetics and efficacy. There have also been models developed within frameworks ranging from molecular receptor binding to whole-body physiology.

Parmar *et al.* developed a physiologically based pharmacokinetic–pharmacodynamic (PBPK–PD) model to characterize CAR-T cell trafficking and anti-tumor activity in solid tumors, explicitly accounting for host immune competition as well as the effects of lymphodepletion. The model described vascular (CV) and extravascular (CEV) CAR-T concentrations across organs, CAR–target complexes (C_{TE}), host T cells (R), antigen-positive (V^{TAA}) and antigen-negative tumor cells (V^{NO-TAA}). In this framework, CAR-T cells extravasate from the blood into tissue interstitial spaces and recirculate via lymphatic flow. Molecular engagement with antigen-expressing tumor cells leads to the formation of CAR–target complexes, driving CAR-T expansion and tumor cell depletion, while recovering host T cells compete for immunological space. [23]

In the work of Singh *et al.*, the objective was similar, aiming to establish a quantitative relationship between CAR affinity, antigen abundance, and tumor depletion in order to characterize CAR-T cell activity. In this mechanistic PK–PD model, effector ($CAR - T_e$) and memory ($CAR - T_M$) cells were assessed in blood and bone marrow, together with CAR–target complexes (C_{plx}), total tumor cells (*Tumor*), and soluble biomarkers like serum M-protein and soluble BCMA. CAR-T cells rapidly distributed to the bone marrow, which represents the site of action, where interaction with BCMA led to complex formation. These interactions drove effector proliferation and differentiation into a persistent memory pool while inducing tumor cell lysis, which was monitored through reductions in biomarker levels. [22]

Salem *et al.* developed a multiscale mechanistic cellular kinetic–pharmacodynamic (CK–PD) model to describe *in vitro* and *in vivo* CAR-T cell dynamics in patients with cancer. The model included CAR-T cells stratified into CD4+ and CD8+ subsets and further differentiated into stem cell memory (T_{SCM}), central memory (T_{CM}), effector memory (T_{EM}), effector (E) and terminal effector (T_{Eff}) immunophenotypes, distributed between peripheral blood (PB) and bone marrow (BM) compartments. Additional state variables included CD19+ tumor cells (*Tumor*), as well as CAR–tumor target complexes (C_{mplx}) formed upon antigen engagement. CAR-T cells interacted with tumor cells through the formation of CAR–target complexes, which simultaneously drove tumor cell killing and CAR-T cell expansion. Expansion was restricted to early immunophenotypes, while CAR-T cells progressively transitioned toward terminal effector states over time. Tumor

depletion reduced antigen availability, thereby modulating CAR-T expansion, contraction, and long-term persistence through feedback between tumor burden and immunophenotypic composition. [19] CAR-T cells were described in a similar manner by Minucci *et al.* in a semi-mechanistic CK–PD model that directly linked molecular-scale receptor binding to systemic T cell kinetics and tumor growth. In this approach, the receptor binding fraction dictated the rates of naïve cell activation and effector-mediated tumor killing. Activated cells underwent multiple divisions to form the cytotoxic effector CAR-T cell pool, which subsequently transitioned into the CAR-T cell memory state. [25]

Population and pharmacometric models of CAR-T kinetics

Finally, population and pharmacometric models prioritize statistical characterization of CAR-T exposure profiles and covariate effects, supporting exposure-response and exposure–safety analyses. Other models focus primarily on pharmacometric and population-based cellular approaches to describe these processes. Wu *et al.* developed a two-compartment population PK model to support exposure–safety and efficacy analyses. Their model assessed CAR transgene copies in a rapidly eliminated CAR-T compartment and a sustained CAR-T compartment. A series of transit compartments and an empirical lag time were used to represent the delay between infusion and measurable transgene levels in the blood. Following expansion, the model described a biphasic decline, in which a fraction of cells was rapidly eliminated while the remaining cells transitioned into a sustained state. [27]

Stein *et al.* developed a nonlinear mixed-effects (NLME) cellular kinetic model to characterize CAR-T cell kinetics and evaluate the impact of anti-inflammatory comedications (tocilizumab and corticosteroids) on expansion rates. The model captured exponential growth of CAR transgene copies driven by antigen binding, followed by biexponential contraction. [26] Similarly, Mueller *et al.* applied noncompartmental cellular kinetic analysis and population modeling to evaluate the impact of CAR-T therapy on clinical outcomes. [16]

Discussion

The utility of computational models represents a significant step toward understanding the complexity of CAR-T cell therapy. The majority of existing models rely on ordinary differential equations (ODEs) to describe interactions between therapeutic and cancer cell populations, spanning a wide range of frameworks, from empirical compartmental models to advanced quantitative systems pharmacology (QSP) and physiologically based pharmacokinetic (PBPK) approaches. Notably, the field has undergone a clear methodological shift from purely descriptive models aimed at fitting observed concentration-time profiles toward mechanistic frameworks that incorporate prior biological knowledge and explicitly account for interindividual variability.

The primary strength of mechanistic models lies in their ability to provide biologically interpretable insights into cellular populations and their interactions within the context of human physiology. These models enable exploration of causal relationships underlying treatment response, persistence, toxicity, and resistance. However, their major limitation remains parameter identifiability, as such models typically require high-quality, information-rich datasets to reliably reproduce clinically relevant outcomes. In contrast, empirical models are structurally simpler and more flexible, allowing them to accommodate heterogeneous datasets collected across different

studies, time points, and patient populations. Nevertheless, their limited biological resolution restricts their ability to support mechanistic interpretation of therapeutic failure, resistance, or disease relapse.

Across modeling frameworks, CAR-T cells are commonly described through phenotypic differentiation into functional subsets, most frequently effector, memory, and exhausted populations. Some models further refine this representation by distinguishing between expanding and persister cells or by explicitly incorporating antigen specificity. Such phenotypic stratification enables accurate representation of the multiphasic CAR-T cell kinetics observed clinically across different malignancies. Moreover, this approach facilitates identification of intrinsic cellular determinants that may influence patient outcomes.

A key challenge in accurately modeling these processes is the limited availability of longitudinal clinical data that directly quantify CAR-T cell phenotypic subsets. Commonly used bioanalytical methods, such as qPCR and flow cytometry, often provide measurements of total CAR-T cell abundance rather than detailed phenotypic resolution. Consequently, many models rely on fixed parameters or literature-based assumptions to resolve identifiability constraints, which may introduce uncertainty regarding model structure and the biological accuracy of the framework.

Conclusions

This review provides a focused synthesis of how CAR-T cell behavior is represented in computational models developed using human clinical data. Across a wide range of mathematical frameworks, from empirical cellular kinetic models to multiscale QSP and PBPK-PD approaches, CAR-T cells are consistently conceptualized as dynamic, interacting populations whose expansion, contraction, persistence, and functional exhaustion critically determine therapeutic outcomes. By systematically comparing these representations, this work highlights both common modeling strategies and key conceptual differences in how CAR-T cell dynamics are formalized.

A central conclusion of this review is that phenotypic stratification of CAR-T cells has emerged as a unifying principle for capturing clinically observed multiphasic kinetics. More detailed frameworks further extend this representation to include functional roles such as antigen-driven state transitions, and compartment-specific behaviors. These modeling choices enable the translation of clinical observations into quantitative descriptors of CAR-T cell functionality and support interpretation of treatment outcomes.

Importantly, this study demonstrates that the choice of CAR-T cell representation, rather than the overall mathematical complexity of the model, largely determines its interpretability and applicability. Models that align the level of CAR-T cell description with available clinical data and study objectives offer the greatest translational value. In contrast, increasing biological detail without adequate data support may limit parameter identifiability and reduce predictive robustness.

Looking forward, the continued refinement of CAR-T cell representations will depend on improved access to longitudinal, phenotype-resolved clinical data and standardized reporting of cellular measurements. Integration of emerging data modalities, combined with modeling strategies, is expected to further enhance the ability of computational models to support optimization and personalization of CAR-T cell therapies. Ultimately, unified frameworks for robust and biologically grounded representations of CAR-T cells will be essential for translating computational modeling into applicable tools for the clinical development and application of cellular immunotherapies.

Conflict of interest

None declared.

Abbreviations

ALL — acute lymphoblastic leukemia
B-ALL — B-cell acute lymphoblastic leukemia
CLL — chronic lymphocytic leukemia
DLBCL — diffuse large B-cell lymphoma
GBM — glioblastoma
HCC — hepatocellular carcinoma
LBCL — large B-cell lymphoma
MCL — mantle cell lymphoma
mCRPC — metastatic castration-resistant prostate cancer
MM — multiple myeloma
MPM — malignant pleural mesothelioma
NHL — non-Hodgkin lymphoma
NSCLC — non-small-cell lung cancer
OVCA — ovarian adenocarcinoma
PDAC — pancreatic ductal adenocarcinoma
PMBCL — primary mediastinal B-cell lymphoma
TFL — transformed follicular lymphoma

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Does newest immunomodulative treatment in patients with psoriasis impact growth and transformation of melanocytic nevi in patients with psoriasis? Preliminary results from a prospective cohort study

Marta Kacprzyk[1,2], Magdalena Masajada[1,2], Aleksandra Kulbat[3,4], Karolina Richter[2,5], Paweł Brzewski[1,2], Wojciech M. Wysocki[2,3,4]

[1] Department of Dermatology and Venereology, Stefan Zeromski Municipal Hospital, Kraków, Poland
[2] Faculty of Medicine and Health Sciences, Andrzej Frycz Modrzewski Krakow University, Poland
[3] Department of Surgical Oncology, 5th Military Clinical Hospital in Krakow, Poland
[4] National Institute of Oncology, Maria Skłodowska-Curie Memorial, Warsaw, Poland
[5] Department of General, Oncological, Metabolic and Emergency Surgery, University Hospital in Krakow, Poland

Corresponding author: Wojciech M. Wysocki, M.D., Ph.D.
Chair of Surgery, Faculty of Medicine, Andrzej Frycz Modrzewski Krakow University
ul. Gustawa Herlinga-Grudzińskiego 1, 30-705 Kraków
Phone: +48 602 137 218; E-mail: wwysocki@mp.pl

Abstract: Introduction: Patients with psoriasis treated with biologic therapy are considered to have increased risk of developing skin neoplasms. The impact of novel therapies on the development and biology of melanocytic lesions is not completely known. The aim of the study was assess if the treatment with biologics exerts effects on the melanocytic nevi.
Material and Methods: Dermoscopy and videodermoscopic imaging of 276 melanocytic lesions were performed at the initial visit and after one year in 13 patients with psoriasis treated with IL-12/23 inhibitors, IL-23 inhibitors or IL-17 inhibitors.
Results: The nevi size significantly increased at the one-year follow-up in the whole study group and in patients treated either with IL-23 inhibitors or IL-12/23 inhibitor. 2% of melanocytic nevi showed an increase in any of the scores (ABCD score, 7-Point Checklist score, CASH score) or at least 1-mm change in the diameter at the one-year follow-up; however, these changes were not statistically significant between the different therapy groups.
Conclusions: Although treatment with novel groups of biologics leads to an increase in nevi size, it does not contribute to significant structural or color changes in melanocytic nevi or to the development of dysplastic nevi or melanoma at the one-year follow-up.

Keywords: psoriasis, melanoma, melanocytic nevus, biologic therapy, dermoscopy.

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Introduction

Psoriasis is a chronic, immune-mediated skin disease affecting approximately 2% of the European and North American populations. [1] A significant proportion of individuals with psoriasis require systemic treatment, such as phototherapy, non-biologic systemic agents (methotrexate, acitretin, ciclosporin A) or biologic therapies. Utilization of these methods of treatment has been associated with an increased risk of skin malignancy in patients with psoriasis, particularly non-melanoma skin cancer (NMSC). [2, 3, 4] Additionally, a higher risk of certain malignancies, including melanoma and NMSC, cannot be ruled out in patients treated with tumor necrosis factor α (TNF-α) inhibitors. [5–7] These risks are much less known in patients treated with novel groups of biologics (interleukin (IL)-23 inhibitors, IL-17 inhibitors). Nevertheless, according to recently published meta-analysis, the risk of melanoma and NMSC seems to be slightly higher in patients treated with targeted therapies when compared to the general population. [8]

Melanoma is an aggressive malignancy that derives from melanocytes and approximately 30% of melanomas arise from a pre-existing nevus. [9] Patients with psoriasis have been reported to have a lower number of melanocytic nevi compared to healthy controls. [10] This phenomenon has been linked to the inhibitory effect of pro-inflammatory cytokines (IL-1α, IL-6, TNF-α and transforming growth factor-β1) on tyrosinase activity, which hinders melanogenesis and melanocytic growth. [10]

Up until now, only few studies have assessed if there are any changes in melanocytic nevi in patients treated with biologics and immunosuppressants and showed conflicting results. In 2015, Koseaglu *et al.* conducted a prospective case-control trial on patients treated with immunosuppressive therapy (including TNF-α inhibitors, cyclosporine, methotrexate and azathioprine) and found that such therapies were associated with increased number of nevi and dermoscopic changes compared to the group of healthy controls. [11] Nevertheless, another study including only patients treated with TNF-α inhibitors and ustekinumab, an IL-12/23 inhibitor, found no significant changes in nevi or development of new dysplastic naevi when compared to controls. [12] To the best of our knowledge, there are no corresponding studies on patients with psoriasis treated with newer biologics, such as IL-17 and IL-23 inhibitors.

Our article presents preliminary results of the study investigating whether treatment with novel groups of biologics (IL-12/23 inhibitors, IL-23 inhibitors, IL-17 inhibitors) leads to changes in the size or dermoscopic scores of existing melanocytic nevi in patients with psoriasis.

Materials and Methods

This prospective cohort study funded by the research grant (WSUB/2023/07/00009) at Andrzej Frycz Modrzewski Krakow University is being conducted at a single academic hospital since October 2023. The recruitment of patients took place between December 2023 and April 2024. The preliminary analysis was limited to patients from the study group who had completed follow-up before the end of January 2025. The inclusion criteria were: 1) age above 18 years, 2) diagnosis of psoriasis (with or without psoriatic arthritis), 3) treatment with IL-12/23 or IL-23 inhibitor (ustekinumab, guselkumab, risankizumab, tildrakizumab) or IL-17 inhibitor (bimekizumab, ixekizumab, secukinumab) for at least 3 months. Patients were excluded, if they concomitantly use other biologic drugs, immunosuppressants (for example methotrexate, cyclosporin A) or phototherapy. Previous use of TNF-α inhibitors or immunosuppressive agents was permitted. In the

control group we included healthy participants without a history of psoriasis or other conditions that necessitated prior or ongoing immunosuppressive treatment, including biologics. Data regarding demographic characteristics, onset of the therapy and previous treatment were collected from all participants in the study and control groups. Additionally, all patients completed questionnaires concerning sun exposure during specific life periods, history of sunburns, clothing and history of sun protection, personal and family history of skin cancer, frequency of previous dermoscopy check-ups. Patients with missing data or those who were lost to follow-up were excluded from the analysis. The ethical committee of Andrzej Frycz Modrzewski University approved the study. Informed consent was obtained from all individual participants included in the study in accordance with the Declaration of Helsinki.

At the initial visit and after one year total body pictures and photographs of each melanocytic nevi were taken by FotoFinder Medicam 1000 (FotoFinder Systems GmbH, Bad Birnbach, Germany). Dermoscopic evaluation at each visit was conducted using Dermlite DL4 (3GEN LLC, San Juan Capistrano, CA) handheld dermoscope. Dermoscopy and videodermoscopic imaging of the melanocytic lesions were performed by a dermatologist trained in dermoscopy. Any doubtful cases were discussed with a senior physician. In a given patient the assessement was performed by the same investigator at all time points. Dermoscopic parameters of each melanocytic nevi assessed during the initial visit and after 12 months were: 1) the largest dimension expressed in millimeters, 2) score according to the asymmetry, border sharpness, colors, different structural components (ABCD) rule, [13] 3) score according to 7-Point Checklist (atypical pigment network, grey-blue areas, atypical vascular pattern, radial streaming, irregular diffuse pigmentation, irregular dots and globules, regression pattern), [14] 4) score according to the color, architecture, symmetry, homogeneity (CASH) algorithm. [15] Changes in scores were defined as any increase in the total score of each rule/algorithm after one year of follow-up. Change in the diameter was defined as at least 1-mm increase or decrease in the largest dimension of the nevi after one-year of follow-up. Suspicious lesion were histopathologically examined or dermoscopically controlled at shorter intervals depending on the clinical evaluation. Melanocytic lesions surgically excised after first assessment were not included in the analysis.

Statistical analysis

STATISTICA 14.0 software (StatSoft Inc., Tulsa, OK, USA) was utilized for the statistical analysis. Categorical variables are presented as numbers and percentages, whereas continuous variables are expressed as mean ± standard deviation (SD) or median and interquartile range (IQR), as appropriate. The Wilcoxon signed-rank test compared continuous variables between related groups. ANOVA was used for normally distributed data with homogeneous variances, while the Kruskal-Wallis test was used for non-normally distributed data. Associations between categorical variables were tested using the Pearson chi-square test. Statistical significance was defined as p-values below 0.05.

Results

In this preliminary analysis a total of 13 patients (8 male and 5 female) with psoriasis were included. Three patients have been treated with ustekinumab (IL-12/23 inhibitor), 5 patients with risankizumab (IL-23 inhibitor), 1 patient with guselkumab (IL-23 inhibitor), 1 patient with tildrakizumab

(IL-23 inhibitor), 1 patient with bimekizumab (IL-17 inhibitor), 1 patient with ixekizumab (IL-17 inhibitor) and 1 patient with secukinumab (IL-17 inhibitor). A cumulative number of analyzed nevi during the initial assessment was 378. Two nevi in a male patient treated with risankizumab were surgically excised after first visit due to melanoma-specific dermoscopic findings (histopathologic reports revealed compound nevi); therefore, the number of nevi included in the analysis was 376. The mean time from the onset of the therapy to the initial visit was 28.5 ± 36.8 months. No significant differences were found between the different therapy groups in terms of baseline characteristics, including age, sex, duration of therapy and follow-up, number of nevi per person. Characteristics of study participants and nevi count are set out in Table 1 and Table 2.

Size of the melanocytic lesions was measured at the initial visit and after one year. Mean and median diameters of all nevi and in subgroups are presented in Table 3. We found a significant increase in the size of nevi in one-year follow-up in the whole study group ($p < 0.001$) and in patients treated either with IL-23 inhibitors ($p = 0.003$) or IL-12/23 inhibitor ($p = 0.02$). The increase was not statistically significant in patients treated with IL-17 inhibitors ($p = 0.76$).

Table 1. Baseline characteristics of the study participants.

Number of patient	Sex	Age (years)	Drug group	Drug name	Therapy duration (months)	Prior biologic therapy	Prior immunosuppressive therapy	Previous phototherapy	Number of nevi analyzed (n)
1	M	38	IL-23 inhibitor	Risankizumab	19	No	No	Yes	17
2	M	36	IL-23 inhibitor	Risankizumab	6	No	Yes	Yes	35
3	M	58	IL-17 inhibitor	Bimekizumab	6	No	Yes	Yes	10
4	F	52	IL-23 inhibitor	Risankizumab	6	Yes	Yes	Yes	84
5	F	50	IL-23 inhibitor	Risankizumab	13	Yes	Yes	No	35
6	F	33	IL-12/23 inhibitor	Ustekinumab	93	Yes	Yes	Yes	14
7	M	38	IL-23 inhibitor	Tildrakizumab	10	Yes	Yes	No	43
8	F	72	IL-17 inhibitor	Ixekizumab	25	Yes	Yes	Yes	24
9	M	46	IL-12/23 inhibitor	Ustekinumab	123	Yes	Yes	No	24
10	M	38	IL-12/23 inhibitor	Ustekinumab	12	Yes	Yes	Yes	46
11	M	63	IL-23 inhibitor	Guselkumab	21	Yes	Yes	No	6
12	M	38	IL-17 inhibitor	Secukinumab	3	Yes	Yes	No	22
13	F	30	IL-23 inhibitor	Risankizumab	32	No	Yes	Yes	16

M — male, F — female, IL — interleukin

Results reporting changes in the analyzed scores and diameter of nevi are presented in Table 4. We found no statistically significant differences in the number of nevi that showed significant changes in any of the scores (p-values 0.92, 0.86, 0.94 for ABCD score, 7-Point Checklist score, CASH score, respectively) or diameter (p-value 0.94) between the different therapy groups. Two lesions

were excised due to enlargement or high dermoscopy scores. Both were described in histopathology reports as compound nevi. Lesions showing minimal or insignificant changes were not surgically removed. Figure 1 presents examples of the changes observed in the nevi in the study group.

Table 2. Characteristics of study participants and number of nevi.

Parameter	All patients (n = 13)	IL-17 inhibitors (n = 3)	IL-23 inhibitors (n = 7)	IL-12/23 inhibitors (n = 3)	p-value
Age (years), mean ± SD	45.5 ± 12.7	56 ± 17.1	43.9 ± 11.5	39 ± 6.6	0.24
Sex, n (% male)	8 (61.5)	2 (66.7)	4 (57.1)	2 (66.7)	0.94
Duration of therapy (months), mean ± SD	28.5 ± 36.8	11.3 ± 11.9	15.3 ± 9.4	76 ± 57.4	0.18
Follow-up time (days), median [IQR]	343 [336–395]	365 [336–410]	336 [333–343]	395 [384–416]	0.09
Total number of nevi, n	376	56	236	84	
Number of nevi per patient, mean ± SD	28.9 ± 20.6	18.7 ± 7.6	33.7 ± 25.8	28 ± 16.4	0.65

IL — interleukin, SD — standard deviation, IQR — interquartile range

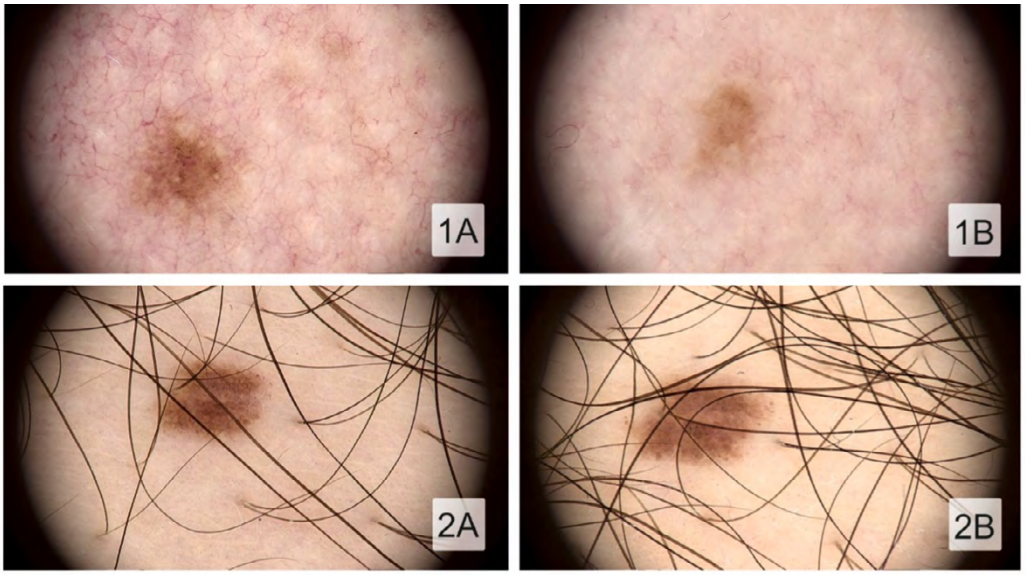

Fig. 1. Examples of the changes observed in the nevi in the study group.
1A and 1B: 34-year old male treated with risankizumab — regression in the size of the nevus, loss of brown globules, lighter appearance.
2A and 2B: 38-year old male treated with tildrakizumab — enlargement of the nevi with the presence of peripheral brown globules, loss of parts of the pigment network replaced by a light brown pigmentation. Histopathology report revealed compound nevus.

Table 3. Changes in nevi size in treatment groups.

	Diameter at baseline		Diameter in 1-year follow-up		p-value
	median [IQR]	mean ± SD	median [IQR]	mean ± SD	
All nevi (n = 376)	2.6 [2.0–3.4]	2.9 ± 1.4	2.6 [2.1–3.6]	3.0 ± 1.4	<0.001
IL-17 inhibitors (n = 56)	2.3 [1.9–2.8]	2.4 ± 0.9	2.4 [1.8–2.8]	2.5 ± 1.0	0.76
IL-23 inhibitors (n = 236)	2.8 [2.2–3.6]	3.1 ± 1.3	2.8 [2.2–4.0]	3.2 ± 1.4	**0.003**
IL-12/23 inhibitors (n = 84)	2.3 [1.8–3.2]	2.7 ± 1.8	2.4 [1.7–3.1]	2.8 ± 1.8	**0.02**

IQR — interquartile range, SD — standard deviation, IL — interleukin

Table 4. Changes in analyzed parameters at one-year follow up.

	ABCD score*		7-Point Checklist*		CASH score*		Nevi diameter**	
	Number of nevi changed, n (%)	p-value	Number of nevi changed n (%)	p-value	Number of nevi changed n (%)	p-value	Number of nevi changed n (%)	p-value
All nevi (n = 376)	7 (1.9)		7 (1.9)		6 (1.6)		6 (1.6)	
IL-17 inhibitors (n = 56)	1 (1.8)	0.92	1 (1.8)	0.86	1 (1.8)	0.78	1 (1.8)	0.94
IL-23 inhibitors (n = 236)	4 (1.7)		5 (2.1)		3 (1.3)		4 (1.7)	
IL-12/23 inhibitors (n = 84)	2 (2.4)		1 (1.2)		2 (2.4)		1 (1.2)	

* Change is defined as any increase in the total score after one-year of follow-up.
** Change is defined as at least 1-mm increase or decrease in the diameter of the nevi after one-year of follow-up.
IL — interleukin, ABCD — asymmetry, border sharpness, colors, different structural components, CASH — color, architecture, symmetry, homogeneity

Discussion

Current evidence suggests that patients with psoriasis are at increased risk of developing melanoma and NMSC due to the usage of specific therapies and environmental factors. The recent introduction of biologics has raised concerns about safety of their use with respect to tumorigenesis. In a recently published meta-analysis, the incidence rates of melanoma and NMSC in patients with psoriasis and psoriatic arthritis treated with IL-12/23 inhibitors, IL-23 inhibitors, IL-17 inhibitors

and Janus kinase inhibitors were 0.08 and 0.45 events per 100 patient-years, respectively, and appeared to be slightly higher than those observed in the general population. [8] Therefore, regular dermoscopic check-ups in patients treated with biologics appear to be reasonable for the early detection of suspicious changes in melanocytic nevi and the development of new lesions.

There is strong evidence that healthy immune system restrains melanocyte proliferation, while immunosuppression promotes the development of eruptive nevi and skin malignancies. [16, 17] Eruptive nevi have been reported in transplant recipients receiving cyclosporin A, prednisolone and azathioprine and been characterized by the presence of symmetrical rim of peripheral brown globules in dermoscopy. [18] This phenomenon is most likely related to the immunosuppressive action of drugs. There are few theories to explain the mechanisms of melanocyte proliferation. Immunosuppression can promote melanocyte-stimulating hormone and melanoma growth-stimulatory activity, which act as growth factors for melanocytes. [16] Another proposed mechanism involves alterations in IL-12 and IL-10 expression leading to inhibition of mitogen-activated protein kinase pathway involved in melanocyte proliferation. [16]

Eruptive nevi have also been described in patients with Crohn disease treated with biologics (infliximab, etanercept and alefacept). [19] Data on the behavior of melanocytic nevi in patients with psoriasis undergoing biological treatment are limited and focus mainly on TNF-α inhibitors. [11, 12, 20] According to Choi *et al.*, treatment with TNF-α inhibitors and ustekinumab was not associated with significant changes in melanocytic nevi in patients with psoriasis when compared to controls. [12] The results of *in vitro* studies on the effects of IL-12, IL-23 and IL-17 on tumorigenesis and the development of nevi are inconsistent. [21] Bernice *et al.* found increase immune activation in Th1/Th2/Th17 axes during the melanomagenesis from common melanocytic nevi to dysplastic nevi to malignant melanoma. [22] On the other hand, IL-23 was found to maintain melanocyte homeostasis and regulate melanomagenesis by activating DNA repair in melanocytes, whereas IL-12 promoted nevi development. [23]

To our knowledge, this is the first study to present changes in melanocytic nevi in patients treated with newest groups of biologics utilized in the treatment of psoriasis (IL-17 inhibitors, IL-23 inhibitors, IL-12/23 inhibitors). We found statistically significant increase in the size of nevi in the whole study group and in patients treated either with IL-23 inhibitors or IL-12/23 inhibitor. This finding is consistent with the results of the study conducted by Koseoglu *et al.*, which indicated that immunosuppressive therapy led to an increase in nevi size. [11] However, according to recent studies, most nevi in the general adult population tend to increase in size rather than decrease. [24] Therefore, an analysis incorporating healthy population is essential to validate this finding. Two percent of melanocytic nevi included in our study demonstrated an increase in any of the scores (ABCD score, 7-Point Checklist score, CASH score) or at least 1-mm change in the diameter at the one-year follow-up. These changes were not statistically significant between the different therapy groups.

The study has several limitations. Firstly, a longer follow-up period would be necessary to reliably assess changes in nevi and the incidence of malignancy. Secondly, since the study presents only preliminary findings from a larger ongoing research, it is limited to a small group of patients and does not include a healthy control group for comparison between the biologics receiving and biologics non-receiving people. On the other hand, this is interim analysis on first patient group included in the study. The final results will include 28 patients in both the study and control groups. What is more, the study does not account for participants' sun protection behaviors or their history of sun exposure, both of which could influence the observed outcomes.

To conclude, preliminary results of our study suggest that treatment with inhibitors of IL-17, IL-12/23 and IL-23 does not cause significant adverse changes in melanocytic nevi in patients with psoriasis. However, we have observed significant increase in nevi size in majority of patients over time. Further larger studies with longer follow-up period may be needed to validate this finding.

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Conflict of interest

M.K. has received honoraria and/or consultation fees from AbbVie, Amgen, Pfizer, Janssen and Eli Lilly. P.B. has received honoraria and/or consultation fees from AbbVie, Amgen, Concert Pharmaceuticals, Eli Lilly, Innovaderm Research, LEO Pharma, Pfizer, Sanofi Genzyme, Samsung, Janssen, and Novartis. W.M.W. has received honoraria and/or consultation fees from Roche, Novartis, Pfizer, Sanofi, BMS, Pierre-Fabre, PCI, J&J.

Authors' contribution

Conceptualization and Study Design: W.M.W. and M.K.; Data Collection: M.K., M.M., P.B.; Statistical Analysis: M.K., M.M., A.K., K.R. and W.M.W.; Data Interpretation: W.M.W., M.K., M.M., A.K., K.R. and P.B.; Manuscript Preparation: W.M.W., M.K., A.K., K.R. and P.B.; Literature Search: M.K., M.M., A.K., K.R., P.B. and W.M.W. All authors have read and agreed to the published version of the manuscript.

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High-fat diet-induced obesity affects sulfurtransferases activity in mice hearts

Dominika Szlęzak[1], Anna Misterka-Kozaka[2], Monika Majewska-Szczepanik[3], Patrycja Bronowicka-Adamska[1]

[1] Chair of Medical Biochemistry, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[2] Center for Biomedicine and Interdisciplinary Sciences, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[3] Chair of Biomedical Sciences, Department of Medical Physiology, Faculty of Health Sciences, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Dominika Szlęzak, Ph.D.
Chair of Medical Biochemistry, Faculty of Medicine
Jagiellonian University Medical College
ul. Kopernika 7, 31-034 Kraków, Poland
E-mail: dominika.szlezak@uj.edu.pl

Abstract: Introduction: Obesity is a chronic disease with complex etiology, characterized by excessive accumulation of fat tissue, which poses a health hazard and increases, among others, risk of cardiovascular diseases. Hydrogen sulfide (H_2S), an endogenous gaseous transmitter, plays an important role in the cardiovascular system, affecting blood pressure and heart function.
Aim: The aim of this study was to determine the effect of high-fat diet-induced (HFD-induced) obesity to hydrogen sulfide metabolism in mice hearts.
Materials and Methods: The research material consisted of hearts collected from male wild-type C57BL/6 mice fed for 8 weeks with a normal diet (ND) or high-fat diet. *Ex vivo* analyses included testing the activity of selected sulfur enzymes (cystathionine gamma-lyase — CGL, 3-mercaptopyruvate sulfurtransferase — MPST, and thiosulfate sulfurtransferase — TST), determining the sulfane sulfur level, and examining the ability of tissues to produce hydrogen sulfide.
Results: Tissues collected from HFD-fed mice were characterized by a lack of CGL activity, increased TST activity, and higher levels of sulfane sulfur compared to group of ND mice. Additionally, the capacity to produce H_2S was higher in the hearts of HFD mice.
Conclusions: Our study shows that HFD-induced obesity affects hydrogen sulfide levels and sulfur enzyme activity in mice hearts. It can be speculated that the increased ability of HFD mice tissues to produce H_2S is a compensatory mechanism for obesity-induced changes. The conducted research indicates the key importance of sulfurtransferases, MPST and TST, in sulfur metabolism in the heart and indicates a new area for further analysis.

Keywords: hydrogen sulfide, thiosulfate sulfurtransferase, 3-mercaptopyruvate sulfurtransferase, high-fat diet, obesity, heart.

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Introduction

Obesity is a disease characterized by the harmful effects of excess body fat on health. In clinical practice, the degree of obesity is usually assessed using the body mass index (BMI) — the ratio of body weight in kilograms to height in square meters (kg/m^2). [1] According to a report by the World Health Organization, in Europe alone, almost 60% of adults and nearly 8% of children are overweight or obese. [2] The accumulation of excess body fat leads to insulin resistance, glucose intolerance, type 2 diabetes, increased blood pressure, dyslipidemia, inflammation, and endothelial damage. All of these factors are considered cardiovascular risk mediators and may result in hypertension, coronary artery disease, heart failure, arrhythmia, atrial fibrillation, and premature sudden death. [3]

There are many causes of obesity, including unhealthy diet, lack of physical activity, genetic, hormonal, and psychological factors (such as stress or sleep disorders), socio-economic factors, and lack of education. [4] In the context of contemporary social changes, the issue of diet deserves special attention, including the increasingly widespread use of the so-called Western diet (WD). WD is high in calories and low in nutritional density. It includes nutrient-poor foods that are high in added sugar, salt, and saturated fat, such as fast food, sugary beverages, and highly processed food. [5] In animal research models, a high-fat diet (HFD) is considered a well-established model of the western diet. [6]

An inappropriate diet not only leads to obesity, but also to disturbances in the homeostasis of the organism and the development of oxidative/nitrosative stress, i.e. increased production of free radicals, such as reactive oxygen species and reactive nitrogen species. Physiological free radicals such as superoxide anion radical ($\bullet O_2^-$) and nitric oxide ($\bullet NO$), and their derivative — peroxynitrite ($ONOO^-$), are important modulators of many diseases, including cardiovascular disease. [7]

In the cardiovascular system, hydrogen sulfide (H_2S), an endogenously produced gaseous signaling molecule, has significant cardioprotective effects related to its vasodilatory properties. [8, 9] H_2S has been shown to protect against heart failure by stimulating angiogenesis, stimulating cardiac mitochondrial biogenesis, and activating endothelial nitric oxide synthase. [9] Therefore, hydrogen sulfide donors appear to be particularly useful therapeutic agents. The presence of sulfur atom/thiol group (-SH) in the structure of antihypertensive drugs from angiotensin-converting enzyme inhibitors (ACEI) group may intensify the cardioprotective effect of these medicine. [10]

In mammalian cells, H_2S is formed from L-cysteine in reactions catalyzed by three enzymes: cystathionine beta-synthase (CBS), cystathionine gamma-lyase (CGL), and 3-mercaptopyruvate sulfurtransferase (MPST). Additionally, hydrogen sulfide can be produced by the reduction of sulfane sulfur-containing compounds. [8] An important role in the metabolism of endogenous sulfur, is played by the enzyme thiosulfate sulfurtransferase (TST), which participates in the transsulfuration reaction. It catalyzes the transfer of sulfane sulfur from thiosulfate to thiol or cyanide, leading to the formation of persulfides or thiocyanates. [11] TST is a protein related evolutionarily to MPST and it is hypothesized that both may cooperate, including in eliminating the effects of oxidative stress. [12]

The aim of this study was to determine the effect of high-fat diet-induced obesity on the ability of mice heart tissue to produce hydrogen sulfide and on the activity of selected enzymes involved in sulfur metabolism. This work is a continuation of our previous experiments focusing on sulfur metabolism in tissues of animals with diet-induced obesity.

Materials and Methods

Animals and tissue collection

This examination was a continuation of our earlier study [13] evaluating the metabolism of sulfur-containing compounds in visceral adipose tissues collected from animals on normal (ND) or high-fat diet (approval no. 43/2017 of the 1st Local Ethics Committee for Animal Experiments in Cracow).

12-week-old wild-type C57BL/6 male mice from Jackson Laboratory (Bar Harbor, Maine, USA) were bred and kept under standardized conditions (light: dark cycle 12:12h, temperature 22°C, humidity 55%) with free access to food and water. The first group of animals (n = 7) were maintained on a standard/normal diet. To induce obesity, a second group of C57BL/6 mice (n = 7) were fed a high-fat diet D12492 from Research Diets, Inc. (New Brunswick, USA) *ad libitum* for 8 weeks. The energy sources in both diets and their caloric values are shown in Fig. 1. Mice body weight was monitored using a digital scale (Scout Pro Electronic Balance, Ohaus).

For tissue collection, the skin of mice anesthetized with a ketamine/xylazine cocktail (90 mg/kg body weight ketamine and 10 mg/kg body weight xylazine) was sterilized with 70% ethanol, and the thorax was incised to expose the heart. The entire heart was removed using sterile scissors and forceps. The tissue was placed in sterile tube, immediately frozen in liquid nitrogen, and stored at –80°C until further use.

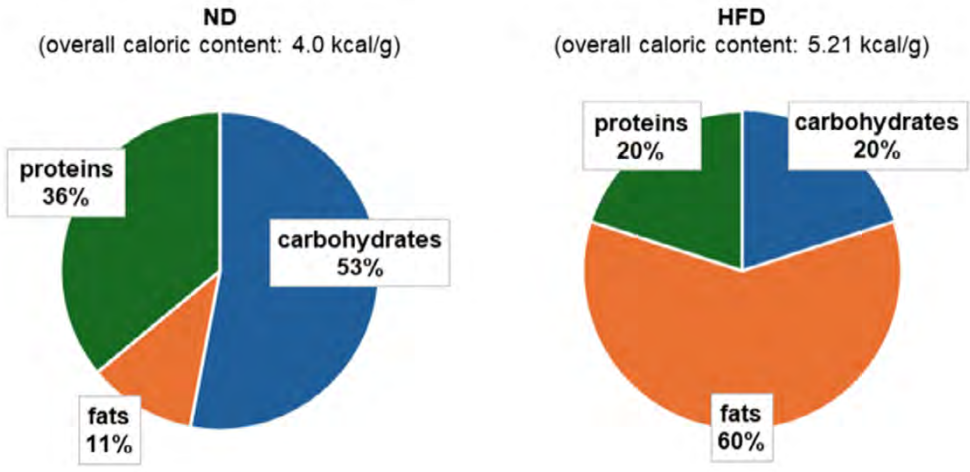

Fig. 1. Caloric composition of normal diet (ND) and high-fat diet (HFD) fed to animals.

Tissue homogenates

Sulfur enzyme activity, sulfane sulfur level, hydrogen sulfide production, and protein content were determined in mice heart homogenates prepared according to the procedures described in our previous work. [14] The obtained preparations were used immediately for further analyses.

Enzyme assay and determination of sulfane sulfur level

Cystathionine gamma-lyase activity was tested using the Matsuo and Greenberg method, [15] 3-mercaptopyruvate sulfurtransferase activity was determined according to the method of Valentine and Frankelfeld, [16] and thiosulfate sulfurtransferase activity was assayed by the Sörbo's method. [17] All these methods were modified by Wróbel *et al.* [18] The full composition of the reaction mixtures used and detailed procedures were described in our previous work. [19]

Sulfane sulfur was determined by Wood's cold cyanolysis method, [20] according to the procedure described previously. [19]

Protein content

Protein content in tissues was determined by the method of Lowry *et al.* [21] Crystalline bovine serum albumin was used as a standard.

H_2S production

The production of H_2S was examined by colorimetric method. [22, 23] The detailed composition of the reaction mixture and the experimental conditions were as described previously. [19]

Statistical analysis

All results are presented as arithmetic means ± standard error of the means. The Shapiro-Wilk test and Levene's test were used to assess the normal distribution of data and to analyze the homogeneity of variance. Statistical analysis was performed in StatSoft Statistica 13 software (Tibco, Palo Alto, California, USA) using the parametric Student's t test or the nonparametric Mann-Whitney test, as appropriate. Differences were considered statistically significant at the first level with a p value <0.05, at the second level when $p <0.01$, and at the third when $p <0.001$.

Results

Animal parameters

Fig. 2 shows the mean body weights of the animals at the zero point of the experiment (12-week-old animals) and after 8 weeks of feeding with a normal diet or a high-fat diet (20-week-old animals). In the ND group, a 31% weight gain was recorded during the experiment. In contrast, the HFD group of mice showed a 75% increase in mean body weight, confirming the occurrence of obesity in these animals.

There were no statistically significant differences in the weight of hearts collected from animals fed the normal diet (mean weight 0.164 ± 0.003 g) and the high-fat diet (mean weight 0.158 ± 0.013 g) (data not shown).

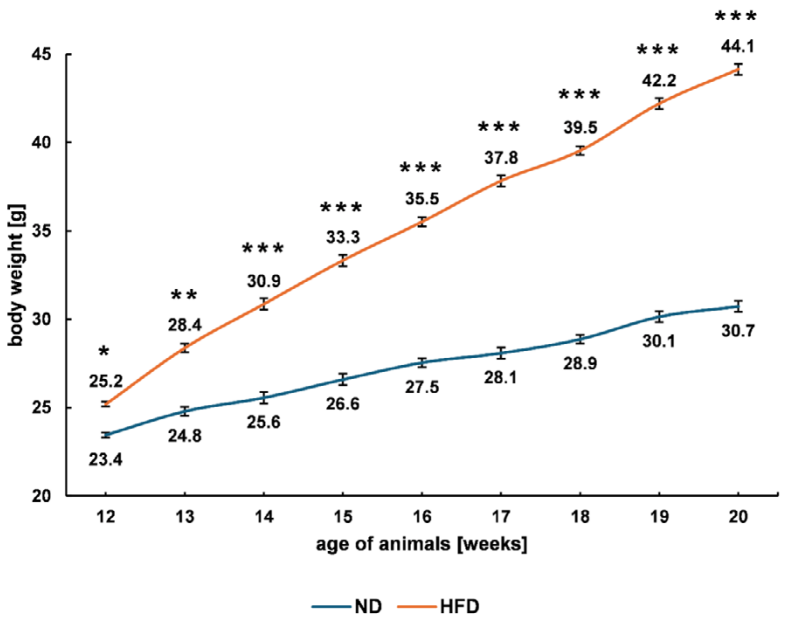

Fig. 2. Body weight changes of C57BL/6 mice fed a normal diet (ND) and a high-fat diet (HFD).
Values are represented as arithmetic mean ±SEM of 7 animals. *p<0.05, **p<0.01, ***p<0.001 high fat diet (HFD) vs. normal diet (ND) (Mann-Whitney test).

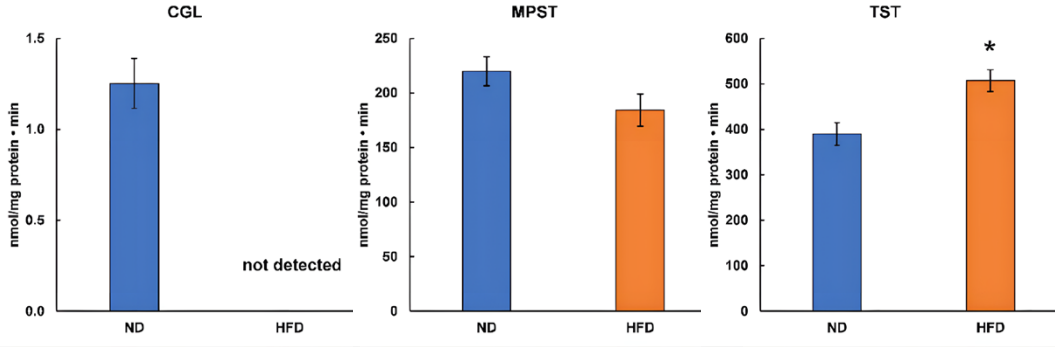

Fig. 3. The activity of cystathionine gamma-lyase (CGL), 3-mercaptopyruvate sulfurtransferase (MPST), and thiosulfate sulfurtransferase (TST) in mice hearts.
Values are presented as the arithmetic mean ±SEM of 3 animals. Each value is the mean of 10–15 repeats. *p<0.05 high fat diet (HFD) vs. normal diet (ND) (Mann-Whitney test).

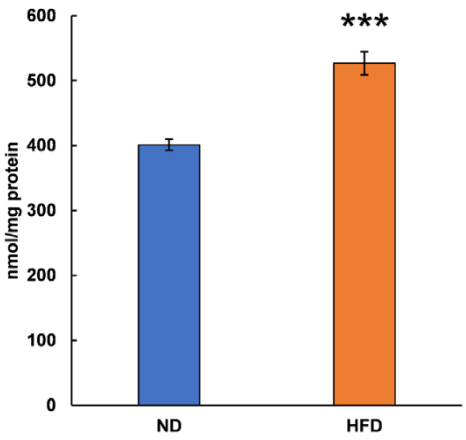

Fig. 4. The level of sulfane sulfur in mice hearts. Values are presented as the arithmetic mean ±SEM of 3 animals. Each value is the mean of 10–15 repeats. ***p<0.001 high fat diet (HFD) vs. normal diet (ND) (Mann-Whitney test).

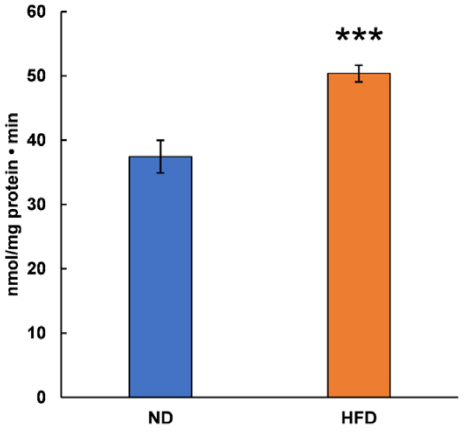

Fig. 5. Hydrogen sulfide (H_2S) production in mice hearts.
Values are presented as the arithmetic mean ±SEM of 4 animals. Each value is the mean of 10–12 repeats. ***p<0.001 high fat diet (HFD) vs. normal diet (ND) (Student's t test).

Enzymes activity and sulfane sulfur level

Enzymes activity in mice hearts are shown in Fig. 3. Low CGL activity was noticed in the tissues of animals fed a normal diet, while no CGL activity was detected in the HFD group. There were no statistically significant changes in MPST activity between the study groups. TST activity was significantly higher in the hearts of HFD mice compared to ND mice. Similarly, sulfane sulfur level increased significantly in the group of animals on the high-fat diet (Fig. 4).

H_2S production

The ability to generate hydrogen sulfide was confirmed in the tissues of animals from both study groups (Fig. 5). Hearts of HFD mice were characterized by significantly higher H_2S production.

Discussion

Obesity has been implicated as an indirect cause of heart disease for decades. Numerous studies in humans and animal models have shown that obesity leads to lipid accumulation in the heart, leading to cardiac steatosis. [24] Cardiac lipotoxicity, cardiomyocyte dysfunction and myocardial contractility dysfunction, is caused particularly by the deposition of saturated free fatty acids in heart cells. [25] The research by Alpert *et al.* [26] confirms the presence of left ventricular hypertrophy and impaired systolic and diastolic function in morbidly obese individuals, with the degree of impairment correlated with the duration of obesity. Left ventricular remodeling, along with increased heart rate and stroke volume, are characteristic of obesity and are thought to be

compensatory adaptations to increased adipose tissue mass. However, over time, they can lead to ischemic dilated cardiomyopathy. [27]

In this study, we focus on the changes in hydrogen sulfide metabolism induced in mice hearts by a high-fat diet. The results show that obesity determines the cardiac activity of sulfur enzymes. The new finding of this work is the important role of the TST enzyme in H_2S metabolism in the hearts of obese mice.

Cystathionine gamma-lyase has been the subject of interest of many research groups for many years as the main enzyme responsible for hydrogen sulfide production in the circulatory system. [28, 29, 30] CGL catalyzes the formation of H_2S from L-cysteine with the release of pyruvate. It can also induce the formation of thiosulfate from L-cystine and is responsible for the catabolism of cystathionine occurring on the pathway of L-cysteine formation from L-methionine. [31] Analysis of enzyme activity showed that the low CGL activity in the hearts of mice fed normal diet was reduced to undetectable level in HFD-fed animals. These results are consistent with immunohistochemical evaluation of CGL performed by Peh *et al*., [32] which showed a significant reduction or absence of CGL in aortic endothelial cells in C57BL/6 mice fed HFD for 16 weeks relative to mice on a standard diet. However, this change was not associated with a reduced ability of these tissues to produce hydrogen sulfide. These results indicate that other enzymes besides CGL are involved in maintaining the H_2S pool in heart cells.

Studies by various groups of scientists indicate the significant importance of 3-mercaptopyruvate sulfurtransferase in maintaining the proper functioning of the cardiovascular system. It has been shown that knockout of the MPST enzyme causes hypertension and cardiac hypertrophy in 18-month-old mice compared to younger animals. [33] Another study [34] showed that coronary rat homogenates produce larger amounts of hydrogen sulfide through the enzymatic pathway using MPST (desulfuration of 3-mercaptopyruvate leading to the formation of sulfane sulfur-rich compounds [35]) than through CGL. Our previous studies [14] in the rat model of hypertension also confirm that 3-mercaptopyruvate sulfurtransferase plays a major role in H_2S production in the heart. However, studies conducted on mice with HFD-induced metabolic syndrome did not show any changes in MPST expression at the protein level in the hearts of these animals compared to control mice. [36] Similarly, no changes were observed in the cardiac levels of free hydrogen sulfide and sulfane sulfur. The same study [36] and study by Katsouda *et al.* [37] showed that MPST knockout in mice resulted in increased pathological changes associated with metabolic syndrome (increased body weight, impaired glucose tolerance) and contributed to vascular inflammation. On the other hand, reports on MPST expression in other tissues of the body are contradictory — Li *et al.* [38] report that MPST expression in the liver at protein level increases in C57BL/6 mice fed a high-fat diet for 8 weeks, while Peh *et al.* [32] indicate a decrease in the hepatic MPST protein level in the same animal model after 8, 12, and 16 weeks of HFD application.

In this study, we did not observe significant changes in MPST activity in mice hearts under the influence of the high-fat diet. However, the obtained results suggest a tendency towards reduced activity of this enzyme in HFD-fed mice, which may be related to its antioxidant potential. Cysteine (Cys) located in the catalytic center of the enzyme and having reducing properties, plays a particularly important antioxidant role. The presence of Cys allows the reduction of oxidizing compounds such as reactive oxygen species, but also causes the inactivation of MPST. [39]

Another important enzyme involved in sulfur metabolism is mitochondrial thiosulfate sulfurtransferase. There are reports suggesting that persistent oxidative stress (especially in the

mitochondrial region) may lead to the induction of TST, which (in cooperation with MPST) regulates the level of sulfane sulfur, reduced glutathione or thioredoxin, causing an antioxidant effect. [12] In the current experiment, we observed a significant increase in cardiac TST activity under the influence of HFD, which may result from the activation of the mentioned antioxidant mechanism. Similarly, the level of sulfane sulfur was significantly higher in the HFD mice group. It is worth emphasizing that TST, although not classified as an H_2S-producing enzyme, catalyzes the reaction of thiosulfate with glutathione to produce hydrogen sulfide. [31] The presented results confirm that TST activity plays an important role in the metabolism of hydrogen sulfide in obese mice.

The increased bioavailability of sulfane sulfur (direct hydrogen sulfide precursor [40]) in HFD mice tissues explains the high capacity of these tissues to generate H_2S and may be due to its physicochemical properties. Elemental sulfur is characterized by high solubility in lipids. [41] Studies on obesity, both in humans and using animal models, [42, 43, 44] confirm that the heart is an important absorber of fatty acids from the plasma due to its high energy demand. At the same time, uptake of fatty acids from the circulation prevents their harmful effects on other tissues such as the pancreas. [43]

A limitation of our study is that we used only a male research model. Since there are reports of gender differences in cardiovascular risk, [45] further studies should be performed with a female research model. In addition, studies using an animal model have some inherent limitations, including the number of animals in each experimental group. The relatively small number of n in our research groups can be considered a limitation of this study.

Conclusions

In summary, high-fat diet-induced obesity affects hydrogen sulfide levels and sulfur enzyme activity in mice hearts. The increased capacity of heart tissue from mice fed a high-fat diet to produce H_2S could be interpreted as a compensatory mechanism aimed at counteracting obesity-induced changes, such as metabolic disorders or oxidative stress. Enzymatic analysis highlights the critical role of sulfurtransferases, MPST and TST, in cardiac sulfur metabolism, suggesting their key role in regulating hydrogen sulfide levels and maintaining redox balance in cardiac tissue. This demonstrates a potential role for these enzymes in cardiac adaptation to obesity and provides a new area for further investigation to understand the mechanisms behind obesity-related cardiovascular disease.

Declaration of competing interest

The authors declare no competing interests related to the present study.

Authorship contribution statement

D.S.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing — original draft, writing — review and editing; A.M.-K.: data curation, formal analysis; M.M.-S.: data curation, funding acquisition, methodology; P.B.-A.: conceptualization, data curation, funding acquisition, project administration, supervision, writing — review and editing.

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Monitoring manual dexterity in dental students — a quasi-longitudinal study and insights from a natural pandemic experiment

Elżbieta Zarzecka-Francica[1], Andrzej Gala[1], Paweł Myciński[2], Małgorzata Pihut[1]

[1] Department of Prosthodontics and Orthodontics, Institute of Dentistry, Jagiellonian University Medical College, Kraków, Poland
[2] Department of Conservative Dentistry with Endodontics, Institute of Dentistry, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Elżbieta Zarzecka-Francica, D.D.S.
Department of Prosthodontics and Orthodontics, Institute of Dentistry
Jagiellonian University Medical College
ul. Montelupich 4, 31-155 Kraków, Poland
Phone: +48 12 424 54 41; E-mail: elzbieta.zarzecka@uj.edu.pl

Abstract: Clinical classes for dental students in Poland start at the beginning of the third year and continue until the end of the fifth year. The implementation of theoretical knowledge tests and trains their manual skills and makes visible the complexity and variability of clinical cases. The COVID-19 pandemic forced a clinical lockdown, interrupting students' ability to train for six months.
The aim of the study was to compare manual dexterity scores measured by the Purdue Pegboard Test (PPT) before and after clinical lockdown among third- and fifth-year dental students and to assess the role of clinical placement. We investigated whether the initial female manual dexterity advantage has changed and whether the subjective assessment of dexterity correlates with objective measurement. The study consisted of students performing PPT subtests, tallying the score and answering additional questions. The cross-sectional (quasi-longitudinal) study included 175 students; 96 before the outbreak of the pandemic and 79 who took a six-month break from full-time classes. Measurements were taken each time for Year III at the very beginning of the start of practical classes, and for Year V at the end.
Analysis comparing cohorts studied before and after the clinical lockdown revealed no significant differences in PPT scores within the same year of study. However, fifth-year students achieved higher scores than third-year students on selected PPT subtest. The t-Welch test showed that year V scores significantly higher on most subtests compared to year III. In a two-factor ANOVA analysis, women scored higher than men on all subtests. Spearman's correlation analysis revealed a lack of correlation between self-esteem and objective PPT scores among third- and fifth-year students.
The findings may suggest a relative stability of the assessed aspects of manual dexterity during a break lasting up to six months, and clinical seniority was associated with higher PPT score. The gender effect persisted, while self-assessment did not coincide with objective measurement supporting the need for objective monitoring of teaching needs.

Keywords: manual dexterity, dental students, Purdue Pegboard Test, fine motor skills, clinical training, COVID-19 pandemic, clinical lockdown.

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Introduction

Dentistry is a field of medicine that requires not only theoretical knowledge, but also precise manual skills, visuomotor coordination and well-developed perception. [1, 2, 3] From the third year of study onwards, dental students begin to apply the theoretical knowledge they have acquired in clinical practice, and the achievement of the intended learning outcomes is also contingent upon adequate manual dexterity.

Manual dexterity is the ability to perform rapid, precise, and coordinated movements of the upper limbs, with particular involvement of the hands and fingers, under visual control and with spatial orientation. [4] Manual dexterity may be classified into gross motor skills, involving the shoulders and elbows, and fine motor skills encompassing precise movements of the fingers and wrists. [5] Dental practice performed in mirror view and with the use of a microscope or magnifying loupes increases the complexity of clinical procedures and requires the operator to fully utilise fine motor skills. [5, 3] Extensive theoretical knowledge does not guarantee nor corresponds to proficiency in clinical procedures; therefore, dexterity tests should be more widely used for the early identification of students' educational needs. [5, 6]

Manual dexterity tests, such as the Purdue Pegboard Test (PPT), the O'Connor Tweezer Test, and the Grooved Pegboard Test, may be used during the admission process for dental studies. They serve as a screening tool used to identify candidates with poor psychomotor skills and to reduce attrition rates during the course of study. [6] In a study conducted in Tel Aviv, two simple tests—the Purdue Pegboard Test (PPT) and the O'Connor Tweezer Test—used to assess manual dexterity under indirect vision predicted, with 80% accuracy, whether a student would pass the preclinical phantom course, highlighting the strength of objective measurement. [4] Due to its ease of administration and proven validation, the Grooved Pegboard Test remains the most widely used tool, and its scores correlate well with the fine motor skills required in clinical practice. [7]

The first lockdown triggered by COVID-19 created the conditions for a natural experiment; an unplanned, simultaneous "intervention" affecting the entire student population. It has caused a surge in stressors among dental students worldwide. [8, 9, 10] Preventive measures reduced the level of psychological burden; however, it did not return to pre-pandemic levels for a prolonged period. [8, 10, 11, 12, 13]

The abrupt transition to online teaching completely precluded the opportunity for clinical practice and for the refinement of manual dexterity through work with patients. [11] The psychomotor domain posed the greatest challenge in remote education, as it requires multisensory feedback, movement coordination, and progressive motor consolidation. [14] It is this domain that remains the least well-documented area of clinical education during the COVID-19 pandemic, and Eroglu *et al.*, in reporting their findings, called for the implementation of objective assessment tools to monitor it. [15] Despite studies evaluating the quality of online education and findings documenting a gradual improvement in dental students' perceptions of e-learning, the majority of pandemic-related articles rely on students' self-assessment of competencies rather than on objective manual tests, thereby creating a research gap. [16, 17, 18]

In our study, through manual dexterity measurements conducted shortly after the resumption of classes, we sought to capture the potential effect of the interruption in clinical training. A study designed in this manner allowed us to determine whether students' subjective discomfort related to the interruption in clinical practice translated into objectively measured manual dexterity. [10]

The study we conducted employed a cohort comparison design (quasi-longitudinal in nature) and included groups of dental students examined before the outbreak of the pandemic and after the interruption enforced by the lockdown. The aim of the study was to answer the questions:

1. whether manual dexterity scores measured using the Purdue Pegboard Test (PPT) differed between cohorts of third- and fifth-year students assessed before the outbreak of the pandemic and cohorts affected by the clinical lockdown (March–September 2020);
2. whether a longer duration of clinical training translates into higher Purdue Pegboard Test scores;
3. whether the female advantage in manual dexterity observed during the initial stage of clinical education persists until the completion of their studies;
4. whether students' subjective assessment of manual dexterity correlates with its objective measurement.

Materials and Methods

The study was a cohort comparison conducted before and after the lockdown-related interruption, with the lockdown treated as an external intervention.

It utilised four measurements performed in two groups of students. Group one (A) consisted of students assessed before the outbreak of the pandemic, whereas group two (B) comprised students who experienced a six-month interruption of in-person classes (from March 2020 to September 2020).

Subgroups were formed within each group:

III A — third-year students (assessed before the pandemic in October 2019, at the beginning of clinical education);

V A — fifth-year students (assessed before the pandemic in June 2019, at the completion of clinical education);

III B — third-year students (assessed after the resumption of clinical classes in October 2020, at the beginning of clinical education);

V B — fifth-year students (assessed in June 2021, at the completion of clinical education).

Owing to this study schedule, third-year students were assessed at the beginning, and fifth-year students at the end of the three-year period of clinical education. This approach allowed for a comparison of results before and after the COVID-19-related lockdown.

Participants first provided information regarding their gender, dominant hand, and subjective assessment of their manual dexterity on a scale from 1 to 5. Self-rated manual dexterity was defined as follows: 1 — poor; 2 — below average; 3 — average; 4 — above average; and 5 — very good. The students then performed the Purdue Pegboard Test. All measurements were collected by one trained examiner according to a standardised protocol, eliminating differences between examiners.

The Purdue Pegboard Test (PPT; model 32020A, Lafayette Instrument Company, Lafayette, IN, USA) consists of a board with four compartments holding pins, washers, and collars, and two parallel rows of holes. The student, seated in a comfortable position with their elbows resting on the table, performs the tasks (subtests) previously explained and presented in a standardised manner. The objective is to place as many individual components or specified assemblies as possible into the holes within a defined time limit. Performance is assessed across four subtests:

subtest 1 — right hand;
subtest 2 — left hand;
subtest 3 — simultaneous use of the right and left hands;
subtest 4 — the sum of the scores obtained in subtests 1. 2. and 3;
subtest 5 — assembly dexterity involving the simultaneous use of both hands (assembly sequence).

The first three subtests each last 30 seconds, whereas the assembly sequence lasts 60 seconds (P, T, I).

For the statistical analysis addressing the first study objective, namely the comparison of cohorts from the same year of study before and after the clinical lockdown, each group was treated separately, maintaining the division into four subgroups: III A, III B, V A, and V B. In contrast, to achieve the second, third, and fourth objectives, differences were analysed between two groups formed from third-year students (subgroups III A and III B) and fifth-year students (subgroups V A and V B).

Statistical analyses were performed in R, version 4.5 (R Core Team, 2025), using the following packages: rio (1.2.1), report (0.5.8), epiR (2.0.75), readxl (1.4.3), dplyr (1.1.4), and tidyr (1.3.1). In addition, analyses were performed using IBM SPSS Statistics, version 29.0 (IBM Corp., Armonk, NY, USA). Distributional assumptions were assessed with the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Categorical variables were compared using the chi-square test, with reporting of Cramér's V/φ. For continuous variables, a two-way ANOVA (measurement: pre-COVID vs post-COVID; year of study: third vs fifth) and Welch's t-test for independent samples were applied, in accordance with the analytical plan and as presented in the tables. In the ANOVA, partial η^2 was reported; in the t-tests, Cohen's d was reported; in all cases, 95% confidence intervals were provided. The relationship between self-assessed manual dexterity and PPT subtest scores was analysed using Spearman's rho with 95% confidence intervals. All tests were two-sided, and the significance level was set at $\alpha = 0.05$; unless otherwise specified, p-values were not adjusted for multiple comparisons, and interpretations were based on effect sizes and 95% confidence intervals.

Participants provided informed consent to take part in the study, and the study (approval no. 1072.6120.124.2019) was approved by the Bioethics Committee.

Results

Characteristics of the study population

The study included 175 students (n = 175), of whom 96 were assessed before the outbreak and 79 after the outbreak of the COVID-19 pandemic. Third-year students comprised a group of 84 participants, and fifth-year students 91. Women accounted for 76%, and over 97% of participants reported right-hand dominance. The proportions of gender and dominant hand did not differ significantly between the groups. Detailed characteristics of the study groups are presented in Table 1.

PPT subtest scores before the COVID-19 outbreak and after the clinical lockdown

Detailed PPT subtest scores obtained by female and male students in the third and fifth years of study, assessed before the COVID-19 outbreak and after the clinical lockdown, are presented in Table 2.

Table 1. Characteristics of the study groups before the COVID-19 pandemic (A and B), after the pandemic outbreak and in the 3rd and 5th year study groups in terms of gender and dominant hand (right or left) (*p-value of the* χ^2 *test,* χ^2 *— value of the* $\chi 2$ *test statistic, φ/V — effect size for the* χ^2 *test*).

	Before COVID (III A)		Pre-COVID (V A)				
Respondents: n 96	n 51	%	n 45	%	χ^2	p	φ/V
Gender							
Women	40	78.4	35	77.8	0.006	0.938	0.008
Men	11	21.6	10	22.2			
Dominant hand							
Right	48	94.1	44	97.8	0.802	0.370	0.091
Left	3	5.9	1	2.2			
	Post-COVID (III B)		**Post-COVID (V B)**				
Participants: n 79	n 33	%	n = 46	%	χ^2	p	φ/V
Gender							
Women	24	72.7	34	73.9	0.14	0.906	0.013
Men	9	27.3	12	26.1			
Dominant hand							
Right	33	100.0	44	95.7	1.472	0.225	0.137
Left	0	0	2	4.3			

	Pre-COVID (III A)		Post-COVID (III B)				
Participants: n 84	n 51	%	n 33	%	$\chi 2$	p	φ/V
Gender							
Women	40	78.4	24	72.7	0.36	0.549	0.06
Men	11	21.6	9	27.3			
Dominant hand							
Right	48	94.1	33	100.0		0.276	0.15
Left	3	5.9	0	0			
	Pre-COVID (V A)		**Post-COVID (V B)**				
Participants: n 91	n 45	%	n 46	%	$\chi 2$	p	φ/V
Gender							
Women	35	77.8	34	73.9	0.19	0.667	0.05
Men	10	22.2	12	26.1			
Dominant hand							
Right	44	97.8	44	95.7		1.000	0.06
Left	1	2.2	2	4.3			

Table 2. Purdue Pegboard Test subtest values in third- and fifth-year student subgroups assessed before the COVID-19 outbreak (III A, V A) and after the clinical lockdown (III B, V B) (*M — mean, Mdm — median, Q1–Q3 — interquartile range, t — t-test statistic comparing women vs. men, df — degrees of freedom, p — p-value of the t-test, 95% CI — confidence interval for the mean difference, LL — lower limit of the interval, UL — upper limit of the interval, Cohen's d — effect size indicating the strength of differences between groups*).

	women			men						95% *CI*		
Dependent variable	M	Mdn	Q1–Q3	M	Mdn	Q1–Q3	t	df	p	LL	UL	Cohen's d
III A												
Right_hand	16.40	17	15.25–17	14.27	14	14–5	6.42[a]	30.95	<0.001	1.45	2.80	1.57
Left_hand	15.00	15	14–16.75	13.45	13	12–15	2.72	49	0.009	0.40	2.69	0.93
Both_hands	12.80	13	11.25–14	11.91	12	11–13	1.51	49	0.138	–0.30	2.08	0.51
Right+left+both	44.25	44	42–47	39.64	40	37–42	3.50	49	0.001	1.96	7.27	1.19
Assembly	41.40	45	38.25–45	36.45	37	33–40	2.80	49	0.007	1.39	8.50	0.95
V A												
Right_hand	16.97	17	16–18	16.70	17	15–17.25	0.51	43	0.610	–0.79	1.34	0.18
Left_hand	15.83	16	14–17	15.70	15.5	15–16.25	0.23	43	0.818	–0.99	1.25	0.08
Both_hands	13.06	13	12–14	12.70	13	12–13.25	0.73	43	0.469	–0.63	1.34	0.26
Right+left+both	45.86	46	43–48	45.10	45	43–47.25	0.64	43	0.526	–1.63	3.15	0.23
Assembly	39.17	40	35–44	38.80	38.5	37–41	0.18	43	0.859	–3.82	4.56	0.06
III B												
Right_hand	16.92	17	16–17.75	15.22	15	13.5–17.5	2.09[a]	9.47	0.065	–0.13	3.52	1.10
Left_hand	14.71	15	13.25–16	14.44	15	11.5–16.5	0.31	31	0.762	–1.50	2.02	0.12
Both_hands	13.38	13	12–14	12.22	12	11.5–13	2.21	31	0.035	0.09	2.22	0.86
Right+left+both	44.75	45	42–47	41.89	41	38–47.5	1.87	31	0.072	–0.27	5.99	0.73
assembly	41.75	43	37.5–44.75	40.89	43	34.5–45.5	0.39	31	0.696	–3.59	5.31	0.15
V B												
Right_hand	17.24	17	16–18.25	15.75	15.5	14–17.75	1.93	44	0.060	–0.06	3.03	0.65
Left_hand	15.38	15	14–17	14.33	14	13–16	1.69	44	0.098	–0.20	2.30	0.57
Both_hands	13.79	14	13–15	12.92	13	11.25–14.75	1.43	44	0.159	–0.36	2.11	0.48
Right+left+both	45.82	45.5	43–49.25	43.00	43.5	38.25–46	1.53	44	0.133	–0.89	6.54	0.51
Assembly	42.00	41.5	38–44.5	39.08	40.5	33–45.25	1.06[a]	13.72	0.307	–2.99	8.82	0.46

The two-way ANOVA (variables analysed: manual dexterity pre-COVID-19 vs. post-COVID-19 across the third and fifth years of study) did not reveal differences in manual dexterity in any of the five PPT subtests among either third- or fifth-year students. Detailed results are presented in Table 3.

Table 3. Comparison of subtest performance between groups III A (third year before the pandemic), III B (third year after the clinical lockdown), V A (fifth year before the pandemic), and V B (fifth year after the clinical lockdown) (*t — t-test statistic, df — degrees of freedom, p — p-value, mean difference — difference of means, std. error difference — standard error of the difference, 95% Confidence Interval of the Difference — confidence interval for the mean difference, lower — lower limit, upper — upper limit*).

Comparison III A with III B	Subtest	t	df	p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	**Upper**
	Right_hand	–1.4	82	0.165	–0.513	0.367	–1.243	0.216
	Left_hand	0.07	82	0.944	0.03	0.434	–0.832	0.893
	Both_hands	–1.243	82	0.217	–0.453	0.364	–1.177	0.272
	Right+Left+Both	–0.761	82	0.449	–0.715	0.94	–2.584	1.154
	Assembly	–0.957	82	0.341	–1.182	1.234	–3.638	1.274

Comparison V A with V B	Subtest	t	df	p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	**Upper**
	Right_hand	0.154	89	0.878	0.063	0.412	–0.755	0.882
	Left_hand	1.916	89	0.059	0.691	0.361	–0.026	1.408
	Both_hands	–1.727	89	0.088	–0.587	0.34	–1.263	0.088
	Right+Left+Both	0.626	89	0.533	0.602	0.961	–1.307	2.511
	Assembly	–1.681	89	0.096	–2.15	1.279	–4.692	0.392

The role of clinical experience in the development of manual dexterity and the effect of gender

Welch's t-test showed that fifth-year students achieved significantly higher PPT scores in three of the five subtests compared with third-year students: Right hand ($p = 0.008$), Left hand ($p = 0.005$), and Right + Left + Both ($p = 0.006$). A detailed analysis of the Purdue Pegboard Test subtests for the third and fifth years is presented in Table 4.

In the two-way ANOVA (gender, year of study), women achieved higher scores than men in all five PPT subtests, and fifth-year students achieved higher scores than third-year students. In the initial stage of clinical practice, the difference between women and men was statistically significant; however, among fifth-year students, the observed female advantage decreased. Statistics in this respect are presented in Table 5.

Table 4. Comparison of manual skills of third- and fifth-year students in Purdue Pegboard Test subtests (*M — mean, SD — standard deviation, t — t-test statistic comparing third- vs. fifth-year students, df — degrees of freedom, p — p-value, 95% CI — confidence interval for the mean difference, LL — lower limit, UL — upper limit, Cohen's d — effect size for the mean difference*).

	Third year (n = 84)		Fifth year (n = 91)					95% CI		
Dependent variable	**M**	**SD**	**M**	**SD**	**t**	**df**	**p**	**LL**	**UL**	**Cohen's d**
Right_hand	16.14	1.65	16.88	1.95	–2.70	171.69	0.008	–1.27	–0.20	0.41
Left_hand	14.65	1.93	15.45	1.75	–2.85	167.61	0.005	–1.35	–0.24	0.43
Both_hands	12.79	1.64	13.27	1.64	–1.97	171.96	0.050	–0.98	0.00	0.30
Right+Left+Both	43.54	4.20	45.38	4.57	–2.79	173.00	0.006	–3.16	–0.54	0.42
Assembly	40.80	5.52	40.18	6.16	0.70	172.85	0.483	–1.12	2.37	0.11

Table 5. Gender dimorphism in Purdue Pegboard Test scores (ANOVA analysis: sex × year of study) (*F — ANOVA test statistic, df — degrees of freedom, p — p-value, η2 — effect size measure*).

	Effect	F	df	p	η2
Right hand	Year of study	10.75	1; 171	**0.001**	0.06
	Sex	21.51	1; 171	**<0.001**	0.11
	Year of study* sex	2.58	1; 171	0.110	0.02
Left hand	Year of study	7.62	1; 171	**0.006**	0.04
	Sex	6.56	1; 171	**0.011**	0.04
	Year of study* sex	0.28	1; 171	0.601	<0.01
Both hands	Year of study	4.22	1; 171	**0.042**	0.02
	Sex	7.54	1; 171	**0.007**	0.04
	Year of study* sex	0.41	1; 171	0.525	<0.01
Right+left+both	Year of study	9.86	1; 171	**0.002**	0.06
	Sex	14.32	1; 171	**<0.001**	0.08
	Year of study* sex	1.61	1; 171	0.206	0.01
Assembly	Year of study	0.05	1; 171	0.823	<0.01
	Sex	5.20	1; 171	**0.024**	0.03
	Year of study* sex	0.51	1; 171	0.476	<0.01

Self-assessment and objective measurement of manual dexterity

Spearman's correlation analysis revealed that subjective self-assessment of manual dexterity did not reflect the actual test results. Both among third- and fifth-year students, no correlation was observed between self-assessment and objectively measured manual dexterity scores. Detailed results are presented in Table 6.

Table 6. Comparison of self-assessed manual skills of third- and fifth-year students with objective subtest measurements of the Purdue Pegboard Test (*rs — correlation coefficient between objective manual skill measurement and self-assessment, p — p-value, 95% CI — confidence interval for the correlation coefficient rs*).

Test	Self-assessment of skills					
	Third year (n = 84)			Fifth year (n = 91)		
	rs	p	95% CI	rs	p	95% CI
Right_hand	–0.01	0.922	–0.23; 0.21	–0.07	0.498	–0.28; 0.14
Left_hand	0.02	0.885	–0.20; 0.24	0.02	0.869	–0.20; 0.23
Both_hands	0.02	0.850	–0.20; 0.24	0.14	0.189	–0.08; 0.34
Right+left+both	0.01	0.954	–0.21; 0.23	0.02	0.832	–0.19; 0.23
Assembly	–0.07	0.526	–0.29; 0.15	0.05	0.614	–0.16; 0.26

Discussion

During the pandemic, the majority of students expressed uncertainty and concerns regarding the forms of education proposed by universities. [16] There were proposals to develop simulators, haptic technologies based on virtual reality (VR)/augmented reality (AR), and tele-dentistry as future solutions in similar crises. [11] The absence of clinical training increased students' concerns about a decline in the manual dexterity required for professional practice. Many students rated both clinical and theoretical classes as worse throughout the entire pandemic period. [19] Even if the lockdown enforced a suspension of patient care, it is important to have objective assessment tools that allow for a reliable evaluation of current manual dexterity levels, the early detection of any regression caused by the interruption in clinical training, and the planning of targeted activities to compensate for identified deficits.

Psychomotor abilities of students at two measurement points: before and after the clinical lockdown

Our proposed study model, based on objective assessment of manual dexterity conducted shortly after the clinical lockdown, may effectively fill the diagnostic gap by facilitating universities' adaptation of curricula in similar situations in the future. It is worth noting that there are reports indicating a regression in clinical skills following a suspension of patient care, and consequently a lack of practice of manual skills. [20] In our quasi-longitudinal study, when comparing pre-pandemic and post-clinical lockdown cohorts, we did not observe statistically significant differences in manual dexterity, which may confirm the stability of psychomotor competences during a break lasting up to six months. Despite this, our other questionnaire-based studies conducted among the same group of students during the pandemic showed that concerns about insufficient practical preparation were among the major stressors. [10, 13] Longer lockdowns would require separate measurements.

Such findings should not constitute a conclusion for universities that there is no necessity to fill the clinical training gap in similar situations. Seymour-Walsh *et al.* demonstrate that, if a transition to remote teaching is necessary, universities are able to develop students' psychomotor skills remotely, provided that online activities (video, distributed practice) are well designed. The authors

criticise condensed courses intended to compensate for missed training and emphasise the necessity of distributing practice over time in order to consolidate the motor trace. Intensive training following a break does not provide time for the consolidation of motor memory. [14] The observational learning strategy (video-based) is consistent with this trend. It appears that even observation of a virtual procedure alone activates motor circuits and supports the maintenance of the memory trace. LeBel *et al.* demonstrated the effectiveness of this strategy in their study. [21] Many conclusions from other studies, including those conducted at Harvard University, favour a hybrid teaching model in the future, comprising 25–50% online content, while manual skill development should definitively be conducted on site in the form of clinical training. [16, 22]

Role of clinical experience in the development of manual dexterity

Compared with third-year students, fifth-year achieved higher Purdue Pegboard Test score, suggesting that tree years of clinical training may contribute to the development of manual dexterity. Fifth-year students achieved significantly higher PPT scores in three of the five subtest compared with third-year students, whereas the both-hands subtest was borderline and the assembly subtest did not differ significantly. This supports the assumption that clinical training and patient contact accelerate motor consolidation. The results of Saeed *et al*. indicate that systematic preclinical training can significantly improve third-year dental students' manual dexterity, as measured, among others methods, by the Purdue PegBoard Test. [23] However, our data did not reveal significant differences between the third-year cohorts studied before and after the pandemic, sugest that the impact of the pandemic break on PPT scores in this group was not confirmed in our studies.

It is also worth citing the assertion that experience shapes resilience. The manual dexterity of fifth-year students at the Catholic University of Rome did not regress despite a six-month interruption, confirming the resilience, stability, and plasticity of manual competencies developed during the course of students' education. [24]

Numerous pre-pandemic studies confirm that senior students outperform junior students in terms of manual skills. [4] The six-month interruption in practising their skills did not eliminate this advantage, as fifth-year students maintained significantly higher PPT scores compared with third-year students. This suggests that clinical experience enhances manual skills and that competencies remain stable provided that practice is maintained. The implementation of tele-dentistry and VR exercises could help maintain competencies during extended lockdowns by improving students' manual skills, supporting future university programs, and providing a buffer for clinical interruptions. [25]

Gender effect independent of clinical experience

There is a common belief that women have greater manual dexterity than men. In our study of third-year students, women achieved higher PPT scores compared with men, confirming this social stereotype. In the overall analysis, women achieved higher mean PPT scores than men; however, in the fifth-year subgroups, these differences were less clear. Because the sex x year-of-study interaction was not significant, the results should not be interpreted as unequivocal evidence of a persisting female advantage until the end of clinical education. Previous evidence indicates that both manual and spatial abilities contribute to the acquisition of dental skills; [3] therefore, the present findings should be interpreted in this broader educational context rather than confimation

of a persistent sex-related advantage. This advantage of women can be explained by smaller hand and finger size, which facilitates grasping small objects, and by women more frequently engaging in daily tasks that require greater precision. Intensive clinical practice for students, both women and men, led to higher scores in most of those assessed. Such results support the concept that clinical training and exposure to stimuli promote neuroplastic compensation, enabling students to achieve better competencies.

A study conducted by Frank *et al.* confirms that dexterity is amenable to training. Moreover, he reported no differences in the quality of scaling performed by students between genders, but only after prior intensive training. [26] From a neurobiological perspective, this can be explained by the presence of mechanisms of long-term motor memory consolidation. Repetition of clinical procedures activates the motor cortex and the mirror system, leading to the remodeling of motor maps.

Intensive, systematic practice distributed over time benefited all students in our study; however, we did not observe a reduction in gender-related differences. Results from Lugassy *et al.* show that even preclinical exposure to complex tasks can improve manual skills, particularly in students who were initially less manually dexterous. [4] Comparative analysis in our study showed that both genders systematically develop manual skills over time and with practice.

Dexterity tests such as the PPT are used at various stages of education and for different purposes. Our results suggest that such tests should serve a diagnostic function rather than a selective one. They allow for early identification of students experiencing regression in manual skills, those with lower manual dexterity, and those requiring additional support.

Discrepancy between self-assessment and objective measurement of manual dexterity

In our analyses, there was no significant correlation between subjective self-assessment of manual dexterity and PPT subtest scores. This self-assessment of manual skills may result from limited experience, insufficient knowledge, lack of comparison with others, or absence of exposure to clinical conditions without supervisor support. These results indicate that self-assessment does not reliably reflect psychomotor competencies, and objective measurements serve as a tool for accurate evaluation in this context. In educational practice, this finding justifies the use of objective tests to monitor progress and quickly identify needs.

If the academic environment rarely evaluates actual skills, for example through practical exams, and does not provide feedback, the illusion of competence may persist, and students themselves may show less motivation to improve their skills. Interestingly, there are publications suggesting that individuals with high manual skills often tend to underestimate their own self-assessment. [27, 28] This may result from awareness of the existing criteria. Clinical experience, and the expertise acquired through it, improves the accuracy of the self-assessment. [29] Despite students expressing anxiety about a decline in manual skills, PPT results did not confirm this. However, in their subjective self-assessment, students rated their skills higher than indicated by objective measurements. This shows a large discrepancy between students' perception and the objective results. At the same time, this highlights the diagnostic limitations of questionnaires alone and points to the usefulness of employing objective and reliable tests.

PPT, due to its relatively low cost, reliability, objectivity, and short completion time, is a suitable test for periodic monitoring of students' manual skills. It allows verification of competency levels in a relatively short time, both in relation to subjective variables (self-assessment) and objective factors (disruptions in education, clinical interruptions, or program reorganization). Its simple

protocol allows evaluation of the effectiveness of alternative training methods (VR/AR simulations), providing data on whether the implemented innovations actually improve manual dexterity. PPT can serve as part of a quality assurance system in education that universities should use, combining its diagnostic value with the possibility of prompt educational intervention.

It is worth emphasizing that our study was based on measurements taken during a unique period of a natural experiment, namely the COVID-19 pandemic. A limitation of our work is the quasi-longitudinal nature of the study, in which we compare different groups across successive manual measurements.

Conclusions

1. In this quasi-longitudinal study, no significant differences in PPT scores were found between students assessed before the pandemic and those assessed after the clinical lockdown.
   The finding may suggest a relative stability of the assessed aspects of manual dexterity in the face of an external factor such as the clinical lockdown. From a practical perspective, in the event of similar interruptions, objective monitoring and staggered clinical training appear more justified than the automatic implementation of catch-up-courses.
2. Clinical experience influences manual skills
   Higher PPT scores among fifth-year students compared with third-year students indicate an relationship between the level of clinical education and better performance of selected manual tasks. However, the study does not allow the mechanism underlying this relationship to be clearly determined. Study programmes should therefore minimize gaps and disruptions in students' real clinical contact, fully utilizing this potential.
3. Gender differences in PPT scores without a significant gender x year of study interaction
   From the perspective of the curriculum, this means that universities should focus on individually monitoring initial results and providing well-targeted support (additional exercises, observational learning, peer-assisted learning).
4. Limited value of self-assessment and the need for objective measurements of manual skills
   The low concordance between students' self-assessment and PPT results highlights the limited value of questionnaires as a diagnostic tool. Universities should therefore systematically monitor progress through objective tests such as the PPT, rather than relying solely on students' self-reported competencies.
5. PPT provides diagnostic value as a tool for monitoring the quality of education
   Thanks to its low cost, high reliability and short completion time, the PPT test proves itself as a diagnostic tool for periodic manual dexterity testing. It enables early detection of regression following interruptions in practical training, objective assessment of alternative forms of training (VR/AR, simulations) and rapid identification of students requiring targeted support without using the test as an exclusionary screening tool.
6. Objective measurements can reduce unnecessary emotional burden on students and correct the illusion of declining manual skills during periodic breaks in clinical practice, while also allowing students to objectively assess their own competencies.

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Conflict of interest

None declared.

Author contribution statements

E.Z.-F. — conceptualization, methodology, data collection, literature review, manuscript preparation, and submission, A.G. — formal analysis and interpretation of data, P.M. — support in clinical data acqusition, M.P. — supervision, review, and editing. All authors have read and approved the final version of the manuscript.

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Arrhythmogenic right ventricular cardiomyopathy — a disease that ends athletic careers

Agnieszka Skrzypek[1], Ian Perera[1,2], Marta Szeliga[1]

[1] Department of Medical Education, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[2] Department of Internal Medicine and Gerontology, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Agnieszka Skrzypek, M.D., Ph.D.
Department of Medical Education, Faculty of Medicine
Jagiellonian University Medical College
ul. Medyczna 7, 30-688 Kraków, Poland
Phone: +48 12 347 69 06; E-mail: a.skrzypek@uj.edu.pl

Abstract: Arrhythmogenic right ventricular cardiomyopathy, commonly detected between the second and fifth decades of life, is caused by mutations that result in the dysfunction of the junctions between cardiomyocytes. However, in 40% of patients, the causative mutation cannot be identified. The detection of arrhythmogenic right ventricular cardiomyopathy is particularly important in endurance athletes, as dangerous ventricular arrhythmias and symptoms of heart failure occur at a younger age in this patient group. The course of arrhythmogenic right ventricular cardiomyopathy involves four stages: no macroscopic changes in the myocardium, clinically overt ARVC, progressive dysfunction of the right ventricle and subsequently the left ventricle and end-stage. Transthoracic echocardiography is a widely available cardiac imaging study. This examination allows for the visualization of segmental or global wall motion abnormalities of the right ventricle. Cardiac magnetic resonance imaging, supplemented by late gadolinium enhancement, is the preferred examination for patients with suspected arrhythmogenic right ventricular cardiomyopathy. An alternative diagnostic study in cases of contraindications is multidetector-row computed tomography. Once a diagnosis of arrhythmogenic right ventricular cardiomyopathy is established, it is recommended to cease sports participation. Only moderate recreational physical activity is permissible.

Keywords: arrhythmogenic right ventricular cardiomyopathy, ARVC, sports medicine, medical examination of athletes.

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Background

Various factors associated with competitive sports can provoke life-threatening arrhythmias in the presence of underlying myocardial diseases, conduction system disorders, ion channelopathies, and heart defects. An example is the effect of ambient pressure changes in individuals

with an atrial septal defect (for instance, during diving or skydiving). It is crucial to perform systematic, periodic examinations of athletes to detect even subtle changes in the subjective assessment (new symptoms not previously reported, such as palpitations or fainting), objective assessment (a new heart murmur for example), and electrocardiographic (ECG) readings by identifying patterns that suggest the emergence of a medical condition which could potentially be life-threatening.

Objective

To define cardiomyopathy and the diagnosis of arrhythmogenic right ventricular cardiomyopathy.

Cardiomyopathies — definition

Cardiomyopathies are a heterogeneous group of pathologies characterized by structural and functional alterations of the heart. [1] Patients with these diseases can be symptomatic or asymptomatic. [2] Presented below are some examples of cardiomyopathies:

— dilated cardiomyopathy, which is one of the main causes of heart failure [3, 4]
— hypertrophic cardiomyopathy (HCM), which is the most common inherited cardiomyopathy caused by mutations in numerous genes
— restrictive cardiomyopathy (RCM), which is a heart-muscle disease characterized by stiffness of the ventricular walls and leads to diastolic dysfunction, raised end-diastolic pressure, and dilated atria [1]
— arrhythmogenic cardiomyopathy (ARCV), which is a pathology characterized by the substitution of the myocardium with fibro-fatty tissue, leading to the development of arrhythmias, reduced systolic function, and sudden cardiac death, especially in young patients [1]
— stress-induced cardiomyopathy, also known as broken-heart syndrome or Takotsubo cardiomyopathy, is connected with an abrupt onset of left ventricular dysfunction in response to severe emotional or physiologic stress. [1, 5]

Arrhythmogenic right ventricular cardiomyopathy — prevalence and clinical course

Arrhythmogenic right ventricular cardiomyopathy is caused by mutations that result in the dysfunction of the junctions between cardiomyocytes (connexons), specifically, impairment of desmosomes, gap junctions, adherens junctions, and ion channels. This leads to a loss of connectivity between cardiomyocytes and, ultimately, to their apoptosis. [6] Consequently, fibro-fatty remodelling of the myocardium occurs. In the final stage, this leads to thinning of the ventricular wall and the formation of aneurysms. [7] However, in 40% of patients, the causative mutation cannot be identified. [8]

Arrhythmogenic right ventricular cardiomyopathy occurs with a frequency of approximately 1:1,300 people. [9] It is most commonly detected between the second and fifth decades of life (and manifests very rarely in childhood). The risk is higher in first-degree relatives, especially siblings. It is more prevalent in males and follows a more severe clinical course in men, which is attributed to the influence of testosterone on the expression of defective genes and typically higher intensity of physical exertion. [7, 8]

The detection of arrhythmogenic right ventricular cardiomyopathy is particularly important in endurance athletes, as dangerous ventricular arrhythmias and symptoms of heart failure occur at a younger age in this patient group. [8, 10]

The course of arrhythmogenic right ventricular cardiomyopathy (ARVC) involves four stages:

I. no macroscopic changes in the myocardium, though arrhythmic episodes leading to sudden cardiac death cannot be ruled out [7, 8]

II. clinically overt ARVC, in which functional and structural changes in the heart muscle are present; patients may report palpitations, dyspnoea, or non-specific chest pain; syncope occurs rarely during this period

III. progressive dysfunction of the right ventricle, and subsequently the left ventricle

IV. end-stage, characterized by the presence of biventricular dilation and systolic dysfunction (which may be misdiagnosed as dilated cardiomyopathy). At this stage, the risk of sudden cardiac death is highest at approximately 4% per year. The primary cause of death is the worsening of heart failure symptoms, which increases the risk of complex ventricular arrhythmias. [7, 8]

The stable phases of ARVC can be interrupted by exacerbations in the form of myocarditis (the so-called "hot phase"). These exacerbations may present as acute coronary syndrome, featuring ECG changes (involving the ST-segment and T-wave) and an increase in markers of myocardial necrosis. [7, 8]

Diagnosis of arrhythmogenic right ventricular cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy — ECG changes

ECG changes in various heart diseases are not visible from birth. The electrocardiographic recording of a healthy child changes with age and differs significantly from that of an adult. Certain ECG patterns that are considered normal for children—including young competitive and amateur athletes—would serve as a basis for further diagnostic testing for life-threatening diseases in adults. A primary example is the presence of negative T-waves in three precordial leads among adolescents under the age of 16; in this age group, these are common and, if they occur as isolated findings, are not indicative of pathology. Thorough, repeatable ECG analysis at annual intervals for individuals regularly training in various sports disciplines allows for the identification of those who require more in-depth cardiological diagnostics. This gives us the chance to prevent disease progression or even forestall sudden cardiac arrest resulting from, for instance, life-threatening heart rhythm disorders. Furthermore, athletes periodically face factors that can increase the risk of dangerous arrhythmias, such as electrolyte imbalances after intense exertion and high-stress situations.

Approximately 81–88% of patients with arrhythmogenic right ventricular cardiomyopathy exhibit abnormalities in their resting ECG, which manifest as the disease progresses. [11] If the diagnosis of ARVC is established using other diagnostic modalities, the ECG recording remains normal in half of the patients. [12] However, six years after the first episode of ventricular arrhythmia, abnormal ECG changes are present in every patient. [13]

ECG Abnormalities in patients with arrhythmogenic right ventricular cardiomyopathy:

I. Changes related to right ventricular depolarization disorders:

1. prolonged S-wave upstroke duration (greater than or equal to 55 ms) in leads V1–V3, measured from the nadir of the S-wave to the isoelectric line; due to possible QRS complex fragmentation or the presence of an epsilon wave, the Terminal Activation Delay (TAD) is used. TAD is measured from the nadir of the S-wave to the end of all QRS complex deflections; this parameter is not applicable in the presence of a right bundle branch block (RBBB). [14]
2. QRS complex fragmentation in leads V1–V3. [15]
3. epsilon wave — a low-amplitude, repetitive signal appearing as a notch on the ascending limb of the S-wave in leads V1–V3 (this finding can also occur in other conditions, such as Brugada syndrome, right ventricular myocardial infarction, sarcoidosis, and others). [16]
4. QRS complex widening greater than or equal to 110 ms in leads V1–V3; however, this is not a formal diagnostic criterion.
5. incomplete intraventricular conduction block, characterized by a QRS duration in leads V1–V3 that is 25 ms longer than the QRS duration in leads V4–V6; this is also not a formal diagnostic criterion. [17]
6. r'/S amplitude ratio < 1 in lead V1, proposed for patients with RBBB. This is considered the most reliable diagnostic parameter in cases of intraventricular conduction disturbances. [17]

II. Abnormalities resulting from right ventricular repolarization disorders:
   1. T-wave inversion (TWI) in leads V1–V3 — this is the most useful diagnostic ECG parameter. The presence of negative T-waves in a greater number of leads may indicate right ventricular dilation and increases the risk of ventricular arrhythmia (however, isolated T-wave inversion occurring only in leads V4–V6 is considered less significant).

III. Low QRS voltage in limb leads: this is characterized by low-amplitude QRS complexes in the limb leads (after excluding other causes such as sarcoidosis, pericardial effusion, emphysema, or obesity). This finding is associated with the presence of subepicardial fibro-fatty scarring located within the wall of the left ventricle. [18]

Holter ECG monitoring in patients with arrhythmogenic right ventricular cardiomyopathy

In Holter ECG recordings, the most common supraventricular tachyarrhythmia is atrial fibrillation, [9] the presence of which worsens the prognosis.

Ventricular arrhythmias may occur with varying degrees of severity, ranging from isolated premature ventricular contractions to complex arrhythmias. Characteristically, these ventricular arrhythmias exhibit a left bundle branch block morphology.

A recording of more than 500 premature ventricular contractions per 24 hours during Holter monitoring is a recognized, sensitive diagnostic criterion, useful in the screening of patients with suspected arrhythmogenic right ventricular cardiomyopathy. [19]

Sudden cardiac death in patients with arrhythmogenic right ventricular cardiomyopathy

Sudden cardiac death may be the first symptom of the disease, which is linked to myocardial dysplasia. ARVC is responsible for approximately 4–23% of sudden cardiac deaths occurring in young individuals under the age of 35. [20] This results from ventricular fibrillation during periods of disease exacerbation. In about 75% of cases, sudden cardiac death occurred during daily

activities unrelated to exertion, while in 3–5% of cases, it occurred during sports participation. [21] In the older patient group, monomorphic ventricular tachycardias (VT) are more common. These are triggered by a re-entry mechanism around areas of damaged myocardium during the chronic phase of the disease. [22]

Imaging studies

Transthoracic echocardiography

Transthoracic echocardiography is a widely available cardiac imaging study. This examination allows for the visualization of segmental or global wall motion abnormalities of the right ventricle. Additionally, it is possible to measure the dimensions of the right ventricular inflow and outflow tracts, which can be useful in prognosticating the course of the disease. [23]

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging (MRI), supplemented by late gadolinium enhancement (LGE), is the preferred examination for patients with suspected arrhythmogenic right ventricular cardiomyopathy. An alternative diagnostic study in cases of contraindications, such as the presence of certain metal implants, is multidetector-row computed tomography (MDCT). [18] Cardiac magnetic resonance imaging has allowed for the genotypic differentiation of arrhythmogenic cardiomyopathy into right-dominant (ARVC), biventricular, and left-dominant (ALVC) forms. Cardiac MRI enables precise assessment of tissue morphology, thereby eliminating the need for endomyocardial biopsy. [23]

Genetic testing

Genetic testing is recommended for patients diagnosed with arrhythmogenic right ventricular cardiomyopathy, [9] as it enables targeted screening of patients' relatives. Mutations associated with the occurrence of ARVC are present in one in five individuals with a structurally healthy heart. [24] A negative genotyping result does not rule out a diagnosis of the disease.

Due to the lack of a pathognomonic sign for arrhythmogenic right ventricular cardiomyopathy, establishing a diagnosis is based on scoring systems. These scales evaluate the overall clinical picture, taking into account ECG changes in the form of repolarization, depolarization, and conduction disorders, heart rhythm disturbances, as well as significant family history and genetic testing. [18]

Management of arrhythmogenic right ventricular cardiomyopathy according to current standards

In cases where a diagnosis of arrhythmogenic right ventricular cardiomyopathy is established, it is recommended to cease participation in sports. Only moderate recreational physical activity is permissible.

Symptomatic treatment primarily involves the use of beta-blockers or amiodarone. Radiofrequency catheter ablation of arrhythmogenic sites and ICD (implantable cardioverter-defibrillator) implantation are also utilized. [25, 26]

Summary

A thorough patient history (including interviews with athletes attending scheduled appointments with sports medicine physicians to obtain medical clearance for continued training and competition) allows for targeted diagnostics based on reported symptoms (such as palpitations, chest discomfort, or syncope).

A meticulous ECG analysis, repeated at least once a year, enables the detection of new changes in the ECG recording.

If ECG abnormalities occur or heart disease is suspected, the athlete should be referred to a cardiology clinic. There, an echocardiogram, stress test, and, if necessary, cardiac magnetic resonance imaging or other essential diagnostic tests will be performed. Once a diagnosis of arrhythmogenic right ventricular cardiomyopathy is established, it is recommended to cease sports participation. Only moderate recreational physical activity is permissible. Thus, obtaining information about diseases occurring in the family of candidates for regular sports training and athletes that may cause sudden cardiac death is important, because it allows for early detection of potential threats. [27]

Conflict of interest

None declared.

Informed consent

Not applicable.

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Haddad Syndrome — more than don't forget to breathe

Katarzyna Kubińska, Stanisław Kwiatkowski, Olga Milczarek

Department of Childrens Neurosurgery, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Olga Milczarek
Department of Pediatric Neurosurgery, Jagiellonian University Medical College
ul. Wielicka 265, 30-663 Kraków, Poland
E-mail: olga.milczarek@uj.edu.pl

Abstract: Congenital central hypoventilation syndrome (CCHS) and Hirschsprung's disease (HD) belong to neurocristopathies — autonomic nervous system defects caused by abnormal neural crest cells migration. Their co-occurrence defines Haddad Syndrome (HS) associated also with increased risk of neural crest origin tumors, mainly neuroblastoma (NBL), and PHOX2B gene mutation. This case concerns a male newborn presenting respiratory failure minutes after birth. Due to sleep apnea and increasing hypercapnia, he required intubation. Genetic testing revealed an anomaly in PHOX2B gene. In subsequent days, the patient did not pass the meconium, which, based on the imaging tests and biopsy, raised a suspicion of HD. The boy was diagnosed with HS and additional diagnostic measures, including cancer screening, were ordered, owing to the syndrome's characteristics. Awareness of such conditions enables clinicians to implement targeted management that may significantly improve long-term outcomes and quality of life for affected children.

Keywords: Haddad Syndrome, Hirschsprung's disease, congenital central hypoventilation syndrome, neurocristopathy.

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Introduction

The autonomic nervous system (ANS) plays a crucial role in controlling essential physiological functions, such as heart rate, blood pressure, pupil width, bowel movements and thermoregulation. [1, 2, 3, 4] Therefore, any damage to the ANS can result in malfunctioning of the body which, if left untreated, can be fatal. Neurocristopathies, such as CCHS and HD, are examples of congenital defects that arise from the disordered migration, differentiation, or destruction of neural crest cells (NCC) and lead to ANS impairment. [1, 2, 3] NCCs are multipotent neuroectodermal cells and can evolve into various cell types. They are only found in humans and tunicates. Prevention, prenatal detection, or complete cure remain elusive, as the disruptions occur during the initial stage of embryogenesis. Medical support therefore focuses on sustaining the patient's life by managing the affected body systems which is possible if the symptoms are detected relatively early after birth. Diseases resulting

from a defect in the ANS are rare in the population. However, if only one has been detected, it is necessary to consider further diagnostics for other ANS defects, because the co-occurrence of CCHS and HD is estimated at 16–20%, and CCHS and neuroblastoma at 5–10%. [1, 2]

Case presentation

A full-term male newborn from an uncomplicated pregnancy was observed to have respiratory failure soon after birth. A non-invasive CPAP ventilation was initiated, but due to sleep apnea and increasing carbon dioxide retention (checked by continuous monitoring of respiratory rate and by capnography), the child was intubated. An unsuccessful extubation attempt was made on the fifth day, and a further three attempts in the third month of life were, too. They resulted in cardiac arrest. Successful resuscitation was undertaken. Organic cases were ruled out via flexible bronchoscopy and brain MRI, which revealed cerebellar vermis hypoplasia and a brainstem cyst unrelated to the patient's condition. Genetic testing was performed revealing abnormalities in the PHOX2B gene. NPARM (Non PolyAlanine Repeat Mutations) was detected in Next-generation sequencing and was considered to be the cause of CCHS. Due to the results of the genetic test, a search for other congenital defects correlated with the detected mutation was initiated. After stabilizing the vital signs, it was decided to create a tracheostomy and to de-escalate respiratory support—furnishing independent breathing during wakefulness and home mechanical ventilation during sleep.

The newborn displayed symptoms of gastrointestinal obstruction, including a lack of meconium and dilated intestinal loops visible on abdominal X-ray, leading to suspicion of HD (Fig. 1). Additionally, a mutation in the PHOX2B gene was identified as a potential contributing factor to the disease. Rectal suction biopsy confirmed the diagnosis. Neural ganglia were located 40 cm ahead of the Bauhin's valve during gastrointestinal track mapping. An ileostomy was created at this point, with subsequent symptoms of short bowel syndrome, such as lack of expected body growth. While a modified diet was attempted, only partial improvement was achieved.

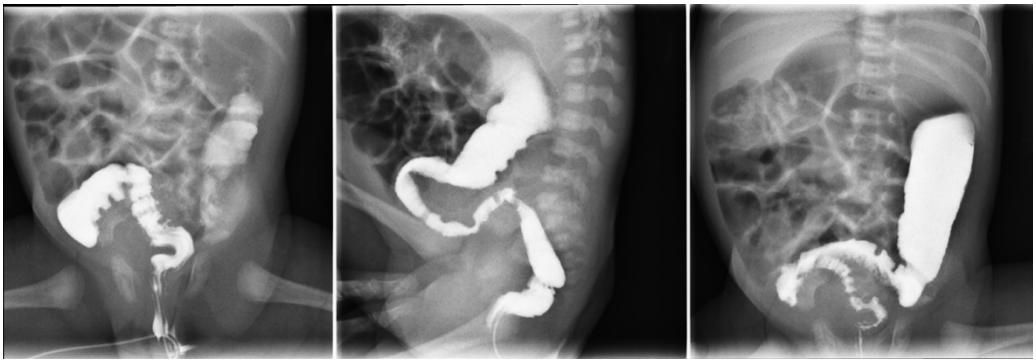

Fig. 1. Barium enema. Sign of HD.

The confirmation of a PHOX2B gene mutation in a patient who presented with both CCHS and HD led to a diagnosis of HS. Owing to the higher cancer risk associated with this syndrome, the patient underwent oncological screening tests at the age of 2 months. During an abdominal ultrasound, bilateral nodular lesions were found on the adrenal glands. A surgical biopsy subsequently confirmed them to be undifferentiated NBL with no expression of the N-MYC gene. Two cycles of

chemotherapy (etoposide + carboplatin) were performed, after which regression of changes was observed in the follow-up CT scan.

During hospitalization, the boy experienced two partial motor seizures (hemilateral facial right) with secondary generalization. Due to the child's anxiety, a valuable EEG test could not be conducted and an administration of carbamazepine was associated with excessive sleepiness.

During the examination, global psychomotor developmental delay, asymmetry in limb muscle tone and hypotonia of the midline were observed. A facial dysmorphia was not detected.

In follow-up imaging tests (transparietal ultrasound, head MRI) performed to check for brain defects detected after birth (cerebellar vermis hypoplasia, brainstem cyst), a gradual dilatation of the cerebral ventricular system was observed without signs of intracranial hypertension. Atrophic nature of the condition was most likely. It was decided to implant a ventriculoperitoneal shunt to prevent further loss of white matter.

Follow-up imaging tests (transparietal ultrasound, head MRI) were conducted to assess brain defects that were previously detected after birth (cerebellar vermis hypoplasia, brainstem cyst). The tests revealed a gradual dilatation of the cerebral ventricular system without signs of intracranial hypertension (Fig. 2). This condition was determined to be atrophic and to prevent further loss of white matter, a ventriculoperitoneal shunt was implanted.

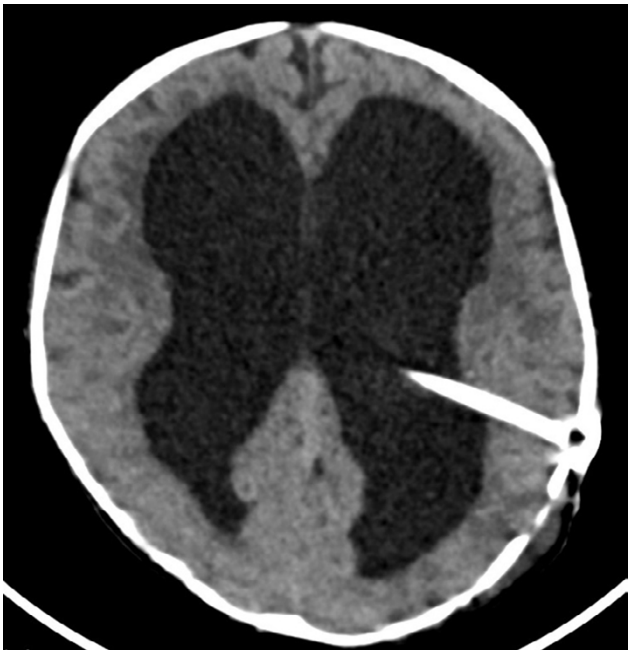

Fig. 2. Dilatation of the cerebral ventricular system without signs of active hydrocephalus. Properly-functioning ventriculoperitoneal shunt.

Due to possible cardiac arrhythmias in HS, a 24-hour Holter ECG monitoring was conducted. The results showed no abnormalities in heart rhythm.

The child underwent numerous hospitalizations complicated by generalized infections. Furthermore, the boy was diagnosed with atopic dermatitis.

The patient requires continuous treatment and life support measures.

Informed consent was obtained from the caregivers for the case report and its publication.

Discussion

Don't forget to breathe

CCHS is a rare condition also known as "Ondine's Curse." According to myth, a nymph cursed her human lover who betrayed her, causing him to forget to breathe whenever he fell asleep. [1] The estimated prevalence of CCHS is 1 in 200,000 live births. [2, 3, 4, 5] The condition is caused by ANS dysregulation, which weakens the response of chemoreceptors to increasing hypercapnia, resulting in central apnea. In the most common form of CCHS, ventilation is normal during wakefulness; but during sleep, especially in the NREM phase, apneas and carbon dioxide retention occur, leading to severe respiratory failure. [6] Typically the disease appears during the neonatal period and an infant will require mechanical ventilation and, over time, will be unable to extubate.

More than don't forget to breathe

The title "more" means that Ondine's Curse is accompanied by HD and a higher risk of cancer—mainly NBL—than in the general population. "more" is therefore HS (1 in 1,000,000 live births). [4]

HD, also called congenital aganglionic megacolon, is the most common of the above-mentioned diseases and affects one out of every 5000 live births. [3] HD is caused by the significant deficiency or absence of ganglion cells in the submucosal and intramuscular plexus of the intestine, causing a paralytic intestinal obstruction. This condition primarily affects the rectum and sigmoid colon, but it can also occur in the small intestine in rare cases. In HS, HD co-occurring with CCHS is more frequently associated with the long-segment form. Total colonic aganglionosis accounts for approximately 5% of HD cases is a rare form and can be difficult to differentiate from other forms of distal intestinal obstruction. [1] The root cause of HD is a disruption in the cranio-caudal migration of vagal crest cells in the intestine, which typically takes place between the fifth and 12th week of pregnancy.

NBL is a malignant tumor originating from neuroblasts. These are neural crest cells that physiologically form the ANS ganglia and the adrenal glands. While most cases of NBL are sporadic, there is also a familial form to which mutations in the PHOX2B and ALK genes are predisposed. The amplification of the N-MYC gene is an important prognostic factor. Patients at a young age (less than 90 days) and the absence of N-MYC gene amplification tend to have a better prognosis. Although NBL in newborns is frequently associated with a tendency toward spontaneous regression, in this case chemotherapy was initiated due to its occurrence within the context of a complex and rare genetic syndrome. This presentation requires a more individualized diagnostic and therapeutic approach compared to the "classic", isolated form of NBL. [7]

Facial dysmorphia is often observed in HS. There are also many defects directly related to ANS dysfunction, such as cardiac arrhythmias leading to bradycardia and even asystole, dysphagia, gastric motility malfunction, the risk of hypoglycemic episodes, thermoregulation disorder or abnormal pupillary light reflex. [4, 6, 8, 9, 10] Therefore, it is necessary to perform additional tests in every patient suspected of HS, aimed at "getting ahead of the diagnosis." For example, detecting life-threatening arrhythmias or hypoglycemia using simple and easily available tests such as ECG Holter or glycemia measurement. A summary of comprehensive assessment of autonomic dysfunctions associated with HS is provided in Table 1. [4, 6, 8, 9, 10]

Table 1. Comprehensive assess ment of autonomic dysfunction associated with HS, which should be undertaken at diagnosis using the methods outlined.

Autonomic dysfunction	Diagnostic methods	Case results
sleep apnea related to CCHS	polysomnography or cardiorespiratory polygraphy	CCHS diagnosed by continuous monitoring of respiratory rate and by capnography
cardiac arrhythmias leading to primarily sinus bradycardia, transient asystole, syncope	regular ECG Holter monitoring	no changes
hypotension	blood pressure monitoring	no changes
dysphagia	oesophageal manometry	no changes
gastric motility malfunction	rectal suction biopsy (the gold standard), contrast enema	HD
risk of hypoglycemia (asymptomatic or manifested by seizures)	glycemia measurement	no changes
thermoregulation disorder		no changes
ocular disorders	a complete ocular examination	no changes
face dysmorphia		no changes

The PHOX2B gene located in the 4p12 locus and plays crucial role in differentiation of the cells of ANS during the initial stages of embryogenesis. Therefore, it may contribute to severe diseases — also those mentioned in the case report. The most common type of PHOX2B abnormalities are PARMs (PolyAlanine Repeat Mutations). NPARMs (Non-PARMs) being frameshift, missense and nonsense mutations are in the minority. While PARMs are usually observed in isolated CCHS, NPARMs are associated with co-ocurance of CCHS, HD and neuroblastoma. Nevertheless, a wide range of phenotypes occurs in patients with HS corelated with NPARMs. [6] The majority of defective PHOX2B variants arise de novo, but around 10–25% of heterozygous asymptomatic carriers pass the mutation to their offspring. [8, 10]

The brain abnormalities detected in the MRI were considered uncorrelated with the patient's condition. They are most likely congenital defects. However, in other cases they may have an impact on the patient's condition. Especially if the changes involve areas responsible for chemoreception, the chemoreception may be impaired.

The title "more" is therefore a warning sign that should alert medical professionals to exercise heightened diagnostic vigilance when identifying only one of the components of the HS symptoms. For instance, in the case of late-onset CCHS, respiratory failure may not be evident, as was observed in the presented case. [6] However, when a pathological mass appears in the abdominal cavity of a patient diagnosed with HD, considering HS and anticipating the occurrence of the first symptoms of respiratory failure with appropriate actions is paramount. In such cases, readiness to provide respiratory support is essential and stoma creation may not be as urgent a procedure as intubation. Tracheotomy should be considered as soon as a diagnosis of CCHS is made, which indicates that extubation will not be possible in the future and that lifelong ventilatory support will be necessary for the patient. [7, 9] The combination of certain images may be key to diagnosing this rare syndrome. Children who exhibit unexplained central hypoventilation should undergo

evaluation for CCHS. [6] While genetic testing plays an important role in the correct diagnostic pathway, the readiness to implement mechanical ventilation seems to be more important in determining patient survival. [6] Currently, the optimal solution for most CCHS patients is a voluntary respirator, which detects episodes of apnea and turns on only when the break in the respiratory cycle is too long.

Conclusion

Children diagnosed with Haddad Syndrome require comprehensive, lifelong medical care due to the dual challenges posed by CCHS and HD, as well as the increased risk of tumors originating from neural crest cells. Early and accurate diagnosis — supported by genetic testing — and timely initiation of multidisciplinary treatment are crucial for improving both survival rates and long-term quality of life. Effective management relies on coordinated efforts from interdisciplinary medical team.

Beyond addressing immediate life-threatening symptoms, long-term care should prioritize routine oncological surveillance, monitoring of autonomic nervous system function, and psychosocial support for both patients and families. The dynamic nature of this rare syndrome also necessitates clinical vigilance and openness to evolving diagnostic and therapeutic strategies. With appropriate support and advances in medical care, patients with HS can experience significantly improved outcomes despite the complexity of their condition.

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Author's contribution

S.K. and O.M. conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed and revised the manuscript. K.K. designed the study, collected data and drafted the initial manuscript.

All authors approved the final manuscript as submitted, and agreed to be accountable for all aspects of the work.

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Conflict of interest

None declared.

Abbreviations

ANS — autonomic nervous system
CCHS — congenital central hypoventilation syndrome

HD — Hirschsprung's disease
HS — Haddad Syndrome
NBL — neuroblastoma
NCC — neural crest cells
NPARM — Non PolyAlanine Repeat Mutations
PARMs — PolyAlanine Repeat Mutations
PEG — Percutaneous Endoscopic Gastrostomy

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Infectious screening and vaccination coverage in patients with inflammatory bowel disease: a single-center observational study

Estera Banasik[1], Agnieszka Dobrowolska[1], Magdalena Roszak[2], Piotr Eder[1]

[1] Department of Gastroenterology, Dietetics and Internal Medicine, Poznań University Clinical Hospital, Poland
[2] Department of Pathophysiology, Poznań University of Medical Sciences, Poland

Corresponding author: Piotr Eder, M.D., Ph.D.
Department of Gastroenterology, Dietetics and Internal Medicine,
Poznań University of Medical Sciences
ul. Przybyszewskiego 49, 60-355 Poznań, Poland
Phone: +48 618 691 142; E-mail: piotreder@ump.edu.pl

Abstract: Introduction: Patients with inflammatory bowel disease (IBD) are at increased risk of infections due to immune dysfunction and use of immunosuppressive and biologic therapies. Therefore, proper screening for latent infections and vaccinations are important to improve treatment safety. The aim of the study was to assess serological screening for hepatitis B and C virus (HBV, HCV) infections and to evaluate vaccination coverage in patients with IBD in routine clinical practice in a tertiary center.
Materials and Methods: A total of 105 patients with IBD were included (Crohn's disease, n = 56; ulcerative colitis, n = 48; IBD unclassified, n = 1). Data were collected using an original questionnaire and laboratory assessment of HBV and HCV markers. A subgroup analysis evaluated COVID-19 vaccination and adverse events.
Results: All patients reported a negative history of HBV and HCV infection. Anti-HBc total antibodies were detected in 5 patients, while none were HBsAg-positive and none tested positive for anti-HCV antibodies. Among 82 patients vaccinated against HBV, 31 (38%) had nonprotective anti-HBs titers. Current IBD treatment was not associated with impaired vaccine response. Vaccination rates for influenza, pneumococci, and meningococci were low. Only 15% of patients reported receiving information about vaccination from the physicians. In the COVID-19 subgroup, 74.7% of patients were vaccinated, with mostly mild adverse events.
Conclusions: Despite existing recommendations, screening and vaccination coverage in patients with IBD remain insufficient, indicating the need for implementation of immunoprophylaxis in routine clinical practice.

Keywords: inflammatory bowel disease, opportunistic infections, vaccination, immunoprophylaxis, hepatitis B.

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Introduction

Infectious complications represent one of the most common and clinically significant adverse outcomes in patients with inflammatory bowel disease (IBD), with opportunistic infections accounting for a substantial proportion of these events. [1, 2, 3] Evidence from studies conducted in European populations indicates that patients with IBD treated with immunosuppressive agents and biologic therapies particularly when used in combination, are at an increased risk of opportunistic infections. [1, 3, 4, 5] In a large European cohort comprising 6914 patients with IBD, the incidence of serious infections was reported to be 3% in the overall population and 5% among patients receiving immunosuppressive therapy. Comparable findings have been reported in other regions of the world; in a cohort study conducted in China among hospitalized patients with IBD between 2014 and 2019, the prevalence of opportunistic infections was estimated at approximately 4.5%. [6]

Opportunistic infections are caused by microorganisms that are ubiquitous in the environment and typically exhibit limited pathogenic potential in individuals with a competent immune system. However, in patients with impaired immune function such as those with chronic inflammatory conditions including those with IBD, individuals undergoing immunosuppressive therapy, or patients with active malignancies these infections may occur more frequently and be associated with atypical and often severe clinical manifestations. [4, 6, 7, 8]

Established risk factors for opportunistic infections include age over 50 years, high disease activity in IBD, steroid refractoriness, the use of biologic therapies, and combination treatment involving immunosuppressive agents. Additional factors contributing to an increased risk of infection include chronic comorbid conditions such as diabetes mellitus, chronic kidney disease, and liver disease, as well as malnutrition and inadequate implementation of recommended primary and booster vaccinations. [5, 6, 7, 8]

In accordance with European Crohn's and Colitis Organisation (ECCO) recommendations, infection prevention in patients with IBD should be based on an individualized assessment of infectious risk and the performance of appropriate screening procedures prior to and during immunosuppressive therapy. [7] Before initiating immunosuppressive or biologic treatment, screening for chronic and latent infections is recommended, including evaluation for tuberculosis (interferon gamma release assays and chest radiography), viral hepatitis B and C (HBsAg, total anti-HBc and anti-HBs antibodies; anti-HCV antibodies with subsequent HCV RNA testing in seropositive individuals), and human immunodeficiency virus (HIV) infection. [7, 9, 10] In patients without a documented history of varicella infection or vaccination, assessment of immunity to varicellazoster virus by measurement of anti-VZV IgG antibodies is also advised. [7] This structured diagnostic approach enables early identification of high-risk patients and significantly enhances the safety of immunosuppressive treatment in individuals with IBD.

Particular attention should be paid to occult hepatitis B virus (HBV) infection, which is characterized by the absence of detectable HBsAg in the presence of anti-HBc antibodies and low or undetectable levels of HBV DNA in serum or liver tissue. In such cases, total anti-HBc antibodies may represent the sole serological marker of previous HBV exposure. [11, 12] Following HBV infection, viral eradication is incomplete, as the viral genome persists in hepatocytes in the form of covalently closed circular DNA (cccDNA). Under conditions of immunosuppression, reactivation of viral replication may occur, leading to an increase in HBV DNA levels and the development of hepatitis, which may occasionally follow a severe clinical course. [11, 12, 13, 14] Although such

cases are relatively uncommon, they constitute a clinically relevant risk in immunosuppressed patients.

It should be emphasized that existing clinical guidelines do not always fully reflect real-world clinical practice. Therefore, the primary objective of the present study was to evaluate adherence to recommended screening and vaccination strategies in routine care of patients with IBD. Our center is among the largest institutions in the country dedicated to the management of IBD, providing an opportunity to assess real life clinical practice in a representative patient population. This analysis aimed to identify areas requiring improvement and to propose potential strategies for optimizing clinical management, with the goal of enhancing patient safety and quality of care.

Additionally, in the context of the recent SARS-CoV-2 pandemic, COVID-19 vaccination rates in patients with inflammatory bowel disease were evaluated, along with the potential impact of vaccination on disease activity and its tolerability. The influence of ongoing IBD treatment on these outcomes was also assessed.

Materials and Methods

The study population consisted of patients diagnosed with Crohn's disease (CD), ulcerative colitis (UC), or IBD unclassified (IBD-U) who were hospitalized at a tertiary referral center for adult patients in the Greater Poland region, either due to exacerbation of IBD or for scheduled administration of biologic therapy.

Data were collected using an original, structured questionnaire that included items related to demographic characteristics (sex, age), disease course (type of IBD, disease duration, current treatment, and history of surgical interventions), as well as questions regarding the implementation of preventive vaccinations. These included vaccinations covered by the current National Immunization Program in Poland (hepatitis B virus and varicella-zoster virus) and recommended (non-mandatory at the time of patients' inclusion to the analysis) vaccinations, such as those against influenza, pneumococci, meningococci, yellow fever, herpes zoster, and human papillomavirus (HPV). The questionnaire also contained questions addressing whether patients had been informed by physicians about the need to initiate or complete recommended vaccinations and about potential additional vaccinations.

Following completion of the questionnaire, the immunization status against hepatitis B and C viruses was assessed in all patients based on laboratory analyses of venous blood samples, including hepatitis B surface antigen (HBsAg), anti-HBs antibody titers, total anti-HBc antibodies, and anti-HCV antibodies. Laboratory testing was performed using two-step chemiluminescent microparticle immunoassays (CMIA) on the Abbott Alinity analyzer (Abbott Diagnostics), in accordance with the standard procedures applied in the hospital's diagnostic laboratory.

An additional analysis was performed with regard to coronavirus disease 2019 (COVID-19). Information was collected regarding COVID-19 vaccination status (number of vaccine doses received), adverse events following vaccination, and the clinical course of SARS-CoV-2 infection, as well as the potential impact of vaccination on the occurrence or exacerbation of gastrointestinal symptoms. The questionnaire was expanded to include questions concerning whether patients had been informed by physicians about the potential risk of a more severe course of COVID-19 in individuals with IBD and whether they had been encouraged by physicians to receive COVID-19 vaccination.

This study had an observational and retrospective design. Patient participation was voluntary, and all data were analyzed anonymously. The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of Poznan University of Medical Sciences (Resolution No. 363/20).

Results

A total of 105 patients with IBD were included in the analysis, comprising 56 patients with CD (53.3%), 48 patients with UC (45.7%), and 1 patient with IBD-U (1%). The study population included 61 men (58.1%) and 44 women (41.9%). The median age was 35 years (interquartile range [IQR]: 25–46 years), and the median disease duration was 6 years (IQR: 2–9 years).

The most commonly used treatments were mesalazine (86.7%) and glucocorticosteroids (75.2%), with glucocorticosteroids being used significantly more frequently in patients with UC compared with those with CD ($p = 0.01079$). Azathioprine was administered to 43 patients (41%), while 13 patients (12.4%) received biologic therapy, including 12 patients treated with tumor necrosis factor inhibitors and 1 patient treated with vedolizumab.

No patients tested positive for hepatitis B surface antigen (HBsAg) or anti-HCV antibodies. Total anti-HBc antibodies were detected in 5 patients (4.8%). Non-protective anti-HBs antibody titers (<10 mIU/mL) were observed in 38 patients (36.2%) in the entire study population (n = 105), including 31 patients (38%) among the 82 individuals who reported previous vaccination against hepatitis B virus. Among vaccinated patients, those with nonprotective anti-HBs titers tended to be older compared with patients who achieved protective titers (40 ± 15 vs. 35 ± 14 years); however, this difference did not reach statistical significance ($p = 0.08$).

The clinical characteristics of the patients, serological status, and treatments used are summarized in Table 1.

Table 1. Serological status, vaccination uptake and clinical characteristics of IBD patients.

Variable	Results
A. Patient characteristics	
Age, median (IQR), years	35 (25–46)
Sex, n (%) Female Male	 44 (41.9) 61 (58.1)
Type of inflammatory bowel disease, n (%) Crohn's disease, n (%) Ulcerative colitis, n (%) Inflammatory bowel disease unclassified, n (%)	 56 (53.3) 48 (45.7) 1 (1)
Disease duration, median (IQR), years	6 (2–9)
B. Serological status	
HBsAg positive, n (%)	0 (0)
Total anti-HBc positive, n (%)	5 (4.8)
Anti-HBs < 10 mIU/mL, n (%)	38 (36.2)

Table 1. Cont.

Variable	Results
C. Treatment	
5-aminosalicylic acid, n (%)	91 (86.7)
Glucocorticosteroids, n (%)	79 (75.2)
Azathioprine, n (%)	43 (41.0)
Biologic therapy, n (%)	13 (12.4)

No significant associations were found between HBV serological status and age, sex, type of IBD, or disease duration ($p > 0.05$).

Analysis of the relationship between current treatment and hepatitis B vaccination status revealed that patients receiving tumor necrosis factor-α (TNF-α) inhibitors were significantly less likely to have been vaccinated against hepatitis B compared with patients not receiving this therapy (6/12 [50.0%] vs. 76/91 [83.5%], p = 0.0199). This association is illustrated in Figure 1.

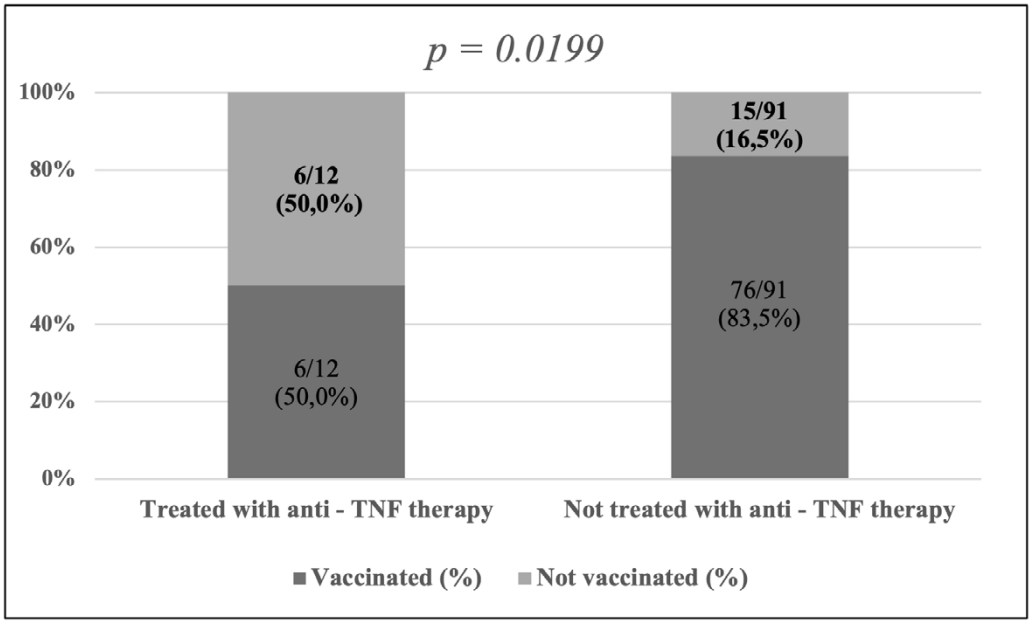

Fig. 1. Hepatitis B vaccination status according to treatment with tumor necrosis factor inhibitors. Patients treated with tumor necrosis factor inhibitors were significantly less frequently vaccinated against hepatitis B compared to patients not receiving this therapy ($p = 0.0199$).

No significant associations were observed between hepatitis B vaccination uptake and the use of other medications (5-aminosalicylic acid, glucocorticosteroids, or azathioprine) or demographic variables ($p > 0.05$).

Analysis of preventive vaccination uptake showed that, despite widespread recommendations for hepatitis B vaccination, 21.9% of patients had not been vaccinated.

Other recommended vaccinations, including those against influenza, pneumococci, meningococci, and varicella-zoster virus (VZV), were administered only sporadically. Detailed data on vaccination uptake are presented in Figure 2.

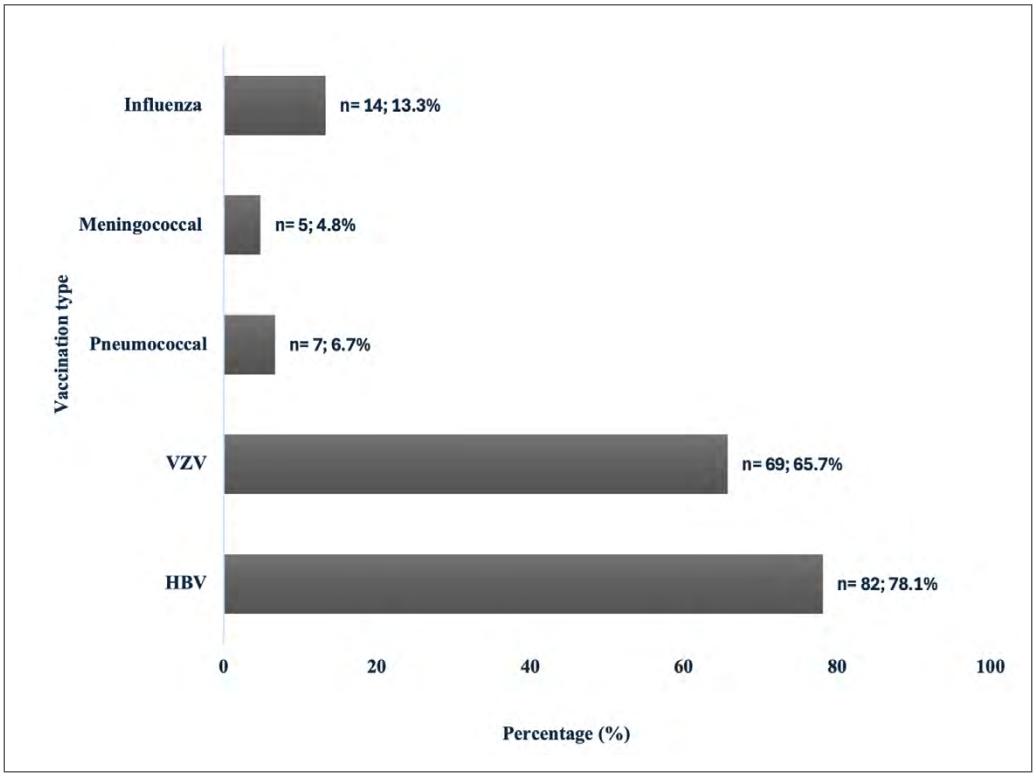

Fig. 2. Uptake of recommended vaccinations among patients with inflammatory bowel disease. Values are presented as percentages with corresponding absolute numbers (n); denominators may differ depending on data availability.
HBV — hepatitis B virus; VZV — varicella-zoster virus.

No significant associations were found between uptake of recommended vaccinations and sex, type of IBD, or current treatment ($p > 0.05$).

According to the collected data, 16 patients (15.23%) reported that gastroenterologists addressed the topic of infectious disease prevention during routine follow-up visits, including active encouragement to receive vaccinations.

In light of the recent COVID-19 pandemic, an additional analysis was performed in a subgroup of 75 patients. In this group, 56 patients (74.7%) reported receiving at least one dose of a SARS-CoV-2 vaccine. The median number of administered vaccine doses was 2 (interquartile range [IQR]: 2–3 doses).

No significant differences in COVID-19 vaccination rates were observed according to age, sex, type of IBD, or ongoing treatment ($p > 0.05$).

Adverse events following COVID-19 vaccination were reported by 12 vaccinated patients (21.4%) and were predominantly mild, including general fatigue (6/56; 10.7%), fever (3/56; 5.4%), and arthralgia (2/56; 3.6%). Transient exacerbation of gastrointestinal symptoms following COVID-19 vaccination was observed in 8 patients (14.3%). No association was found between IBD treatment and the occurrence of adverse events.

Among patients who reported being encouraged by a gastroenterologist to receive COVID-19 vaccination, 18 of 24 individuals (75.0%) were vaccinated, compared with 38 of 51 patients (74.5%) in the non-encouraged group; this difference was not statistically significant ($p > 0.05$).

Discussion

In the present study, we evaluated the practical implementation of immunoprophylaxis in patients with inflammatory bowel disease (IBD). The obtained results indicate that, despite existing guidelines, there remains room for improvement in everyday clinical practice. Diagnostic management in this group of patients should be comprehensive and include multiple aspects, such as the assessment of latent and chronic infections, as well as strategies to prevent infectious complications associated with immunosuppressive and biologic therapies. Immunoprophylaxis should therefore be regarded as an integral component of IBD management rather than merely a pre-treatment step. [1, 2, 3, 4, 5, 6, 7] This is consistent with previous observations highlighting the importance of a comprehensive approach to IBD treatment, including modifiable factors influencing diseases outcomes. [15]

One of the important findings of the present study was the presence of total anti-HBc antibodies in patients without a documented history of HBV infection, suggesting that the risk of occult HBV infection in this population is clinically relevant and should not be overlooked. [9, 10, 11, 12, 16] Furthermore, despite self-reported vaccination against hepatitis B virus, a substantial proportion of patients exhibited anti-HBs antibody titers below the protective threshold. [17] These findings support routine assessment of anti-HBs titers in vaccinated individuals, not only prior to planned initiation of immunosuppressive or biologic therapy but also in patients not currently receiving such treatment. This approach is justified by the possibility of sudden disease exacerbation, the need for urgent hospitalization or surgical intervention, and situations in which there may be insufficient time to complete vaccination. [18, 19]

In our cohort, older age showed a tendency toward lower anti-HBs titers among vaccinated patients, although this association did not reach statistical significance. Previous studies, including those conducted in pediatric populations, indicate that the risk of an inadequate post-vaccination response increases with age, which may represent an additional factor to consider when planning immunization strategies in patients with IBD. [19, 20]

In the present study, no negative impact of immunosuppressive or biologic therapy on the immune response to HBV vaccination was observed. Similar conclusions have been reported in some previous studies, [19, 21] as well as in the meta-analysis by Jiang *et al.*, which demonstrated substantial heterogeneity in the immune response to HBV vaccination among patients with IBD and did not provide conclusive evidence of a dominant negative effect of immunosuppression. [20] At the same time, other reports indicate reduced vaccine efficacy in patients receiving intensive immunosuppressive treatment or anti-TNF therapy, particularly in older age groups,

highlighting the importance of administering vaccination prior to treatment intensification. [19, 20, 21]

We also evaluated the implementation of recommended vaccinations, including those against influenza, pneumococci, meningococci, varicella-zoster virus (VZV), human papillomavirus (HPV), and yellow fever. The results indicate a low level of adherence to these vaccination recommendations despite existing guidelines, which may reflect limited awareness of recommendations and insufficient communication regarding immunoprophylaxis among both patients and physicians. [22, 23, 24] Similarly low vaccination rates have been reported in other observational studies, in which insufficient knowledge among patients and healthcare providers, as well as limited awareness of the risk of opportunistic infections, were identified as major barriers. [22, 23, 24] Available evidence further suggests that even among patients receiving biologic therapy, adherence to recommended vaccinations remains suboptimal, which is consistent with our observations.

In the analysis of COVID-19 vaccination, 74.7% of patients received at least one dose of the vaccine. This finding indicates a relatively high level of vaccine acceptance among patients with IBD; however, approximately 25% remained unvaccinated, which continues to represent an area requiring attention. In a global meta-analysis conducted by Bianchi *et al.*, similar results were reported, with 72% of patients having received at least one vaccine dose. [25, 26] Comparable vaccination rates (70–90%) have been described in other population-based studies and meta-analyses. [27, 28] Vaccination was well tolerated and was not associated with a significant risk of worsening intestinal symptoms. Adverse events were reported in 21.4% of vaccinated patients and were predominantly mild and flu-like in nature (headache, fatigue, myalgia, and arthralgia). Transient exacerbation of intestinal symptoms was observed in 14.3% of patients. Similar cohort studies have demonstrated that the frequency of adverse events in patients with IBD is comparable to that observed in the general population and that vaccination is not associated with an increased risk of disease flare. [29, 30] No association was observed between immunosuppressive or biologic therapy and the frequency of adverse events, which is consistent with findings from other analyses involving patients with IBD. [25, 26]

Patients who reported receiving a vaccination recommendation from their gastroenterologist were not significantly more likely to be vaccinated than those in whom the topic had not been addressed during the visit. This may suggest that vaccination decisions are influenced by multiple factors beyond physician recommendation alone. However, it should be emphasized that the proportion of patients reporting such recommendations was low, which limits the ability to draw definitive conclusions.

Systematic reviews indicate that physician recommendation may facilitate vaccine acceptance; therefore, physicians — particularly gastroenterologists should actively engage in promoting immunoprophylaxis among patients with IBD. [23, 24]

The results of our analysis are consistent with the 2021 ECCO guidelines, which emphasize the need for screening for chronic and latent infections prior to initiation of immunosuppressive therapy in patients with IBD. [7] Similar positions are presented in the guidelines of the World Health Organization (WHO) and other institutions involved in infection prevention, which highlight the importance of systematic assessment of infectious risk in this population. [9, 10]

In our view, one of the key factors limiting vaccination uptake is concern regarding vaccine safety and potential adverse events. This phenomenon may have intensified in recent years due to extensive public debate on vaccination, particularly in the context of COVID-19, potentially contributing to increased scepticism toward other preventive vaccines. [23, 24] An additional challenge

appears to be the lack of clearly defined responsibility for initiating and coordinating vaccinations among physicians of different specialties, which may lead to fragmentation of preventive efforts.

Failure to implement recommended vaccinations is associated with increased and avoidable risk related to immunosuppressive and biologic therapies, including atypical infection courses and reactivation of latent infections. [1, 6, 7] The findings of our study underscore the need for a systematic approach to immunoprophylaxis in patients with IBD. The implementation of simple, standardized tools such as a dedicated vaccination calendar in paper or electronic form could facilitate rapid and reliable verification of vaccination status. Within such a framework, the gastroenterologist, as the specialist planning therapies associated with increased infectious risk, could play a central role in coordinating preventive measures.

This study has several limitations. Its single center design may limit the generalizability of the results. Moreover, part of the data was based on patient completed questionnaires, which may be associated with reporting bias beyond our control. We did not account for the time elapsed since vaccination or for factors such as educational level or socioeconomic status, which may influence both antibody titers and vaccination uptake.

In conclusion, despite existing recommendations regarding immunoprophylaxis of infectious diseases in patients with IBD, gaps in everyday clinical practice remain. The findings of this study highlight deficiencies in infection prevention and may support efforts aimed at improving screening and vaccination strategies in patients with inflammatory bowel disease. Analogous to vaccination schedules used in pediatric populations, consideration should be given to the introduction of a dedicated vaccination calendar for patients with IBD — a population that continues to grow and evolve, partly due to the dynamic development of biologic therapies.

Author Contributions

E.B. and P.E. conceived the study and developed the methodology. E.B. performed the investigation and collected the data. M.R. performed the statistical analysis. E.B. prepared the first draft of the manuscript. P.E. and A.D. critically revised the manuscript for important intellectual content. P.E. supervised the work. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

Abbreviations

anti-Hbc — antibody to hepatitis B core antigen
anti-Hbs — antibody to hepatitis B surface antigen
HBsAg — hepatitis B surface antigen
IQR — interquartile range
mIU/mL — milli-international units per milliliter

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Elevated mycophenolic acid levels after kidney transplantation: avoiding unnecessary dose reduction through team-based TDM interpretation

Angelika Śmiertka[1,2], Agnieszka Cios[1], Łukasz Hońdo[1,2], Agnieszka Supernak[2], Anna Grzywa[2], Katarzyna Sobczyńska[3], Katarzyna Krzanowska[3], Anna Wesołowska[1]

[1] Department of Clinical Pharmacy, Jagiellonian University Medical College, Kraków, Poland
[2] Clinical Pharmacy Unit, University Hospital, Kraków, Poland
[3] Department of Nephrology and Transplantology, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Agnieszka Cios, Ph.D.
Department of Clinical Pharmacy, Jagiellonian University Medical College
ul. Medyczna 9, 30-688 Kraków, Poland
Phone: +48 12 620 56 54; E-mail: agnieszka.cios@uj.edu.pl

Abstract: Mycophenolate mofetil (MMF) is a cornerstone immunosuppressive agent used in combination with calcineurin inhibitors and corticosteroids to prevent acute allograft rejection in solid organ transplant recipients. Long-term transplant outcomes depend not only on the pharmacological efficacy of immunosuppressive regimens but also on individualized dosing strategies and sustained medication adherence. Owing to the complex and highly variable pharmacokinetics of MMF's active metabolite, mycophenolic acid (MPA), therapeutic drug monitoring (TDM) has become an important tool for optimizing treatment safety and efficacy.

We report a case of a kidney transplant recipient with unexpectedly elevated MPA exposure in the absence of clinical toxicity. A detailed pharmacokinetic assessment combined with a comprehensive clinical pharmacist-led medication review indicated that the increased MPA concentrations and elevated $AUC_{0\text{-}12h}$ were most likely related to impaired renal elimination of mycophenolic acid glucuronide (MPAG). Reduced MPAG clearance may enhance enterohepatic recirculation (EHR) of MPA, resulting in increased systemic exposure. This case highlights the importance of interpreting elevated MPA concentrations within a broader clinical context and underscores the critical role of clinical pharmacists, as members of an interdisciplinary team, in integrating TDM with comprehensive pharmacokinetic assessment to ensure safe and effective long-term immunosuppressive therapy.

Keywords: kidney transplant recipient, clinical pharmacist, enterohepatic recirculation, therapeutic drug monitoring, limited sampling strategy.

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Introduction

Mycophenolate mofetil (MMF) is an ester prodrug and a cornerstone immunosuppressive agent administered in combination with calcineurin inhibitors, most commonly tacrolimus, in solid organ transplant recipients. [1] Following oral administration, MMF is rapidly absorbed and undergoes extensive presystemic hydrolysis to its active metabolite, mycophenolic acid (MPA). [2, 3] MPA exerts its immunosuppressive effect through potent, reversible, and non-competitive inhibition of inosine-5′-monophosphate dehydrogenase, the rate-limiting enzyme in de novo guanosine nucleotide synthesis. Because activated T and B lymphocytes rely predominantly on this pathway for proliferation, MPA selectively suppresses lymphocyte expansion while sparing other cell types capable of utilizing salvage pathways. Further, MPA undergoes hepatic glucuronidation to the inactive metabolite mycophenolic acid glucuronide (MPAG). MPAG is primarily eliminated renally; therefore, reduced graft function may decrease MPAG clearance and lead to MPAG accumulation. Increased MPAG availability can enhance enterohepatic recirculation (EHR) through biliary excretion of MPAG, intestinal deconjugation, and subsequent reabsorption of MPA, thereby increasing systemic MPA exposure despite unchanged MMF dosing. [2, 4, 5]

Despite its well-established efficacy, the clinical management of MMF-based immunosuppression in kidney transplant recipients remains challenging. MMF exhibits a relatively narrow therapeutic window: insufficient exposure is associated with an increased risk of acute and late allograft rejection, whereas excessive exposure predisposes patients to toxicity. [6, 7] Gastrointestinal adverse effects are common and frequently lead to dose modification or treatment discontinuation. Hematological complications, particularly leukopenia and anemia, require close laboratory monitoring and may necessitate dose reduction or temporary discontinuation; notably, up to 50% of patients require MMF dose adjustment or discontinuation within the first year after transplantation. [8] In addition, MMF therapy increases susceptibility to infectious complications, including opportunistic viral and fungal infections, further complicating post-transplant care. [2]

MMF pharmacokinetics is characterized by substantial inter- and intra-patient variability and is influenced by multiple clinical and pharmacological factors. Clinically relevant drug-drug interactions play a key role, particularly with calcineurin inhibitors. Cyclosporine interferes with EHR of MPA and may significantly reduce systemic exposure, in contrast to tacrolimus-based regimens. [9] Additional sources of variability include non-linear absorption, high and variable plasma protein binding (approximately 97% albumin-bound), genetic polymorphisms, changes in graft function, and variability in EHR. [4, 5, 10, 11, 12, 13, 14, 15, 16] Importantly, routine clinical markers such as serum creatinine, creatinine clearance, or liver transaminase levels do not consistently correlate with MPA exposure in stable transplant recipients, limiting their utility as predictors of individual pharmacokinetic exposure. [5, 17, 18, 19]

Although MMF was initially prescribed using fixed dosing regimens, it is now well recognized that MPA exposure, expressed as the area under the concentration-time curve (AUC), may vary more than tenfold between patients, ranging from less than 10 mg·h/L to over 100 mg·h/L. [4, 10, 11, 12] Single-point concentration measurements, particularly trough concentration (C_0), correlate poorly with overall exposure and clinical outcomes. [4, 20, 21] Consequently, AUC has emerged as the preferred pharmacokinetic parameter for assessing MPA exposure and guiding dose individualization.

For kidney transplant recipients, a therapeutic target range for MPA $AUC_{0\text{-}12h}$ of 30–60 mg·h/L is generally accepted. [4, 11, 22] However, full 12-hour pharmacokinetic profiling is logistically

demanding and impractical in routine clinical practice. To address this limitation, limited sampling strategy (LSS) has been developed and validated to estimate AUC using a small number of strategically timed blood samples, including abbreviated equations based on concentrations collected within the first 6 hours post-dose ($AUC_{0\text{-}6h}$) to predict the full $AUC_{0–12h}$. [20, 22, 24] The transition from fixed-dose MMF administration to exposure-guided dosing based on therapeutic drug monitoring (TDM) represents a critical step toward personalized immunosuppressive therapy, integrating pharmacokinetic principles with individualized clinical decision-making.

Case description

A 47-year-old man with end-stage renal disease underwent kidney transplantation in 2020. He had been maintained on stable maintenance immunosuppression for approximately three years post-transplant, consisting of MMF 750 mg twice daily, prolonged-release tacrolimus 7 mg once daily, and prednisone 5 mg once daily. The patient was admitted due to biopsy-confirmed recurrence of membranous nephropathy in the transplanted kidney. On the day of admission, laboratory tests revealed a serum creatinine level of 181 μmol/L, an estimated glomerular filtration rate (eGFR) of 37 mL/min/1.73 m^2 calculated using the MDRD equation, and a urine protein-to-creatinine ratio of 79.1 mg/mmol. Urinalysis demonstrated hematuria.

The patient's medical history was significant for arterial hypertension, gout, a past episode of tuberculosis, and atrial fibrillation. Concomitant medications included amlodipine, ramipril, bisoprolol, allopurinol, apixaban, doxazosin, magnesium supplementation, and vitamin D. The patient had also been diagnosed with moderate intellectual disability, necessitating an individualized and closely supervised approach to long-term pharmacotherapy.

On the day of MPA AUC measurement, the patient reported that he had not taken the morning dose of MMF prior to blood sampling and denied any recent changes in medication or supplementation. No clinical signs or symptoms suggestive of MMF toxicity were observed, including gastrointestinal complaints (diarrhea or abdominal pain), infectious symptoms, dysuria, or haematological adverse effects. Laboratory evaluation revealed stable renal function and no clinically relevant abnormalities in liver enzymes, serum albumin, haemoglobin, or white blood cell count.

MPA exposure was assessed using an LSS, and plasma MPA concentrations were determined by an enzymatic immunoassay method. The estimated $AUC_{0\text{-}12h}$ was calculated using a validated LSS equation based on C_{1h}, C_{4h}, and C_{6h} concentrations ($AUC_{0\text{-}12h} = 14.9 + 1.3 \cdot C_{1h} + 3 \cdot C_{4h} + 3.7 \cdot C_{6h}$), [24] yielding an apparently supratherapeutic value of 86 mg·h/L, exceeding the recommended therapeutic range of 30–60 mg·h/L.

A comprehensive medication reconciliation and pharmacotherapy review were conducted by a clinical pharmacist. Potential causes of increased MPA exposure — including hepatic dysfunction, hypoalbuminemia, gastrointestinal adverse effects, drug-drug interactions, and infectious complications — were systematically assessed and excluded. In the context of recurrent membranous nephropathy and documented graft pathology, altered elimination of MPAG was considered a potential explanation for the increased MPA exposure. Subtle alterations in MPAG elimination and EHR related to graft pathology were therefore considered a plausible contributing mechanism. In the absence of clinical toxicity or modifiable pharmacokinetic factors, the MMF dose was maintained, and MPA exposure was reassessed after three weeks.

At follow-up, the patient remained clinically stable with preserved graft function and no signs of MMF-related toxicity. Serum creatinine was 178 μmol/L, with an eGFR (MDRD) of

38 mL/min/1.73 m². A full pharmacokinetic profile was obtained at steady state, with nine blood samples collected over a 12-hour dosing interval (C_0, C_{40min}, C_{1h}, C_{2h}, C_{3h}, C_{4h}, C_{6h}, C_{8h}, and C_{10h}). Individual pharmacokinetic parameters were calculated using non-compartmental analysis (Phoenix WinNonlin v. 8.4, Certara, USA). The $AUC_{0\text{-}12h}$ was 45.25 mg·h/L (Fig. 1), within the therapeutic range, and the remaining pharmacokinetic parameters were consistent with published data for stable kidney transplant recipients.

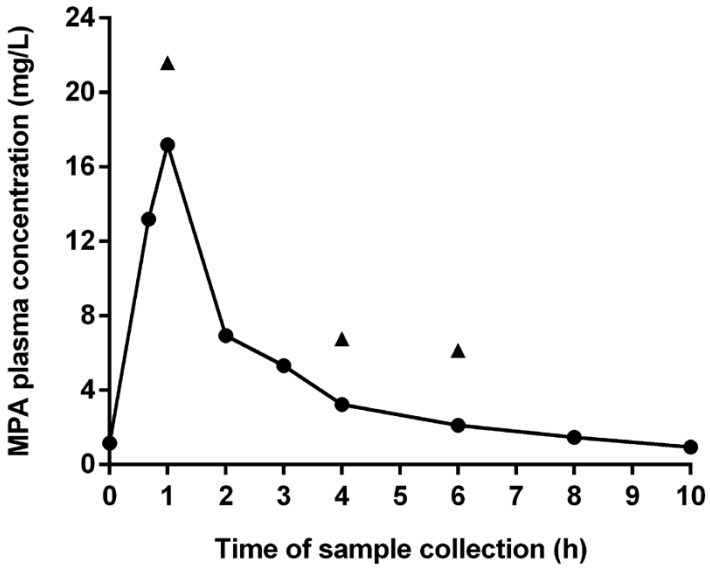

Fig. 1. Comparison of the full 0–10 h steady-state plasma MPA concentration-time profile (nine sampling points) with the selected time points (C_{1h}, C_{4h}, C_{6h}) applied for LSS-derived $AUC_{0\text{-}12h}$ estimation in a kidney transplant recipient. Black circles represent the full concentration-time profile; triangles indicate sampling points used for LSS-based AUC estimation.

Discussion

This case illustrates the clinical complexity of interpreting elevated MPA exposure in the kidney transplant recipient and underscores the importance of integrating TDM with comprehensive pharmacokinetic, pharmaceutical and clinical assessments. A single supratherapeutic AUC value, particularly when derived from LSS, should not automatically prompt dose reduction in the absence of clinical toxicity. LSS models relying predominantly on early post-dose concentrations (0–4 h) may be especially sensitive to shifts in T_{max}, delayed absorption, and coexisting variability in EHR, potentially leading to biased AUC estimates.

In the present case, the initially elevated LSS-derived $AUC_{0\text{-}12h}$ estimate was most plausibly explained by impaired renal elimination of MPAG. Although MPAG concentrations were not measured in this case, reduced MPAG clearance in the setting of graft dysfunction may enhance

EHR and increase systemic MPA exposure; this interpretation remains indirect and is based on the clinical context and available literature. [5] This mechanism has been previously described in kidney transplant recipients and represents an important contributor to interindividual variability in MPA pharmacokinetics. Accordingly, elevated MPA exposure may not necessarily reflect inappropriate dosing but may instead be related to altered metabolite handling and enhanced EHR associated with graft dysfunction.

This approach allows comparison of LSS-derived $AUC_{0\text{-}12h}$ estimates with the reference full $AUC_{0\text{-}12h}$, enabling assessment of bias, precision, and predictive performance of simplified monitoring strategies. [25] This methodology is particularly relevant for MPA, for which full $AUC_{0\text{-}12h}$ is considered the most reliable marker of systemic exposure in solid organ transplant recipients. [26, 27]

Intensive pharmacokinetic assessment is also valuable in cases of unexplained variability in drug concentrations despite stable dosing. Substantial intraindividual fluctuations in MPA concentrations may occur without dose modification and may reflect alterations in absorption kinetics, impaired renal elimination of MPAG, reduced hepatic glucuronidation capacity, variability in EHR, clinically relevant drug-drug interactions, and interindividual genetic differences affecting metabolizing enzymes UGT (e.g. UGT1A9, UGT2B7) and transporters (e.g. MRP2). [4, 26, 28] In such situations, characterization of the full concentration-time profile and calculation of $AUC_{0\text{-}12h}$ enable a more accurate evaluation of systemic exposure and the underlying pharmacokinetic mechanisms. Concurrent comparison with LSS-derived $AUC_{0\text{-}12h}$ provides insight into the applicability and limitations of abbreviated sampling strategies under conditions of increased pharmacokinetic variability.

In the present case, the initially estimated LSS-derived $AUC_{0\text{-}12h}$ was markedly elevated, whereas subsequent full pharmacokinetic profiling demonstrated an $AUC_{0\text{-}12h}$ within the therapeutic range. This discrepancy suggests that transient pharmacokinetic alterations, rather than true sustained overexposure, influenced the abbreviated estimate. It is unlikely that this difference is attributable solely to analytical variability and is more plausibly explained by transient pharmacokinetic perturbations. The absence of MMF-related adverse effects further supports the interpretation that the initial supratherapeutic estimate did not reflect clinically meaningful overexposure.

These findings highlight both the strengths and limitations of LSS in routine practice. Although LSS-based monitoring is validated and cost-effective, its accuracy may be reduced in the setting of altered pharmacokinetics, particularly enhanced EHR. Unexpected TDM results should therefore prompt careful evaluation rather than immediate therapeutic modification.

A recent study [29] has highlighted limitations of conventional LSS models in kidney transplant recipients receiving tacrolimus, particularly under conditions of variable EHR. Model performance appears strongly influenced by this variability, and approaches incorporating later post-dose sampling time points demonstrate improved predictive accuracy. In this context, inclusion of a 6-hour post-dose concentration likely contributed to a more reliable estimation of exposure compared with models based exclusively on early sampling. The marked discrepancy between the initially elevated LSS-derived $AUC_{0\text{-}12h}$ estimate based on abbreviated sampling points and the subsequent full $AUC_{0\text{-}12h}$ within the therapeutic range further suggests that such pharmacokinetic alterations may influence abbreviated sampling-based estimates. Collectively, these observations indicate that LSS-derived AUC values should be interpreted cautiously in patients with altered pharmacokinetics. When clinically indicated, confirmation by full pharmacokinetic profiling may prevent unnecessary dose modification.

More recently, novel LSS models have been proposed to specifically address the limitations imposed by variable EHR. In the study by Mohamed *et al.* [24] involving kidney transplant recipients, LSS models were developed to simultaneously predict $AUC_{0\text{-}12h}$ of MPA, its metabolites (MPAG and acyl mycophenolic acid glucuronide), as well as the extent of MPA EHR. Importantly, the models were developed using a balanced representation of patients with high and low EHR and included late post-dose concentration time points. The best-performing model accurately and reproducibly predicted the $AUC_{0\text{-}12h}$ of MPA and its metabolites, while a separate model enabled reliable estimation of EHR extent. Taken together, these findings indicate that LSS specifically designed to account for EHR and incorporating at least one late post-dose sampling time point (≥5 hours after drug administration) can substantially improve the accuracy of MPA exposure assessment.

In light of these data, selecting an LSS model tailored to the predominant pharmacokinetic mechanisms in a given patient, rather than routinely applying schemes based solely on early time points, is crucial. This case indicates that in patients with significant variability in EHR and altered MPAG metabolism, early MPA concentrations may not reflect true exposure over the 0–12-hour interval, potentially leading to systematic errors in AUC estimation.

An important practical implication is that discrepancies between LSS results and full pharmacokinetic profiling may themselves serve as a clinical signal suggesting the presence of enhanced EHR or altered MPA distribution and albumin binding. In such situations, the LSS result should not automatically be attributed to analytical error but rather considered a cue for more comprehensive pharmacokinetic assessment, including later sampling time points and metabolite analysis. Furthermore, this case highlights the potential role of MPAG measurements as a supportive component of TDM interpretation, particularly when observed AUC values are unexpected in the context of the administered dose and clinical presentation. Finally, these findings suggest that future MPA TDM strategies should move toward adaptive models that account for late sampling points and such variability rather than relying on universal LSS equations. Such an approach may reduce the risk of unjustified MMF dose modification and improve therapeutic safety in patients with atypical pharmacokinetics.

The interpretation of these findings relied on close collaboration within an interdisciplinary transplant team. Clinical pharmacists, physicians, and nurses each contributed essential expertise: pharmacists applied pharmacokinetic principles to contextualize TDM results; physicians integrated these insights with clinical assessment and graft function monitoring; and nurses ensured accurate sample collection and vigilant patient observation. This coordinated approach was crucial in preventing unnecessary modifications to immunosuppressive therapy, optimizing patient safety, and preserving long-term graft function. This report reinforces the importance of interdisciplinary teamwork in managing complex pharmacotherapy in kidney transplant recipients.

Conclusions

This case highlights the complexity of interpreting elevated MPA exposure in kidney transplant recipients and demonstrates that increased MPA concentrations and AUC values may result from impaired renal elimination of MPAG and enhanced EHR, particularly in patients with unstable graft function.

Importantly, elevated MPA exposure should not automatically prompt dose reduction, especially when based on a single abbreviated pharmacokinetic assessment and in the absence of

clinical toxicity, as inappropriate dose modification may increase the risk of under-immunosuppression and graft rejection. Although LSS models are clinically feasible and cost-effective, this case underscores their limitations in the presence of altered pharmacokinetic processes and highlights the need for careful interpretation within the broader clinical and pharmacokinetic context.

A personalized, multidisciplinary approach, combining TDM with comprehensive clinical and pharmacokinetic assessments, remains pivotal for optimizing immunosuppressive therapy and improving long-term transplant outcomes. Clinical pharmacists, as part of the interdisciplinary care team, play an essential role in embedding advanced monitoring strategies, including TDM of MMF and LSS, into routine clinical practice.

Author contribution

A.Ś. — conceptualization, pharmaceutical patient care, pharmacokinetic analysis, writing, editing, discussion; A.C. — conceptualization, pharmacokinetic analysis, writing, discussion, corresponding author; Ł.H. — pharmaceutical patient care, discussion; A.S., A.G. — pharmaceutical patient care, discussion; K.K., K. S. — medical patient care, review, consulting; A.W. — conceptualization, supervision, writing.

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Conflict of interest

None declared.

Abbreviations

AUC	— area under the concentration-time curve
$AUC_{0\text{-}12h}$	— area under the curve concentration-time from 0–12 h after drug administration
$AUC_{0\text{-}6h}$	— area under the concentration-time curve from 0–6 h after drug administration
C_0	— concentration before drug administration
C_{40min}–C_{10h}	— concentration from 40 min up to 10 h after drug administration
DDIs	— drug-drug interactions
eGFR	— estimated glomerular filtration rate
EHR	— enterohepatic recirculation
LSS	— limited sampling strategy
MMF	— mycophenolate mofetil
MPA	— mycophenolic acid
MPAG	— mycophenolic acid glucuronide
PK	— pharmacokinetic
TDM	— therapeutic drug monitoring
Tmax	— time to maximum concentration

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Positive effects of probiotics in people living with HIV: a narrative review

Wiktoria Sobocińska[1,2], Gabriela Kubicka[2], Łukasz Kołodyński[2]

[1] Student Scientific Group of Pathophysiology, Jagiellonian University Medical College, Kraków, Poland
[2] Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Wiktoria Sobocińska
Student Scientific Group of Pathophysiology, Jagiellonian University Medical College
ul. Brzegi 163, 32-040 Wrząsowice, Poland
Phone: +48 661 565 767; E-mail: wiktoria.sobocinska@student.uj.edu.pl

Abstract: Despite effective antiretroviral therapy (ART), no clear guidelines exist on microbiome-targeted adjuncts for people living with HIV (PLWH). Human immunodeficiency virus (HIV-1) infection damages the gastrointestinal tract by altering the mucosal surface and prompting dysbiosis. Gut microbial imbalance, characterized by the predominance of opportunistic bacteria, leads to increased microbial translocation, which, in turn, results in chronic inflammation, digestive tract symptoms, and reduced quality of life. PLWH have substantial dysbiosis compared to healthy controls. In addition, gastrointestinal complications occur as a side effect of ART treatment. Gut-associated lymphoid tissue (GALT) harbors the majority of $CD4^+$ lymphocytes, and this population is significantly depleted during HIV-1 infection. Antiretroviral therapy does not facilitate $CD4^+$ lymphocyte restoration in GALT tissue. Even if the general $CD4^+$ lymphocyte count increases, the GALT $CD4^+$ count, despite therapy, remains stable and low due to unknown causes. Research assessing probiotics in PLWH on ART has yielded mixed results. Some studies report that probiotics may increase $CD4^+$ T cell counts, improve gut barrier integrity, and reduce the incidence of diarrhea in HIV-positive patients. These benefits may be linked to specific probiotic strains that possibly enhance immune function by promoting regulatory T cell populations, thus decreasing microbial translocation. On the other hand, multiple studies did not show any consistent or significant benefits from probiotic supplementation for PLWH. This article assesses critical clinical trials and systematic reviews on this topic, based on limited existing research data. More data from large clinical trials on this topic is needed to assess whether this is or is not a justified new clinical approach for HIV-1-positive patients.

Keywords: probiotics, HIV infection, CD4, gut barrier, PLWH (People Living with HIV).

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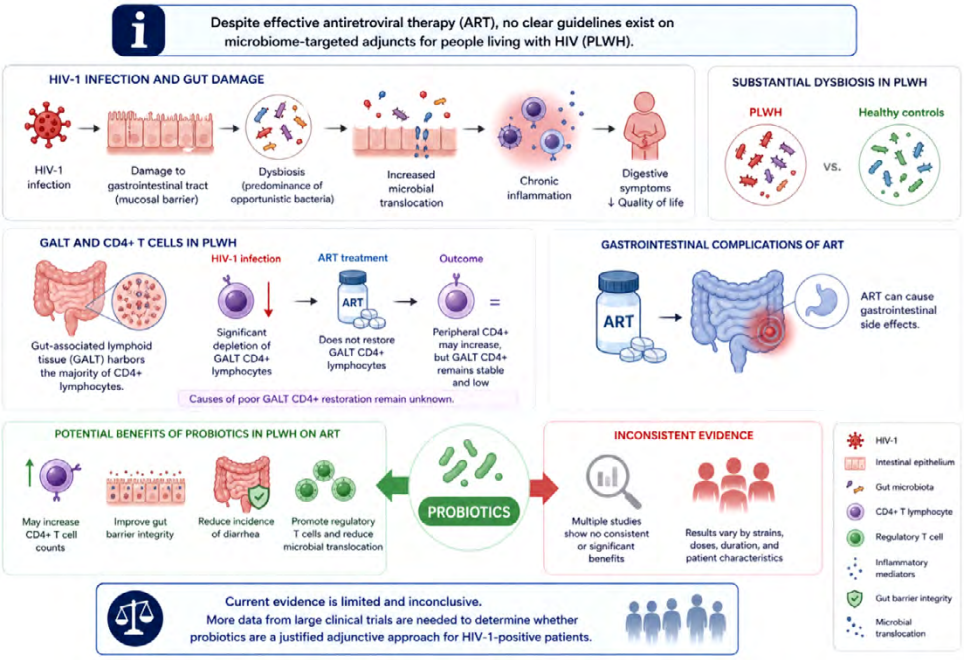
Introduction

According to the World Health Organization, there are 40 million People Living with HIV (PLWH). In Poland, up to the end of 2024, thirty-five thousand patients have been diagnosed as HIV positive. Gut-associated lymphoid tissues (GALT) contain multifollicular Peyer's patches of the ileum, the vermiform appendix, and the isolated lymphoid follicles (ILF). [1] $CD4^+$ cells,

including different types of T-helper cells, [2] are the population pivotal for adaptive immune response [3] and make up a significant percentage of GALT lymphocytes.

Human Immunodeficiency Virus (HIV) is associated with systemic immunological changes, resulting in a decrease in $CD4^+$ lymphocyte count and enteropathy. [4] The gut is an HIV reservoir and is a major virus replication site. [5] Infection leads to inflammatory and immuno-metabolic consequences in the gastrointestinal tract. [6] Weakened gastrointestinal mucosal surface and bacterial translocation play a part in HIV induced immunological activation. [7] The GALT $CD4^+$ cell population is significantly depleted in the HIV-1 positive population. Due to poorly understood reasons, $CD4^+$ GALT compartment reconstruction remains incomplete despite the use of combined antiretroviral therapy (cART). [8, 9] Analysis of gut microbiota in PLWH from multiple studies revealed substantial dysbiosis. [10] Highly active antiretroviral therapy (HAART) has been a major advancement in the treatment of HIV-1 infection; however, knowledge is scarce concerning complementary interventions that restore intestinal structural and functional integrity. Noninfectious diarrhea occurs in 25% of patients on HAART, [11] making it a current and important issue. Individual probiotics, co-infections, and patient age may contribute to the response to treatment and the quality of life of PLWH. Herein, we would like to present data from worldwide scientific research with various endpoints, including gut barrier integrity, $CD4^+$ count, stool consistency, and the effect on diarrhea occurrence. Specific probiotics are not recommended for people with HIV in current guidelines; [12] more data from large clinical trials are needed, and specific strains should be recommended. Zhen Wu *et al.* state that there is a justified recommendation for researchers to explore the potential of probiotics as an adjunctive treatment for HIV. [13]



Graphical abstract. Illustration was made with ChatGPT.

Current status of knowledge: There is not a single HIV guideline that currently recommends routine prebiotic supplementation as part of the standard HIV positive adult and pediatric patient care protocols. The guidelines are primarily focused on early and sustained ART, prevention (PrEP/PEP), and reduction of comorbidity. Probiotic therapies are considered investigational, and more evidence is needed from large clinical trials. Besides general good tolerance and safety of probiotics, practitioners must exercise caution in severely immunocompromised patient groups, as theoretical risks of bloodstream infection have been reported in case reports, clinical trials, and experimental models. [14, 15]

Materials and Methods

A comprehensive narrative review was conducted to analyze evidence from the existing literature on general outcomes of people living with HIV (PLWH) and probiotic therapy, utilizing a PubMed search (probiotics AND HIV). Prospective and experimental studies involving human participants were prioritized for inclusion. The search for reputable publications focused on general probiotic therapy outcomes in PLWH, with various restrictions such as age, ethnicity, clinical status, or other characteristics. Clinical trials were considered essential for evaluating the effects of probiotics. Clinical trial databases were searched; however, many trials were discontinued. The objective is to compare medical studies examining the impact of probiotic use on antiretroviral therapy (ART) and CD4 lymphocyte counts. This review evaluates whether probiotic supplementation benefits HIV-1-positive patients and clarifies the current evidence.

The effect of probiotics supplementation on the gastrointestinal tract of PLWH, immunology, and gut permeability

Research on probiotics demonstrated a reduction in inflammation in antiretroviral-treated individuals. Supplementing combined Antiretroviral Therapy (cART) with probiotics may improve gastrointestinal tract immunity, [16] enhance gut barrier function, and even improve immunity in HIV-infected subjects. [17] Gabriella d'Ettorre *et al.* [18] found benefits from probiotic administration in PLWH on the gut barrier at both gut and immune system levels. Ten patients on ART, aged 18 years or older, with $CD4^+$ T-cell counts above 400 cells/mm^3 and low viral count were included. These patients received probiotics twice daily for 6 months. These probiotics contained: *Lactobacillus plantarum DSM24730, Streptococcus thermophilus DSM24731, Bifidobacterium breve DSM24732, L. paracasei DSM24733, L. delbrueckii subsp. bulgaricus DSM24734, L. acidophilus DSM24735, B. longum DSM24736, B. infantis DSM24737*. The team assessed immune cell activity and T-cell immunophenotype. Following probiotic administration, there was reduced immune activity as indicated by fewer activated T-cells and a higher proportion of Th17 cells in both blood and gut tissues. Tc1 and Tc17 cell levels were not significantly affected, while Th1 cell levels increased in the gut tissue. Stool samples were collected after two and six months. Histopathological assessment of colon samples linked probiotic administration to a healthier gut lining, characterized by fewer immune cells in the lining, a more robust cell structure, and reduced cell death. HIV infection increases gut permeability in PLWH. Schunter *et al.* [19] examined the effect of synbiotics on gut permeability in PLWH in a randomized, placebo-controlled trial with 38 HIV infected women on ART. These patients received a synbiotic product with fibers or fibers alone (placebo). Probiotics were detected in the stool, indicating

bacterial colonization and proliferation. However, there were no changes in inflammation, bacterial invasion into the bloodstream, or immune system activity. Rodney K. Rousseau *et al.* [20] also achieved similar results in their randomized, double-blind, placebo-controlled study using the De Simone Formulation Probiotic (DSFP; Visbiome, with eight bacterial strains). Thirty-six patients with $CD4^+$ T-cell counts under 350/mm^3, despite therapy, were randomly assigned to the probiotic or placebo group in a 2: 1 ratio. $CD4^+$ T-cell activation increased in the probiotic group, while inflammation and immune activation markers remained unchanged. Gut leakage and bacterial migration also remained unchanged. To summarize, studies by Schunter *et al.* and Rodney K. Rousseau *et al.* found successful probiotic colonization but did not observe significant improvements in inflammation, immune activation, or gut permeability markers. On the other hand, Gabriella d'Ettorre *et al.* demonstrated improvement in the gut barrier and immune system. These variations indicate that while some studies report positive effects on gut health and immune activation, others show no consistent or significant benefits for immune or gut permeability markers (Fig. 1).

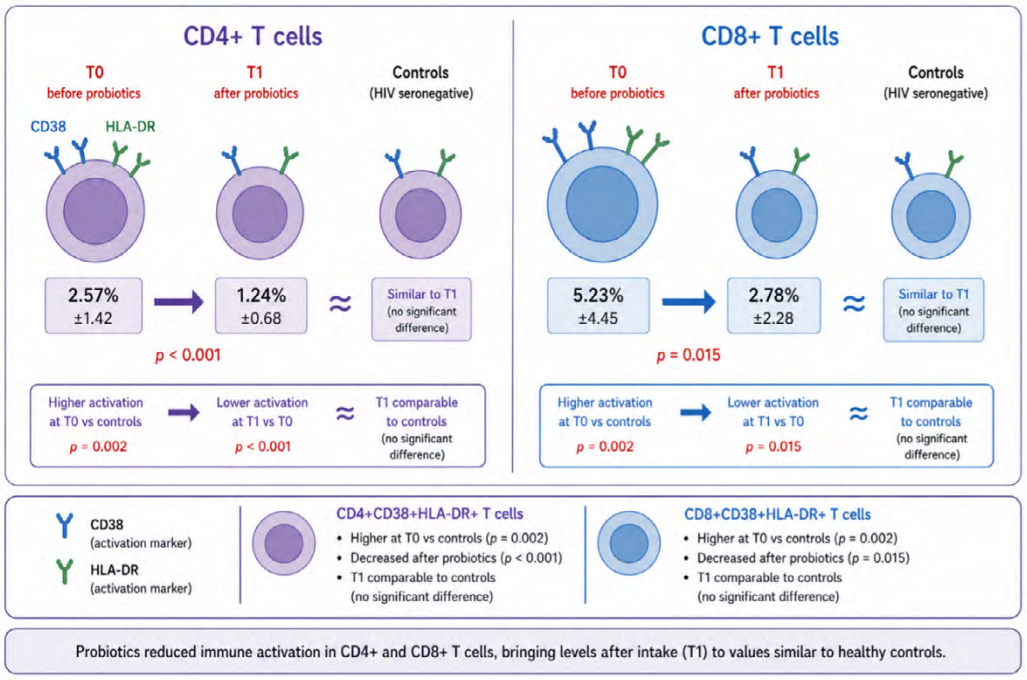


Fig. 1. Immune activation on T-lymphocytes Gabriella d'Ettorre [16]. Assessment of activation markers ($CD38^+HLA\text{-}DR^{+)}$ was performed on $CD4^+$ cells and on $CD8^+$ cells before (T0) and after (T1) probiotic supplementation. Compared with T0, the percentage of activated lymphocytes was significantly lower after probiotic supplementation (T1) in both groups ($CD4^+CD38^+HLA\text{-}DR^+$ T) and ($CD8^+CD38^+HLA\text{-}DR^+$ T). P values <0.05 were considered statistically significant (Fig. 2).
Illustration was made with ChatGPT.

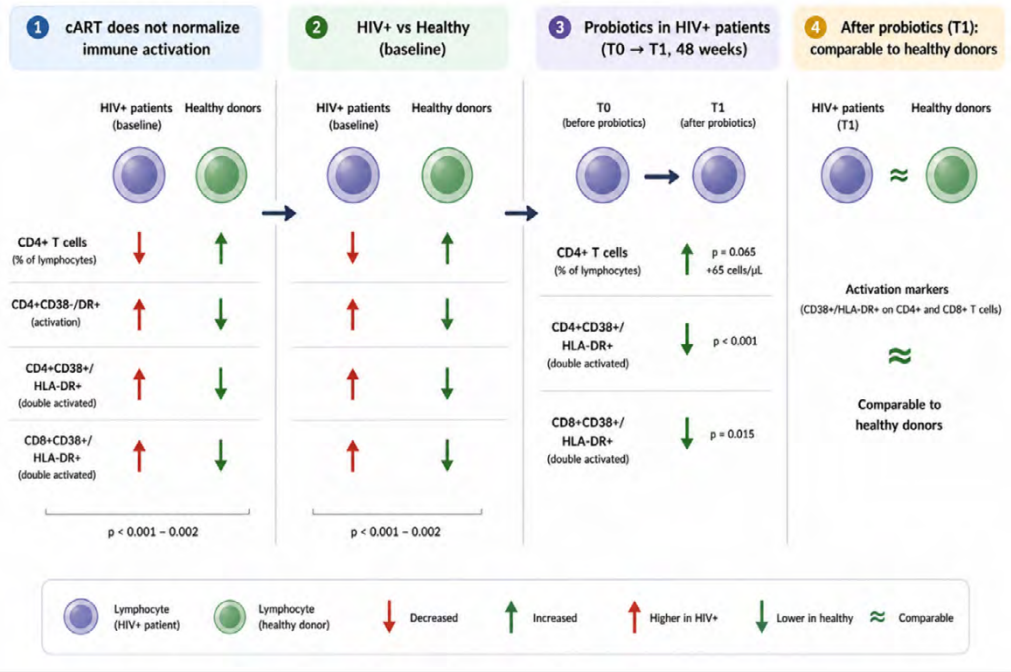


Fig. 2. Summary of Gabriella d'Ettorre's [16] findings.
Illustration was made with ChatGPT.

Clinical evidence on the use of probiotics for HIV-related diarrhea

Non-infectious diarrhea remains a relevant symptom in PLWH, often impairing the quality of life despite highly active antiretroviral therapy (HAART) administration.

While opportunistic diarrhea has become less common in the era of antiviral regimens, functional and treatment-associated gastrointestinal symptoms persist and continue to represent a therapeutic challenge for both patients and clinicians, Rodger D MacArthur. [21] Several interventional studies have investigated the role of probiotics and synbiotics in the treatment of HIV associated diarrhea. Heiser *et al.* [22] presented significant evidence of reduction of diarrhea or complete resolution among HIV positive patients on antiretroviral agents. Enrolled patients were HIV positive males experiencing chronic diarrhea while receiving either lopinavir/ritonavir (8 patients) or nelfinavir (27 patients). The study group consisted of 28 participants randomized to receive dietary supplementation consisting of probiotics and soluble fiber, while the control group consisted of 7 participants who received standard care. Study results showed that supplementation significantly reduced diarrhea. Resolution was found in 36% of patients (10 of 28 participants) in both antiretroviral drug groups. There was no significant change in HIV RNA levels, $CD4^+$ T-cell counts, or weight. Anukam *et al.* [23] have observed diarrhea, flatulence, and nausea resolution within two days after the initiation of probiotic supplementation among HIV patients without antiretroviral therapy. In this study, 24 HIV-positive women were enrolled with moderate diarrhea, $CD4^+$ counts >200 cells/μL without ART. This study

was conducted in Nigeria, where only a select group of patients are receiving ART during the AIDS sub-Saharan epidemic. The investigated probiotics were either 100 mL/day of conventional yogurt (fermented with Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus) supplemented with Lactobacillus rhamnosus GR-1 and L. reuteri RC-14 or unsupplemented yogurt. Hematologic parameters remained unchanged in both groups. Among the participants treated with probiotics, 11 of 12 demonstrated either increased or stable $CD4^+$ counts, compared with 3 of 12 in the control arm. In contrast, S. Guinane [24] stated in her review article that current evidence remains insufficient to recommend or contraindicate the use of probiotics for diarrhea. However, safety and the lack of drug-drug interactions make it a valid option for some patients. Possible evidence of probiotics may improve the immune reconstruction among HIV patients.

Probiotic supplementation and its influence on $CD4^+$ T-cell recovery in HIV-infected adult patients

Several studies have demonstrated that probiotic supplementation may increase $CD4^+$ T-cell levels in the PLVH population. Contrary to these findings, multiple studies have shown no change in $CD4^+$ T-cell counts. Here, we would like to discuss the available findings from clinical trials and secondary studies focused on this issue. Research demonstrating positive outcomes of probiotic intervention on CD4 T cell levels include a notable investigation by Masoud Mortezazadeh *et al.*, [25] a randomized double-blind clinical trial that enrolled 30 patients receiving antiretroviral therapy who exhibited immunologic failure despite sustained viral suppression. The study showed a statistically significant increase in $CD4^+$ T-cell counts in the probiotic group compared with the control group after a seven-month follow-up. However, discontinuation of probiotic supplementation nevertheless showed a subsequent decline in symptoms, and $CD4^+$ T-cell counts at the end of the study remained significantly higher than baseline values. Another study, the 'RECOVER' clinical trial conducted by Blázquez-Bondia *et al.*, [26] investigated the novel probiotic formulation 'i3.1', consisting of two strains of *L. plantarum and one of P. acidilactici.* HIV positive individuals on combination antiretroviral therapy (cART) with $CD4^+$ T-cell counts below 500 cells/mm^3 were enrolled. Subjects were randomized 1: 2: 2 to receive a placebo, a probiotic, or a probiotic plus prebiotic (synbiotic). Only symbiotic (probiotic + prebiotic) supplementation resulted in moderate increases in the CD4/CD8 ratio, as well as minor reductions in soluble CD14 (sCD14). However, the clinical significance of these changes remains unclear. Probiotic supplementation alone did not affect immune parameters or fecal microbiome composition.

The HIV/AIDS epidemic still remains a global health challenge. In 2023, an estimated 25.6 million individuals in sub-Saharan Africa were living with HIV/AIDS and its complications. To address this issue, Jaimie Caitlin Hemsworth [27] and colleagues developed an affordable and tasty nutritional supplement containing probiotics for HIV positive patients. Twenty-one PLWH on HAART were enrolled in a randomized, double-blind, three-period, crossover-controlled trial. The tested formulations were Formulation A (micronutrient plus probiotic), Formulation B (micronutrient alone), and Formulation C (probiotic alone). All the participants received each formulation for 30 days, separated by a 14-day elimination phase. Micronutrient alone (B) was linked to the greatest increase of CD4 lymphocyte count by 41 cells/μL (SD 221). Additionally, micronutrient plus probiotic (A) was associated with a 19 cell/μL increase in CD4 lymphocyte count (SD 142). Unexpectedly exclusive probiotic (C) was linked with a decrease in the CD4

lymphocyte count of 7 cells/μL (SD 154). This study presents the superiority of a combination of micronutrients and probiotics over probiotics alone.

Consistent with these findings González-Hernández *et al.* [28] demonstrated in a randomized, double-blind controlled study a positive effect of probiotic supplementation in the HIV positive AR naive study group. Twenty enrolled patients were divided into three subgroups and assigned to receive a probiotic, a prebiotic, a symbiotic (a combination of probiotics and prebiotics), or a placebo for 16 weeks. In the symbiotic study arm, the $CD4^+$ lymphocyte population increased significantly (median: +102 cells/μL; $p = 0.05$) with a decrease of interleukin-6, the studied inflammation marker. Probiotic supplementation led to a positive bacterial shift in the feces characterized by an increase in Bifidobacterium and a decrease in Clostridium populations. Serrano-Villar *et al.* took a complementary approach [29] when in 2016 they researched the efficiency of a synbiotic-based formulation in a pilot multicenter, randomized, double-blind, placebo-controlled trial. Seventy eight HIV positive, ART-naive patients with a $CD4^+$T count below 350/uL or acquired immunodeficiency syndrome (AIDS) were enrolled. Patients received either PMT25341 (a combination of synbiotics, amino acids, and omega-3/6 fatty acids) or a placebo daily for 48 weeks, either with or without first-line antiretroviral therapy. The mean change in $CD4^+$ T-cell count and the change in CD4/CD8 ratio between treatment groups were not statistically significant. Immunological benefits of ART were not improved by this nutritional intervention targeting the GALT and microbiota. In a subsequent study, Hummelen *et al.* (2011) [30] did not observe any differences in immune markers or diarrhea incidence as a result of supplementing with a probiotic containing Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri for 25 weeks, twice daily, in the study group, with no adverse effects observed. Similarly, Hummelen *et al.* [31] reported in 2014 a good tolerance of probiotics, but without improvement in $CD4^+$ counts. After one month, an average decline in $CD4^+$ count of 70 cells/μL (95% CI: –154 to –15) was observed in the probiotic group compared with a decline of 63 cells/μL (95% CI: –157 to –30) in the control group ($P = 0.9$). One hundred and twelve ART-naive HIV patients were enrolled in this randomized, double-blind, controlled trial. Patients were randomized to receive either micronutrient-fortified yogurt alone or micronutrient-fortified yogurt supplemented with *Lactobacillus rhamnosus* GR-1 for 4 weeks. We need to emphasize the fact that there are some safety concerns regarding the use of probiotics in the PLWH population, such as blood infiltration by a probiotic. [32]

Wolf *et al.* [33] investigated the safety of *Lactobacillus reuteri*, demonstrating no adverse side effects. Supplementation did not affect the safety markers measured and was well-tolerated.

Table 1. Summary of secondary research on the effect of probiotic intervention on $CD4^+$ lymphocyte population.

Author	Type of research	Outcome
Fu *et al.* 2020 [34]	systematic review and meta-analysis	Evidence of a positive outcome on the CD4 count is insufficient.
Zhang *et al.* 2021 [35]	systematic review and meta-analysis	Probiotics had no effect on CD4 cell counts in HIV/AIDS patients.
Kazemi *et al.* 2018 [36]	systematic review and meta-analysis	Supplementation with probiotics may not change CD4 counts. A significant increase in CD4 counts was observed in females following synbiotic treatment compared with pro- or prebiotic treatment alone.

Table 1. Cont.

Author	Type of research	Outcome
Oyadiran *et al.* 2024 [37]	Systematic review and meta-analysis	Meta-analysis reported no consistent effect on CD4; evidence for other outcomes was also inconsistent.
Wen *et al.* 2025 [38]	Meta-analysis	The results of this meta-analysis suggested that probiotics supplementation may have no effect on $CD4^+$ T cell counts. The adverse events reported appeared to have no correlation with the probiotic treatment.

Taken together, evidence from randomized trials and systematic reviews suggests that probiotic supplementation in PLWH is generally safe and well-tolerated, although its effects on $CD4^+$ T-cell counts remain inconsistent. Synbiotic formulations may show greater potential in specific subgroups, such as women, though further large-scale and long-term studies are required to establish their clinical significance.

Studies on pediatric HIV-1 positive population

Every year, around 120,000 new cases of pediatric HIV-1 infection are reported. Vertical infection and infant infection result in the initiation of antiretroviral therapy from the first days of life. We lack data on the influence of HIV-1-related dysbiosis and enteropathy on the development and maturation of the microbiota. [39] Infection in children living with HIV (CLWH) results in bacterial translocation, loss of gut epithelial integrity, $CD4^+$ T lymphocyte cell depletion, systemic immune activation, and viral reservoir establishment; these are identical consequences as in the adult population. Herein, we would like to present a few clinical trials that have been conducted on the effect of probiotics in the pediatric population.

Firstly, we would like to mention the Sachdeva *et al.* [40] systematic review and meta-analysis of randomized control trials (RCTs) of the HIV positive pediatric population. Data from those RCTs showed that in children, probiotic use is associated with improved $CD4^+$ cell counts with no adverse side effects. The study emphasizes the need for future research on this topic on a large scale as well as through international clinical trials. Patients had increased CD4 cell counts in both the ART-treated population and the ART-naive arm, as observed in the subgroup population analysis. A mean difference of 123.92 $CD4^+$ T-cell counts (95% CI: 104.36-143.48) was shown in the pooled analysis of the included trials, $p < 0.0001$, with no heterogeneity ($I2 = 0$). The Cochrane risk of bias tool and Egger's test for publication bias were used for evaluation. In 2014, Gautam *et al.* [41] conducted an open-label randomized control study in Agra (India). Enrollment criteria included HIV infection and age below 15 years. One hundred and twenty-seven pediatric patients were randomized to receive either probiotics for 3 months or micronutrients for 6 months. In children aged 5 years or older receiving probiotic supplementation, CD4 counts increased significantly compared with the control group ($p = 0.0022$). After 6 months in the micronutrient supplementation group (without ART), a statistically significant improvement in WHO clinical staging ($p = 0.049$) was observed. To summarize, this study suggests that probiotic supplementation can be beneficial in the pediatric population, and micronutrient supplementation (including vitamins B, C, E, and folic acid) may slow the progression of disease. However, according to the author, because of the lack of objective data on HIV mortality or progression, these data must be expanded with a larger randomized controlled

trial. New combinations of probiotic strains are an interesting topic. [42] Lívia Trois Trois *et al.* investigated whether the formula containing *Bifidobacterium bifidum* and *Streptococcus thermophilus* (2.5×10^{10} CFU) would be beneficial in comparison with the standard formula (control group). A randomized, double-blind, controlled trial was conducted, enrolling 77 pediatric patients aged 2–12 years. Children were randomized to receive either a studied probiotic formula or a standard formula (control group) for 2 months. The study group demonstrated a significant increase in mean $CD4^+$ T-cell counts (+118 cells/mm^3), whereas the control group exhibited a decline (−42 cells/mm^3; $p = 0.049$) at the end of intervention. Both groups showed a significant improvement in stool consistency ($p < 0.01$), and the occurrence of loose or soft stools decreased slightly in both groups ($p = 0.955$). Similarly, Ishizaki *et al.* [43] assessed the beneficial properties of probiotic strain *Lactobacillus casei Shirota* (LcS) in a 2017 study conducted in Vietnam. Sixty HIV-1 positive children were enrolled, 29 receiving ART for a median of 3.5 years, and 31 without antiretroviral therapy. Twenty HIV-1-negative children were in the control group. Participants consumed fermented milk containing probiotics for 8 weeks. After intervention, there were significant increases in peripheral $CD4^+$ lymphocyte counts and in the Th2 ($CXCR3^-CCR6^-CD4^+$) subset in both HIV-positive arms. Notably, Th17 lymphocyte ($CXCR3^-CCR6^+CD4^+$) rates increased across all groups. On the contrary, regulatory T-cell ($CD25highCD4^+$) counts decreased in the HIV positive ART group and the control group. Bacterial translocation was not detected, and no significant differences in bacterial 16S/23S rRNA were found in patient plasma. The intervention did not lead to any serious adverse events, confirming the safety of LcS supplementation. On the other hand, in Iran, Alpha Fardah Athiyyah *et al.* [44] studied the effect of *L. plantarum* IS-10506 supplementation, and the intervention did not show any beneficial effect on lymphocyte count. Twenty-one pediatric patients aged from 2 to 18 years treated with ART for more than 6 months were enrolled and completed the study. Participants were randomly assigned to two groups: a study group and a placebo group. The study group received 2.86 × 10^10 cfu/day of probiotic L. plantarum IS-10506 for 6 weeks. Results revealed no difference in the absolute $CD4^+$ T cell count, percent $CD4^+$ cells, absolute $CD8^+$ T cell count, $CD4^+/CD8^+$ T cell ratio, and faecal sIgA. However, the bacterial translocation marker, blood lipopolysaccharide (LPS) level, was statistically significantly decreased in the study group ($p = 0.001$).Together, these findings demonstrate that probiotic supplementation in HIV-infected children is well tolerated and may enhance $CD4^+$ T-cell recovery, exert an immunostimulatory effect, and improve stool consistency. While clinical outcomes varied between studies, the evidence supports a potential adjunctive role for probiotics in pediatric HIV care. Larger, long-term randomized trials are needed to validate these findings and determine their clinical relevance in relation to disease progression and quality of life.

On the other hand, some studies emphasize that antiretroviral therapy alone can mildly improve Inflammation and Gut Permeability and microbial translocation induced by HIV infection. [45, 46]

Conclusions

In summary, collectively, these studies suggest that probiotic interventions are safe and may alleviate gastrointestinal symptoms, particularly diarrhea, in HIV-positive populations. While improvements in immune activation and $CD4^+$ T-cell counts have been inconsistent, evidence supports symptomatic relief and enhanced quality of life, highlighting the potential of probiotic-based nutritional therapies as adjunctive strategies in HIV guidelines. Larger, longer-term, and international randomized controlled trials are needed to confirm these benefits and clarify their immunological and clinical significance.

Author's contribution

W.S. — conception of work, formal analysis, writing — original draft, project administration. G.K. — formal analysis, writing — original draft. Ł.K. — formal analysis, writing — original draft.

Conflict of interest

None declared.

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Preliminary clinical association between the Nerve-Sparing Quality (NSQ) Score and early functional outcomes after robot-assisted radical prostatectomy

Jakub Kempisty, Krzysztof Balawender, Oskar Dąbrowski, Karol Burdziak

Clinic of Urology and Urological Oncology, Fryderyk Chopin University Clinical Hospital in Rzeszów, Rzeszów, Poland

Corresponding author: Jakub Kempisty, FEBU
Clinic of Urology and Urological Oncology
Fryderyk Chopin University Clinical Hospital in Rzeszów
ul. Szopena 2, 35-055 Rzeszów, Poland
Phone: +48 605 608 503; E-mail: jakub.kempisty@gmail.com

Abstract: Introduction: Nerve-sparing during robot-assisted radical prostatectomy (RARP) plays a critical role in postoperative functional recovery, particularly urinary continence and erectile function. Although several tools exist for assessing surgical performance in robotic surgery, objective intraoperative evaluation of nerve-sparing quality remains limited. The Nerve-Sparing Quality (NSQ) Score was recently introduced as a structured intraoperative scoring system designed to evaluate the technical quality of neurovascular bundle preservation. The aim of the present study was to explore the preliminary clinical correlation between NSQ Score and early functional outcomes following RARP.

Methods: This retrospective observational study included 20 patients who underwent RARP and had available intraoperative NSQ Score assessment together with 6-month postoperative functional follow-up. The NSQ Score is a 10-point intraoperative scoring system based on five domains: dissection plane, bleeding control, bundle manipulation, continuity of dissection, and symmetry. Functional outcomes were assessed at 6 months using data obtained from routine postoperative follow-up records, using pad use (pads per day) and the International Index of Erectile Function-5 (IIEF-5). Associations between NSQ Score and functional outcomes were evaluated using Spearman correlation analysis. Exploratory subgroup comparisons were performed using an NSQ Score threshold of ≥8.

Results: The mean NSQ Score was 7.95 ± 1.61. Mean pad use at 6 months was 0.75 ± 0.91 pads/day, and mean IIEF-5 score was 18.05 ± 6.60. Higher NSQ Score correlated with lower pad use (Spearman $\rho = -0.56$, $p = 0.011$) and higher IIEF-5 ($\rho = 0.87$, $p < 0.001$). Patients with NSQ Score ≥8 demonstrated significantly better erectile function compared with those with lower scores (21.38 ± 4.46 vs 11.86 ± 5.40, $p = 0.001$) and lower pad use (0.38 ± 0.51 vs 1.43 ± 1.13 pads/day, $p = 0.035$).

Conclusions: Higher NSQ Score was associated with improved early functional outcomes after RARP, particularly regarding erectile function and postoperative pad use. These findings provide preliminary clinical support for the construct validity of the NSQ Score as a structured intraoperative measure of nerve-sparing quality. Larger prospective studies incorporating baseline functional parameters are required to determine the independent predictive value of the NSQ Score.

Keywords: robot-assisted radical prostatectomy, Nerve-Sparing Quality Score, nerve-sparing technique, functional outcomes, erectile function, urinary continence, surgical quality assessment.

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Introduction

Nerve-sparing during radical prostatectomy is one of the most important determinants of postoperative functional recovery, particularly urinary continence and erectile function, which are key contributors to patient quality of life following prostate cancer treatment. [1, 2, 3] Since the original anatomical description of nerve-sparing prostatectomy by Walsh and Donker, numerous refinements in surgical technique have aimed to preserve the neurovascular bundles responsible for erectile function while maintaining oncologic efficacy. [4]

The introduction of robotic surgical platforms has significantly improved visualization and dexterity during prostatectomy, allowing more precise dissection in proximity to the neurovascular bundles. [5] As a result, robot-assisted radical prostatectomy (RARP) has become the predominant surgical approach for localized prostate cancer in many countries. [6] Despite these technological advances, substantial variability in postoperative functional outcomes persists. Large prospective studies have demonstrated significant heterogeneity in continence and potency recovery following RARP, suggesting that surgical technique remains a key determinant of postoperative results. [7, 8]

Several tools have been developed to assess surgical performance in robotic surgery. The Global Evaluative Assessment of Robotic Skills (GEARS) evaluates general robotic surgical competence, including depth perception, dexterity, and efficiency. [9] The Objective Structured Assessment of Technical Skills (OSATS) provides a broader framework for structured surgical skill evaluation across multiple surgical disciplines. [10] Procedure-specific assessment tools such as the Prostatectomy Assessment and Competency Evaluation (PACE) score have also been proposed for evaluating robotic prostatectomy performance. [11] However, these instruments primarily focus on overall surgical performance and do not specifically evaluate the quality of nerve-sparing dissection.

To address this limitation, the Nerve-Sparing Quality (NSQ) Score was recently introduced as a structured intraoperative scoring system designed to evaluate the technical quality of neurovascular bundle preservation during RARP. The NSQ Score has been developed as a structured intraoperative scoring system for evaluating nerve-sparing quality during robot-assisted radical prostatectomy. A detailed description and methodological validation of the score are presented in a separate methodological study. The score incorporates five domains - dissection plane, bleeding control, bundle manipulation, continuity of dissection, and symmetry, and provides a standardized framework for assessing nerve-sparing technique. [12]

While the feasibility and reproducibility of the NSQ Score have been previously demonstrated, its clinical relevance in relation to postoperative functional outcomes has not yet been explored. Establishing such a relationship would provide important support for the clinical utility of the score. Therefore, the aim of this study was to perform a preliminary exploratory analysis of the association between NSQ Score and early functional outcomes after RARP, including urinary continence and erectile function at 6 months.

Materials and Methods

Study design

This retrospective observational study evaluated the relationship between intraoperative nerve-sparing quality and postoperative functional outcomes following robot-assisted radical prostatectomy.

The study was conducted at a high-volume tertiary referral center with an established robotic surgery program performing more than 600 robotic procedures annually, including a substantial number of robot-assisted radical prostatectomies. Robot-assisted radical prostatectomy has been routinely performed at our institution for several years and represents a standardized component of the surgical treatment of localized prostate cancer.

Intraoperative assessment

The assessment was performed through retrospective review of anonymised, recorded operative videos focusing on the nerve-sparing dissection phase and applying the predefined NSQ Score criteria.

Nerve-sparing quality was evaluated using the Nerve-Sparing Quality (NSQ) Score, a structured 10-point intraoperative scoring system based on five domains.

- continuity of dissection
- bleeding control
- dissection plane
- bundle manipulation
- symmetry

Each domain is scored from 0 to 2 points, resulting in a total score ranging from 0 to 10.

In bilateral NS, both sides were rated separately and averaged to obtain a composite score. In unilateral NS procedures, symmetry was always scored as 0, ensuring that the total score more accurately reflected the actual quality and extent of nerve preservation.

The scoring definitions for each parameter were standardized and are presented in Table 1.

Table 1. Nerve-Sparing Technique Scoring System.

Parameter	0 points	1 point	2 points
Continuity of dissection	Interrupted, chaotic	Moderate continuity	Smooth, uninterrupted
Intraoperative bleeding	Massive, obscuring field	Moderate, controlled	Minimal or none
Dissection plane	Outside anatomical layer	Partially correct	Fully anatomical
Bundle manipulation	Grasping, stretching and/or extensive monopolar or bipolar cauterization close to the bundle	Light touching/tension and/or limited or indirect cautery near the neurovascular bundle	None or very delicate manipulation, only pinpoint coagulation
Symmetry	Significant difference/unilateral	Minor differences	Both sides comparable

Functional outcome assessment

Functional outcomes were assessed at 6 months after surgery using data obtained from routine postoperative follow-up visits documented in the outpatient clinic medical records.

Two outcome measures were collected:

Urinary continence

Continence was evaluated using pad use per day, which represents a commonly used clinical measure of urinary continence following radical prostatectomy. [2]

Erectile function

Erectile function was evaluated using the International Index of Erectile Function-5 (IIEF-5) questionnaire, a validated instrument widely used for assessing erectile function in clinical studies. [13]

Statistical analysis

Statistical analysis was performed using standard exploratory methods appropriate for small sample size studies. Continuous variables were summarized as mean ± standard deviation (SD) as well as median with interquartile range (IQR), to provide a comprehensive description of data distribution.

Given the limited sample size and potential non-normal distribution of variables, non-parametric statistical methods were used throughout the analysis. Associations between the Nerve-Sparing Quality (NSQ) Score and functional outcomes were assessed using Spearman rank correlation coefficients. Correlation strength was interpreted as weak ($\rho < 0.3$), moderate ($\rho = 0.3–0.6$), or strong ($\rho > 0.6$).

Exploratory subgroup analyses were performed using a predefined NSQ Score threshold of ≥8. Between-group comparisons were conducted using the Mann–Whitney U test for continuous variables. Categorical variables were analyzed using Fisher's exact test, as appropriate.

In addition to correlation analysis, descriptive distribution analyses were performed, including frequency distribution of NSQ scores and continence status (0 vs ≥1 pad/day), to better characterize clinical outcomes.

All statistical tests were two-sided, and a p-value < 0.05 was considered statistically significant. Due to the exploratory nature of the study and the limited sample size, no adjustment for multiple comparisons was applied, and no multivariable analysis was performed.

Statistical analyses were conducted using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and verified using R software (R Foundation for Statistical Computing, Vienna, Austria).

Results

Twenty patients with available intraoperative NSQ Score assessment and complete 6-month functional follow-up were included in the analysis.

The baseline characteristics of the study cohort are presented in Table 2. The mean NSQ Score was 7.95 ± 1.61, with a median of 8 (IQR 7–9), indicating generally high-quality nerve-sparing dissection within the cohort.

At 6 months after surgery, the mean pad use was 0.75 ± 0.91 pads/day (median 0.5, IQR 0–1), while the mean IIEF-5 score was 18.05 ± 6.60 (median 21.5, IQR 13.5–23), reflecting variability in functional recovery.

Table 2. Baseline characteristics of the study cohort.

Variable	Mean ± SD	Median (IQR)	Range
NSQ Score	7.95 ± 1.61	8 (7–9)	5–10
Pad use (pads/day)	0.75 ± 0.91	0.5 (0–1)	0–3
IIEF-5 score	18.05 ± 6.60	21.5 (13.5–23)	6–25

Correlation analysis

Higher NSQ Score was significantly associated with improved functional outcomes. A moderate negative correlation was observed between NSQ Score and pad use (Spearman $\rho = -0.56$, $p = 0.011$), indicating that better nerve-sparing quality was associated with improved urinary continence. The correlation analysis is summarized in Table 3.

A strong positive correlation was found between NSQ Score and erectile function (Spearman $\rho = 0.87$, $p < 0.001$), suggesting a substantial relationship between intraoperative nerve preservation and postoperative erectile recovery.

Table 3. Correlation between NSQ Score and functional outcomes.

Variable	Spearman ρ	p-value
NSQ Score vs Pad use	−0.56	0.011
NSQ Score vs IIEF-5 score	0.87	<0.001

Subgroup analysis

Patients were stratified using an NSQ Score threshold of ≥8.

Patients with higher NSQ Scores demonstrated significantly better functional outcomes. Mean pad use was 0.38 ± 0.51 pads/day in the NSQ ≥8 group compared with 1.43 ± 1.13 pads/day in the NSQ <8 group ($p = 0.035$). Mean IIEF-5 score was 21.38 ± 4.46 in the NSQ ≥8 group versus 11.86 ± 5.40 in the NSQ <8 group ($p = 0.001$). Functional outcomes according to NSQ score threshold are presented in Table 4.

At 6 months, 10 patients (50%) achieved complete continence (0 pads/day), while 10 patients (50%) required at least one pad per day.

Table 4. Functional outcomes according to NSQ score threshold.

Outcome	NSQ <8 (n = 7)	NSQ ≥8 (n = 13)	p-value
Pad use (pads/day)	1.43 ± 1.13	0.38 ± 0.51	0.035
IIEF-5 score	11.86 ± 5.40	21.38 ± 4.46	0.001

Distribution analysis

The distribution of NSQ Scores demonstrated that procedures were generally performed with moderate-to-high technical quality, with 16 of 20 patients (80%) achieving an NSQ Score of 7 or higher. This reflects the overall distribution of technical performance, while a threshold of ≥8 was used for subgroup analysis.

Discussion

This study provides the first preliminary clinical evidence linking the Nerve-Sparing Quality (NSQ) Score with early functional outcomes following robot-assisted radical prostatectomy. The main finding is that higher NSQ Scores were associated with both lower pad use and improved erectile function at 6 months after surgery.

The observed relationship between NSQ Score and postoperative erectile function is biologically plausible. The score specifically evaluates technical aspects of nerve-sparing dissection that are directly related to preservation of the neurovascular bundles, including accurate identification of the dissection plane, minimal traction on the bundles, and continuity of dissection. These factors have previously been shown to influence postoperative erectile recovery. [14]

In recent years, increasing attention has been directed toward identifying objective metrics that can link surgical performance with patient outcomes in robotic surgery. Although global assessment tools such as GEARS or OSATS provide valuable information regarding overall surgical skill, they do not specifically capture the technical nuances of nerve-sparing dissection during prostatectomy. Procedure-specific tools such as the PACE score partially address this limitation; however, they still evaluate the overall performance of the procedure rather than focusing specifically on neurovascular bundle preservation. The NSQ Score was therefore designed to fill this gap by focusing exclusively on the key technical components of nerve-sparing dissection.

From a biological perspective, the strong correlation observed between NSQ Score and postoperative erectile function is consistent with the current understanding of neurovascular bundle preservation during radical prostatectomy. Damage to the cavernous nerves may occur through several mechanisms including traction injury, thermal injury from electrocautery, and disruption of the anatomical dissection plane. Each of these mechanisms is indirectly captured within the NSQ Score domains, including bundle manipulation, bleeding control, and dissection plane accuracy. Consequently, higher NSQ Scores may reflect a surgical technique that minimizes mechanical and thermal trauma to the neurovascular structures responsible for erectile function.

Another important implication of the present findings relates to the potential role of the NSQ Score in surgical training and quality assessment. Modern surgical education increasingly emphasizes objective assessment of procedural performance. In robotic surgery, structured assessment tools have been shown to facilitate skill acquisition and improve the standardization of surgical training. A focused intraoperative metric specifically evaluating nerve-sparing quality could therefore provide a useful educational tool, particularly during the learning phase of robotic prostatectomy.

In addition to its potential educational value, the NSQ Score may also contribute to quality monitoring in robotic prostatectomy programs. Variability in functional outcomes after RARP remains substantial even among experienced surgeons. Objective intraoperative metrics capable of capturing technical quality may therefore provide an additional layer of quality control and allow more precise investigation of the relationship between surgical technique and postoperative outcomes.

Finally, the concept of structured intraoperative assessment may have broader implications for surgical research. Many outcome studies in prostatectomy rely primarily on postoperative functional results without direct evaluation of intraoperative technique. Incorporating standardized technical metrics such as the NSQ Score could help bridge this gap and improve our understanding of how specific surgical maneuvers translate into patient outcomes.

The correlation with urinary continence was less pronounced but remained consistent in direction. Continence recovery after prostatectomy is multifactorial and may depend on additional surgical factors such as urethral length preservation, bladder neck reconstruction, and pelvic floor function. [15] Therefore, although nerve-sparing contributes to continence recovery, it represents only one component of the overall functional outcome.

Interestingly, several cases were observed in which patients achieved excellent continence outcomes despite poor postoperative erectile function, even in the presence of high NSQ Scores. Further follow-up revealed that some of these patients had impaired erectile function prior to surgery. This observation highlights the importance of baseline erectile status when interpreting postoperative outcomes and emphasizes that intraoperative technical quality alone cannot fully explain postoperative erectile recovery.

The present study should therefore be interpreted as an exploratory clinical correlation rather than a definitive validation of the NSQ Score. Nonetheless, the results provide an early signal suggesting that structured intraoperative evaluation of nerve-sparing quality may have clinical relevance. If confirmed in larger cohorts, the NSQ Score may represent a useful tool for surgical training, quality monitoring, and research on the relationship between surgical technique and patient outcomes.

Future studies should incorporate prospective data collection including preoperative erectile function, patient age, comorbidities, and oncological variables, enabling multivariable analyses and more robust validation of the NSQ Score as a surgical quality metric.

Study limitations

This study has several limitations that should be acknowledged. First, the sample size was relatively small and the analysis was conducted at a single institution, which limits the generalizability of the findings. The present study should therefore be considered exploratory and hypothesis-generating.

Second, important baseline patient characteristics were not available for analysis. In particular, preoperative erectile function assessed by IIEF-5 and patient age were not systematically collected. Both factors are well-established determinants of postoperative erectile recovery after radical prostatectomy and may confound the observed relationship between intraoperative nerve-sparing quality and postoperative functional outcomes.

Furthermore, pad use, although widely used as a clinical indicator of continence recovery, may not fully capture the complexity of postoperative urinary function.

Fourth, no multivariable analysis was performed due to the limited sample size and the absence of key baseline covariates. Consequently, the observed associations between NSQ Score and functional outcomes should be interpreted as preliminary correlations rather than evidence of an independent predictive relationship.

Finally, the present analysis focused on early functional outcomes at six months after surgery. Longer follow-up will be required to determine whether the observed associations persist over time.

Conclusions

Higher Nerve-Sparing Quality (NSQ) Score was significantly associated with improved early functional outcomes following robot-assisted radical prostatectomy, particularly in relation to erectile function and urinary continence at 6 months.

The strong correlation observed between NSQ Score and postoperative erectile function supports the clinical relevance of structured intraoperative assessment of nerve-sparing quality. These findings suggest that technical precision during neurovascular bundle dissection may play a critical role in functional recovery.

The NSQ Score may represent a promising tool for objective intraoperative evaluation, with potential applications in surgical training, quality assurance, and outcome prediction. By providing a standardized framework for assessing nerve-sparing technique, it may facilitate better understanding of the relationship between surgical performance and patient outcomes.

However, given the exploratory design and limited sample size, these results should be interpreted as hypothesis-generating. Further prospective studies incorporating baseline functional parameters and multivariable analyses are required to validate the independent predictive value of the NSQ Score.

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Author contributions

J.K. conceived the study, analyzed data, and drafted the manuscript. K.Ba., O.D., and K.Bu. contributed to data interpretation, manuscript revision, and final approval.

Conflict of interest

None declared.

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Current evidence of gut microbiota dysbiosis and its role in atrial fibrillation and cardiovascular diseases

Maria Szwarkowska[1,2], Kamil A. Sobieszek[1,2], Krzysztof Bartus[1,3], Jerzy Sadowski[2,3,4]

[1] Jagiellonian University Medical College, Faculty of Medicine, Krakow, Poland
[2] Foundation of Prof. J. Sadowski "HEART for KNOWLEDGE" Krakow, Poland
[3] Department of Cardiovascular Surgery and Transplantology, Jagiellonian University Medical College, Krakow Specialist Hospital Named After St. John Paul II, Krakow, Poland
[4] Academy of Applied Sciences in Nowy Sącz, Department of Cardiology and Cardiac Surgery, Nowy Sącz, Poland

Corresponding author: Prof. Jerzy Sadowski, M.D., Ph.D.
Department of Cardiovascular Surgery and Transplantology,
Jagiellonian University Medical College, Krakow Specialist Hospital Named After St. John Paul II
ul. Prądnicka 80, 31-202 Kraków, Poland
Phone: +48 504 299 302; E-mail: jerzysadowski3344@gmail.com

Abstract: The human gut microbiota has emerged recently as an important regulator of cardiovascular physiology through its effects on metabolism, immune signaling, and systemic inflammation. Increasing evidence suggests that alterations in intestinal microbial composition (dysbiosis) may contribute to the development and progression of atrial fibrillation (AF). This review summarizes current evidence on gut microbiota alterations, microbiota-derived metabolites, and their mechanistic links to atrial fibrillation pathogenesis and potential therapeutic strategies. Patients with AF demonstrate reduced microbial diversity and characteristic shifts in bacterial taxa, including depletion of short-chain fatty acid–producing bacteria and enrichment of potentially pro-inflammatory microorganisms. Gut microbiota–derived metabolites appear to play a key role in mediating these effects. Trimethylamine N-oxide (TMAO), lipopolysaccharide (LPS), and phenylacetylglutamine (PAGln) promote oxidative stress, endothelial dysfunction, and activation of inflammatory pathways such as NF-κB and the NLRP3 inflammasome, leading to atrial fibrosis, electrical remodeling, and increased arrhythmogenic susceptibility. In contrast, short-chain fatty acids exert anti-inflammatory and cardioprotective effects that may stabilize cardiac electrophysiology. Importantly, the relationship between gut microbiota and atrial fibrillation appears to be bidirectional, as cardiovascular disease itself may influence intestinal barrier integrity and microbial composition. Understanding the gut–heart axis may open new perspectives for risk stratification and therapeutic strategies, including dietary interventions and microbiome-targeted therapies. Further clinical studies are required to determine whether modulation of the gut microbiota can effectively prevent or modify the course of atrial fibrillation.

Keywords: atrial fibrillation, gut microbiota, gut–heart axis, dysbiosis, microbiota-derived metabolites, inflammation.

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Introduction

The human gut microbiota constitutes a complex microbial ecosystem that plays a fundamental role in host metabolism, immune regulation, and maintenance of intestinal barrier integrity. Increasing evidence indicates that intestinal microorganisms influence not only gastrointestinal physiology but also systemic processes, including inflammation, metabolic homeostasis, and cardiovascular function. [1, 2, 3] This concept has led to the emergence of the gut–heart axis, which describes potential bidirectional interactions between the intestinal microbiome and the cardiovascular system.

Dysbiosis, defined as a disruption of microbial composition characterized by reduced diversity and expansion of pro-inflammatory taxa, has been recently associated with cardiometabolic disorders such as hypertension, coronary artery disease, heart failure, and atrial fibrillation (AF). [1, 2, 3, 4] Several mechanisms have been proposed to explain this association, including systemic inflammation, oxidative stress, metabolic disturbances, and autonomic nervous system modulation. [1, 3, 4, 5, 6]

Atrial fibrillation, the most common sustained cardiac arrhythmia worldwide, remains a major cause of stroke, heart failure, and cardiovascular mortality. While classical mechanisms of AF pathogenesis include atrial fibrosis, electrical remodeling, and autonomic dysregulation, accumulating evidence suggests that systemic metabolic and inflammatory pathways may also contribute to arrhythmogenesis. In this context, alterations in gut microbiota composition have recently emerged as a potential contributor to AF development and its progression. [1, 3, 5]

Clinical studies have demonstrated distinct microbial signatures in patients with AF, including reduced microbial α-diversity and depletion of short-chain fatty acid (SCFA)–producing bacteria such as *Faecalibacterium*, *Alistipes*, and *Bifidobacterium*, accompanied by enrichment of potentially pathogenic taxa including *Ruminococcus*, *Streptococcus*, and *Enterococcus.* [1, 5, 7] These changes may promote intestinal barrier dysfunction and systemic translocation of microbial components, thereby triggering inflammatory signaling pathways involved in atrial structural and electrical tissue remodeling.

Microbiota-derived metabolites, including trimethylamine N-oxide (TMAO), lipopolysaccharide (LPS), and phenylacetylglutamine (PAGln), have been implicated in endothelial dysfunction, inflammation, and myocardial fibrosis, whereas short-chain fatty acids exert protective anti-inflammatory effects. [1, 2, 3] Despite growing interest in this field, the mechanistic links between gut microbiota alterations and cardiac arrhythmogenesis remain incompletely understood.

Therefore, this review summarizes current evidence regarding gut microbiota dysbiosis, microbiota-derived metabolites, and their potential role in the pathogenesis of atrial fibrillation.

Gut microbiota alterations in atrial fibrillation

Microbial diversity and global dysbiosis

The human gut microbiota is dominated by bacteria belonging primarily to the Firmicutes and Bacteroidetes phyla, which together account for the majority of microbial species in healthy individuals. A balanced microbial composition is essential for maintaining metabolic homeostasis, immune regulation, and intestinal barrier integrity. In contrast microbial imbalance has been increasingly associated with cardiometabolic diseases, including atrial fibrillation (AF). [1, 2, 3, 4]

Several clinical studies have demonstrated that patients with AF exhibit reduced gut microbial diversity, a hallmark feature of dysbiosis. Lower α-diversity is generally considered an indicator of impaired microbial ecosystem stability and has been associated with increased systemic inflammation and metabolic dysfunction. [1, 5, 7] In addition to reduced diversity, AF-related dysbiosis is characterized by alterations in the relative abundance of specific bacterial taxa and shifts in the overall microbial community structure.

One of the commonly reported features of dysbiosis in cardiovascular disease is an altered Firmicutes-to-Bacteroidetes (F/B) ratio, which has been associated with metabolic disturbances and systemic inflammatory responses. Although the exact significance of this ratio remains debated, changes in the F/B balance may reflect broader microbial ecosystem disruption contributing to cardiometabolic pathology. [6, 8]

Specific taxonomic changes in AF

Beyond global changes in microbial diversity, several studies have identified distinct microbial signatures associated with atrial fibrillation. These alterations include both depletion of beneficial commensal bacteria and enrichment of potentially pathogenic taxa.

In particular, AF has been associated with reduced abundance of short-chain fatty acid (SCFA)-producing bacteria, including genera such as *Faecalibacterium*, *Alistipes*, and Bifidobacterium. These microorganisms are key producers of short-chain fatty acids (SCFAs), which play an important role in maintaining intestinal and systemic homeostasis (Fig. 1). Their depletion may therefore contribute to increased intestinal permeability and systemic inflammatory activation. [1, 5, 7]

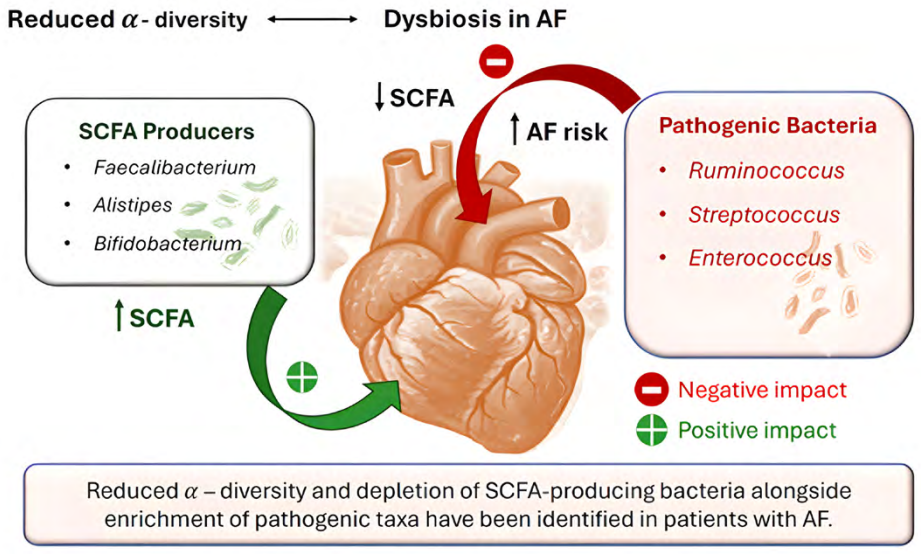

Fig. 1. Microbial signatures associated with atrial fibrillation. Schematic overview of gut microbiota alterations associated with atrial fibrillation, including reduced microbial diversity, depletion of SCFA-producing bacteria, and enrichment of potentially pathogenic taxa.

Conversely, enrichment of potentially pro-inflammatory or opportunistic taxa has been observed in patients with AF. These include genera such as *Ruminococcus*, *Streptococcus*, and *Enterococcus*, which have been associated with inflammatory signaling, altered host metabolism, and increased production of microbial metabolites involved in cardiovascular pathology (Fig. 1). [1, 5, 7]

Some studies have also reported increased abundance of trimethylamine (TMA)-producing bacteria, including *Prevotella* and *Bacteroides*. These taxa are of particular interest because TMA serves as a precursor of trimethylamine N-oxide (TMAO), a gut microbiota–derived metabolite implicated in cardiovascular disease and arrhythmogenesis. [6, 8]

Microbiota alterations and AF phenotype

Emerging evidence suggests that the degree of gut microbiota dysbiosis may correlate with clinical characteristics and duration of atrial fibrillation. For example, patients with persistent or long-standing AF appear to exhibit more pronounced alterations in gut microbial composition compared with individuals with paroxysmal AF. [6, 9]

This observation supports the hypothesis that sustained atrial remodeling and systemic inflammatory activation may interact with microbial dysbiosis in a self-reinforcing pathological cycle. Chronic AF may lead to hemodynamic disturbances, intestinal hypoperfusion, and congestion, which in turn can impair intestinal barrier function and promote microbial imbalance. [3, 9]

Additionally, gut microbiota composition in patients with AF may be influenced by several confounding factors frequently present in this population, including age, metabolic syndrome, obesity, diabetes, medication use, and dietary patterns. These factors may further complicate the interpretation of microbiome studies and highlight the need for carefully designed longitudinal investigations. [3, 6]

Clinical evidence linking gut dysbiosis and AF

Multiple observational studies have provided evidence supporting an association between gut microbiota alterations and atrial fibrillation. For example, population-based analyses have demonstrated that specific microbial genera are independently associated with incident AF, suggesting that gut microbiome composition may serve as a potential biomarker for arrhythmia risk. [2]

Furthermore, alterations in microbial metabolic profiles have been reported in patients with AF, including increased production of metabolites linked to inflammation, thrombosis, and myocardial remodeling. These findings support the concept that gut microbiota alterations may contribute not only to AF development but also to disease progression and recurrence after therapeutic interventions such as catheter ablation. [6, 10, 11]

Despite these consistent associations, most available evidence derives from observational human studies and experimental animal models, and a direct causal relationship between gut dysbiosis and atrial fibrillation has not yet been definitively established. Further prospective studies and mechanistic investigations are therefore required to clarify the clinical significance of gut microbiota alterations in AF pathophysiology.

Microbiota-derived metabolites in atrial fibrillation

Gut microbiota influences cardiovascular physiology not only through alterations in microbial composition but also through the production of bioactive metabolites that interact with host metabolic, inflammatory, and neurohumoral pathways. Increasing evidence suggests that these microbiota-derived metabolites play a central role in linking intestinal dysbiosis with cardiovascular diseases, including atrial fibrillation (AF). Several compounds generated by microbial metabolism have been implicated in arrhythmogenesis through their effects on inflammation, oxidative stress, endothelial function, autonomic regulation, and myocardial structural remodeling. [1, 2, 3, 6]

Among the most extensively studied metabolites associated with AF are trimethylamine N-oxide (TMAO), lipopolysaccharide (LPS), phenylacetylglutamine (PAGln), bile acids, and short-chain fatty acids (SCFAs). While some of these metabolites exert pro-inflammatory and proarrhythmic effects, others appear to play protective roles in maintaining cardiovascular homeostasis.

Trimethylamine N-oxide (TMAO)

Trimethylamine N-oxide (TMAO) is one of the most extensively investigated gut microbiota–derived metabolites in cardiovascular research. TMAO is generated through a two-step metabolic process in which dietary nutrients such as choline, phosphatidylcholine, and L-carnitine are metabolized by gut bacteria into trimethylamine (TMA), which is subsequently oxidized in the liver by flavin-containing monooxygenases to form TMAO. [1, 2, 12]

Elevated circulating TMAO levels have been associated with increased risk of cardiovascular events, including myocardial infarction, stroke, and atrial fibrillation. [1, 2, 11] Mechanistically, TMAO promotes systemic inflammation, oxidative stress, endothelial dysfunction, and myocardial fibrosis**,** processes that contribute to the formation of an arrhythmogenic substrate within the atria. [2, 6, 12]

Experimental studies suggest that TMAO may activate the NLRP3 inflammasome and the NF-κB signaling pathway, leading to increased production of pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). [4, 6, 10, 13, 14] In addition, TMAO has been shown to influence cardiac electrophysiology by promoting calcium-handling abnormalities, connexin remodeling, and atrial fibrosis, thereby increasing susceptibility to atrial arrhythmias. [5, 6, 10]

TMAO may also affect autonomic nervous system regulation. Experimental studies have demonstrated that TMAO can enhance sympathetic activity through stimulation of cardiac autonomic ganglia, potentially facilitating both supraventricular and ventricular arrhythmias. [6, 15]

Lipopolysaccharide (LPS)

Lipopolysaccharide (LPS) is a major structural component of the outer membrane of Gram-negative bacteria and represents a potent pro-inflammatory endotoxin. In conditions of intestinal dysbiosis, increased intestinal permeability often referred to as "leaky gut" may allow LPS to translocate from the intestinal lumen into systemic circulation. [3, 4, 16]

Circulating LPS activates Toll-like receptor 4 (TLR4) on immune cells and cardiomyocytes, triggering downstream signaling pathways involving MyD88 and NF-κB activation. [3, 10, 17, 18, 19, 20] These pathways stimulate the release of pro-inflammatory cytokines and promote

activation of the NLRP3 inflammasome, a key molecular complex involved in atrial inflammation and fibrosis. [4, 10, 16, 21, 22]

In the context of atrial fibrillation, LPS-induced inflammation may contribute to both structural and electrical remodeling of the atria. Experimental studies suggest that inflammatory signaling can alter calcium channel expression, shorten atrial effective refractory periods, and disrupt gap junction organization through changes in connexin proteins, including connexin 40 and connexin 43. [1, 3, 6, 10] These electrophysiological alterations promote conduction heterogeneity and facilitate the formation of re-entry circuits that sustain atrial fibrillation.

Phenylacetylglutamine (PAGln)

Phenylacetylglutamine (PAGln) is a gut microbiota–derived metabolite generated from the microbial metabolism of phenylalanine into phenylacetic acid, which is subsequently conjugated with glutamine in the host liver. [1, 23] Recent studies have identified PAGln as a metabolite with significant cardiovascular relevance.

PAGln has been shown to interact with adrenergic receptors, including α2A, α2B, and β2 receptors, thereby enhancing sympathetic signaling pathways involved in cardiovascular regulation. [24] Through this mechanism, PAGln may contribute to increased cardiac excitability and facilitate the initiation and maintenance of atrial fibrillation.

In addition to its electrophysiological effects, PAGln has been associated with platelet hyperreactivity and prothrombotic activity, linking gut microbial metabolism to thromboembolic complications in cardiovascular disease. [24, 25] Elevated circulating PAGln concentrations have been reported to predict major adverse cardiovascular events, including myocardial infarction and stroke. [24] These findings suggest that PAGln may represent an important metabolic mediator connecting gut microbiota with arrhythmia-related cardiovascular risk.

Bile acids

Bile acids represent another important group of metabolites influenced by gut microbiota activity. Primary bile acids synthesized in the liver undergo microbial transformation in the intestine, leading to the formation of secondary bile acids, which can exert systemic metabolic and signaling effects. [3, 5]

Alterations in bile acid metabolism have been reported in patients with atrial fibrillation. Certain primary bile acids, such as chenodeoxycholic acid (CDCA), may promote oxidative stress and inflammatory signaling through activation of NADPH oxidase and generation of reactive oxygen species (ROS). [3, 18] ROS production may subsequently trigger ATP release and activation of the NLRP3 inflammasome, contributing to atrial fibrosis and structural remodeling.

Some bile acid conjugates, including taurocholic acid (TCA), may also influence cardiac electrophysiology by modulating ion channel activity and intracellular calcium dynamics, potentially shortening the atrial effective refractory period and increasing susceptibility to arrhythmias. [3, 6, 18]

In contrast, certain secondary bile acids, such as ursodeoxycholic acid (UDCA), appear to exert protective effects by stabilizing cardiomyocyte membrane potential and reducing oxidative stress. [3, 18, 22] These findings highlight the complex and sometimes opposing effects of bile acid metabolism within the gut–heart axis.

Short-chain fatty acids (SCFAs)

Short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate**,** are produced through microbial fermentation of dietary fiber in the colon. These metabolites play an important role in maintaining intestinal barrier integrity, regulating immune responses, and modulating systemic metabolic processes (Fig. 2). [9, 10, 26]

SCFAs exert potent anti-inflammatory effects by activating G protein–coupled receptors such as GPR41 and GPR43 and by inhibiting histone deacetylases (HDAC), which promotes differentiation of regulatory T cells and suppresses pro-inflammatory cytokine production. [6, 20, 25, 26, 27] By strengthening intestinal barrier function, SCFAs may also reduce systemic exposure to endotoxins such as LPS.

In addition to their immunomodulatory effects, SCFAs may influence cardiac electrophysiology. Experimental studies suggest that SCFAs can stabilize cardiac conduction by preventing pathological remodeling of gap junction proteins and improving intracellular calcium handling. [10, 18, 25] These mechanisms may reduce atrial fibrosis and electrical instability, suggesting a potential protective role of SCFAs in atrial fibrillation (Fig. 2).

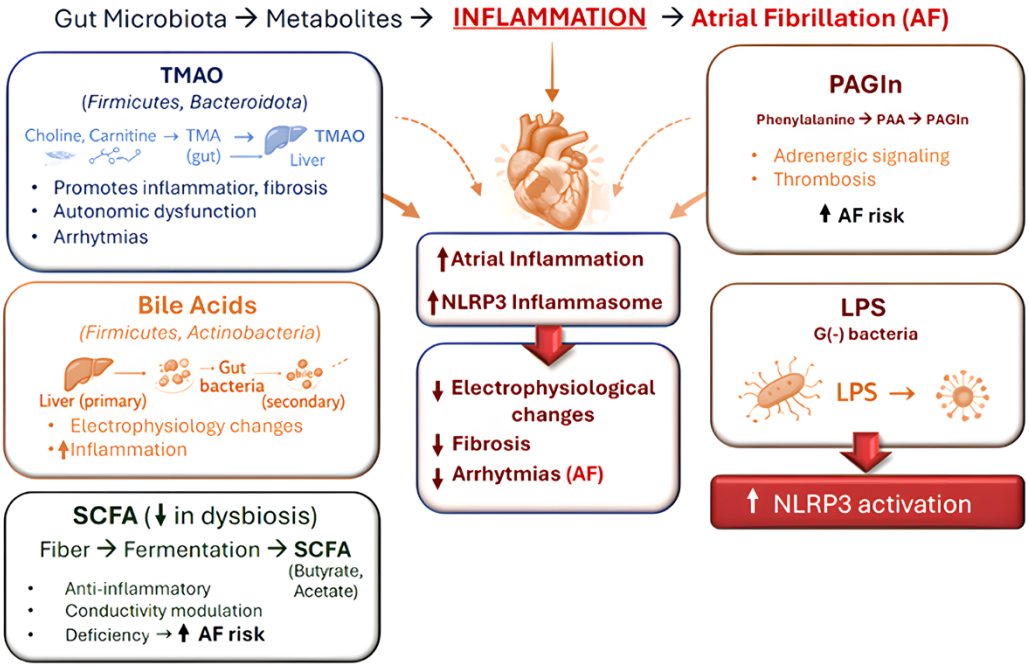

Fig. 2. Gut microbiota-derived metabolites and atrial fibrillation. This figure schematically presents the key gut microbiota-derived metabolites discussed in the manuscript (TMAO, secondary bile acids, SCFAs, PAGln, and LPS) and their proposed links to atrial fibrillation. The diagram illustrates the pathway from gut dysbiosis to systemic and atrial inflammation particularly via NLRP3 inflammasome activation leading to electrophysiological remodeling, fibrosis, and increased AF risk.

Summary of microbiota-derived metabolites

Collectively, microbiota-derived metabolites represent a key mechanistic link between gut dysbiosis and atrial fibrillation. Pro-inflammatory metabolites such as TMAO, LPS, and PAGln appear to promote atrial inflammation, fibrosis, and electrical remodeling, whereas metabolites such as SCFAs and certain secondary bile acids may exert protective cardiovascular effects. These findings highlight the complex role of microbial metabolism in arrhythmogenesis and suggest that targeting microbial metabolic pathways may represent a promising therapeutic strategy for atrial fibrillation.

Mechanisms linking gut dysbiosis and atrial fibrillation

The association between gut microbiota dysbiosis and atrial fibrillation (AF) is mediated by a complex network of metabolic, inflammatory, and neurohumoral mechanisms. Microbial imbalance may influence atrial electrophysiology and structural remodeling through the production of bioactive metabolites, activation of inflammatory pathways, modulation of autonomic nervous system activity, and disturbances in intracellular calcium handling. Together, these processes contribute to the development of an arrhythmogenic substrate that facilitates both the initiation and maintenance of atrial fibrillation. [1, 2, 3, 6]

Inflammation and immune activation

Chronic systemic inflammation represents one of the central mechanisms linking gut dysbiosis with atrial fibrillation. Disruption of intestinal barrier integrity may lead to increased translocation of microbial components such as lipopolysaccharide (LPS) into the systemic circulation. LPS activates Toll-like receptor 4 (TLR4) expressed on immune cells and cardiomyocytes, triggering downstream signaling pathways involving MyD88 and nuclear factor κB (NF-κB) activation. [3, 10, 17, 18, 19, 20]

Activation of these inflammatory pathways stimulates the production of pro-inflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). These mediators contribute to systemic endotoxemia and promote inflammatory remodeling of atrial tissue. [1, 3, 10, 20, 25] A key molecular mediator in this process is the NLRP3 inflammasome, which has been increasingly recognized as an important driver of atrial inflammation and arrhythmogenesis. [2, 3, 10, 16, 21, 22]

Activation of the NLRP3 inflammasome leads to increased production of inflammatory cytokines and may promote cardiomyocyte injury, fibroblast activation, and extracellular matrix deposition, all of which contribute to structural remodeling of the atria and increased susceptibility to atrial fibrillation.

Atrial fibrosis and structural remodeling

Structural remodeling of the atria, particularly the development of atrial fibrosis, is a key factor in the pathogenesis of atrial fibrillation. Several microbiota-derived metabolites, including TMAO and LPS, have been shown to promote pro-fibrotic signaling pathways.

Experimental studies suggest that TMAO can stimulate macrophage polarization toward a pro-inflammatory phenotype and activate molecular pathways such as MAPK and NF-κB, leading to increased cytokine production and inflammatory cell infiltration in atrial tissue. [4, 6, 10, 14] In addition, TMAO may trigger cardiomyocyte pyroptosis and release of damage-associated molecular patterns (DAMPs), which further stimulate fibroblast activation and differentiation into myofibroblasts. [10]

Activated myofibroblasts produce excessive extracellular matrix proteins through pathways including TGF-β1/Smad3 and Wnt/β-catenin signaling, resulting in progressive atrial fibrosis. [1, 25, 28, 29] Fibrotic remodeling disrupts the structural integrity of atrial tissue and creates regions of conduction heterogeneity that facilitate the formation of re-entry circuits sustaining atrial fibrillation.

Electrophysiological remodeling and calcium handling

Gut microbiota dysbiosis may also contribute to atrial fibrillation through disturbances in cardiac tissue electrophysiology and intracellular calcium homeostasis. Inflammatory signaling and microbial metabolites have been shown to affect ion channel expression and gap junction organization in cardiomyocytes.

For example, LPS-induced inflammatory signaling may reduce the expression of L-type calcium channels, leading to decreased calcium current and shortening of the atrial effective refractory period (ERP). [1, 3, 6, 15] Shortened ERP facilitates the formation of re-entry circuits, which represent the fundamental electrophysiological mechanism sustaining atrial fibrillation.

Inflammatory mediators may also disrupt the function and distribution of gap junction proteins, including connexin 40 and connexin 43. Abnormal lateralization of connexins reduces conduction velocity and increases electrical heterogeneity within atrial tissue, further promoting arrhythmogenesis. [1, 10]

In addition, microbiota-derived metabolites such as TMAO and PAGln may influence intracellular calcium regulation by modulating CaMKII kinase activity and ryanodine receptor (RyR2) function, leading to abnormal calcium release from the sarcoplasmic reticulum and the generation of triggered electrical activity. [10]

Autonomic nervous system modulation

The autonomic nervous system plays an important role in the initiation and maintenance of atrial fibrillation. Emerging evidence suggests that gut microbiota may influence autonomic regulation through both neural and metabolic pathways. [15, 18, 24]

Microbiota-derived metabolites such as TMAO and PAGln have been shown to interact with adrenergic signaling pathways, enhancing sympathetic nervous system activity and increasing cardiac excitability. [5, 15, 24] Experimental studies indicate that TMAO may stimulate autonomic ganglia, including the left stellate ganglion and ganglionated plexuses, thereby promoting sympathetic overactivation.

Increased sympathetic tone may shorten atrial refractoriness, enhance triggered activity, and facilitate the initiation of atrial fibrillation. Conversely, beneficial microbial metabolites such as short-chain fatty acids (SCFAs) may exert protective effects by reducing systemic inflammation and modulating autonomic balance. [18, 25]

Integrated pathophysiological model

Taken together, gut microbiota dysbiosis contributes to atrial fibrillation through several interconnected mechanisms, including systemic inflammation, atrial tissue fibrosis, electrophysiological remodeling, and autonomic nervous system dysregulation. These processes interact to create a proarrhythmic substrate characterized by structural and electrical instability of atrial myocardium.

Understanding these mechanistic pathways provides important insight into the emerging concept of the gut–heart axis and suggests that modulation of gut microbiota and its metabolites may represent a promising strategy for the prevention and treatment of atrial fibrillation.

Table 1. Overview of gut microbiota-derived mechanisms in the pathogenesis of cardiac arrhythmias.

Arrhythmia Type	Microbiota-Related Mechanisms	Key Metabolites/Pathways	Evidence/Notes
Atrial Fibrillation (AF)	Structural and electrical remodeling; atrial fibrosis; NLRP3 inflammasome activation	**TMAO, PAGln, LPS** (Pro-arrhythmic); **SCFAs** (Protective)	Strong correlation with gut dysbiosis; TMAO predicts AF recurrence after ablation [6, 10, 11, 30, 31]
Ventricular Arrhythmias	Ca^{2+} handling instability; Cx43 lateralization; autonomic nervous system (ANS) modulation	**Propionate (SCFA)**, Phenylacetylglutamine (**PAGln**)	PAGln enhances adrenergic signaling; SCFAs stabilize electrical conduction via GPR41/43 [15, 18]
Bradyarrhythmias	Vagus nerve overstimulation; metabolic interference with pacemaker cells	**Bile Acids**, **TMAO**	Secondary bile acids can modulate sinoatrial node (SAN) function and heart rate. [3, 18]

Bidirectional relationship between atrial fibrillation and gut dysbiosis

Increasing evidence suggests that the relationship between gut microbiota dysbiosis and atrial fibrillation (AF) could be bidirectional. While alterations in gut microbial composition and microbial metabolite production may contribute to the development and progression of atrial fibrillation, the presence of AF and its associated cardiovascular conditions may in turn influence intestinal physiology and microbiota composition. [1, 3, 9]

On the one hand, gut dysbiosis may promote arrhythmogenesis through several interconnected mechanisms. Changes in microbial composition may increase the production of pro-inflammatory metabolites such as trimethylamine N-oxide (TMAO), lipopolysaccharide (LPS), and phenylacetylglutamine (PAGln), which have been implicated in systemic inflammation, oxidative stress, endothelial dysfunction, and myocardial structural remodeling. [1, 2, 3, 6, 32] These processes may contribute to atrial fibrosis, electrophysiological instability, and autonomic nervous system imbalance, thereby facilitating the initiation and maintenance of atrial fibrillation.

On the other hand, atrial fibrillation and its associated comorbidities that may themselves influence gut microbiota composition. Cardiovascular conditions frequently accompanying AF, including heart failure, hypertension, metabolic syndrome, and diabetes, are known to affect

intestinal perfusion, intestinal barrier function, and immune responses. [3, 9] Hemodynamic disturbances, reduced intestinal blood flow, and venous congestion may impair intestinal barrier integrity, promoting microbial translocation and dysbiosis.

In addition, several clinical factors common in patients with atrial fibrillation may further modify the gut microbiome. These include advanced age, polypharmacy, dietary patterns, and the use of medications such as antibiotics, proton pump inhibitors, and cardiovascular drugs. Such factors may alter microbial diversity and metabolic activity, potentially amplifying inflammatory and metabolic disturbances linked to cardiovascular disease.

Importantly, observational studies have suggested that the degree of gut microbiota dysbiosis may correlate with the severity and duration of atrial fibrillation. For example, patients with persistent or long-standing AF have been reported to exhibit more pronounced alterations in microbial composition and metabolic profiles compared with individuals with paroxysmal AF. [6, 9] This observation supports the hypothesis that chronic atrial remodeling and systemic inflammation may interact with microbial imbalance in a self-reinforcing pathological cycle.

Despite growing evidence supporting this bidirectional interaction, most currently available data are derived from observational human studies and experimental animal models. Therefore, while the association between gut dysbiosis and atrial fibrillation appears consistent, a definitive causal relationship has not yet been fully established. [8, 9] Further longitudinal studies and interventional trials are required to clarify whether modulation of the gut microbiota can influence the incidence, progression, or recurrence of atrial fibrillation.

Therapeutic implications and microbiome-targeted strategies in atrial fibrillation

The growing understanding of the gut–heart axis has generated increasing interest in therapeutic strategies aimed at modulating gut microbiota composition and microbial metabolism. Although the role of microbiome-targeted interventions in atrial fibrillation (AF) remains an emerging field, several approaches have been proposed that may influence arrhythmia risk through anti-inflammatory, metabolic, and electrophysiological mechanisms. [3, 22, 33]

These strategies primarily include dietary interventions, probiotics and prebiotics, fecal microbiota transplantation (FMT), and pharmacological modulation of microbiota-derived metabolites**.**

Dietary interventions

Diet is one of the most important determinants of gut microbiota composition and metabolic activity. Diets rich in dietary fiber, such as the Mediterranean diet, promote the growth of beneficial bacteria that produce short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate. [22, 33]

SCFAs exert multiple cardioprotective effects, including reduction of systemic inflammation, improvement of endothelial function, and stabilization of intestinal barrier integrity. These mechanisms may reduce atrial structural remodeling and inflammatory activation associated with AF. [3, 22, 33, 34] In addition, high-fiber diets have been associated with improvements in several AF risk factors, including hypertension, obesity, and metabolic dysfunction.

Some dietary compounds may also directly influence microbial metabolite production. For example, 3,3-dimethyl-1-butanol (DMB), a compound naturally present in extra virgin olive oil and

balsamic vinegar, has been shown to inhibit microbial trimethylamine (TMA) production, thereby reducing circulating levels of trimethylamine N-oxide (TMAO) in experimental models. [26, 28, 29]

Probiotics and prebiotics

Probiotics and prebiotics represent another potential strategy for modulating gut microbiota composition. Probiotics, defined as live microorganisms that confer health benefits to the host, may improve intestinal barrier function and reduce systemic inflammation.

Supplementation with bacterial strains such as Lactobacillus and Bifidobacterium has been shown to strengthen intestinal barrier integrity and limit translocation of endotoxins such as lipopolysaccharide (LPS) into systemic circulation. [3, 15] By reducing systemic inflammatory burden, probiotics may indirectly influence cardiovascular risk factors associated with atrial fibrillation, including obesity, hypertension, and atherosclerosis. [1, 35]

Prebiotics, which are non-digestible dietary substrates that selectively promote the growth of beneficial bacteria, may increase the abundance of SCFA-producing microorganisms. Although clinical data specifically addressing AF remain limited, prebiotics have been shown to improve metabolic parameters and blood pressure regulation, suggesting a potential indirect benefit for arrhythmia prevention. [3, 34]

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) represents a more direct method of restoring a healthy microbial ecosystem by transferring intestinal microbiota from a healthy donor to a recipient. Although primarily used for the treatment of recurrent *Clostridioides difficile* infection, FMT has recently gained attention as a therapeutic approach in cardiometabolic diseases. [3, 10, 18, 20, 36, 37, 38, 39]

Experimental studies have demonstrated that transplantation of microbiota from healthy donors to animal models with atrial fibrillation may reduce atrial fibrosis, decrease NLRP3 inflammasome activation, and improve cardiac electrophysiological stability. [3, 15, 33, 34, 36, 37, 38, 39] These findings suggest that restoration of microbial diversity may mitigate inflammatory and structural mechanisms involved in AF pathogenesis.

However, clinical evidence in humans remains limited, and further studies are required to determine the safety, feasibility, and therapeutic efficacy of FMT in cardiovascular disease.

Targeting microbiota-derived metabolites

Another emerging therapeutic strategy involves targeting specific microbial metabolic pathways implicated in cardiovascular pathology. Among these, inhibition of trimethylamine (TMA) production represents one of the most actively investigated approaches.

As mentioned previously, compounds such as 3,3-dimethyl-1-butanol (DMB) can inhibit microbial enzymes responsible for TMA formation, thereby reducing circulating TMAO levels and attenuating inflammatory and fibrotic remodeling in experimental cardiovascular models. [28, 29, 40]

Additionally, omega-3 fatty acids have been suggested to influence gut microbiota composition and increase SCFA production while reducing pro-inflammatory microbial metabolites. These effects may contribute to improved endothelial function and reduced arrhythmia susceptibility. [41, 42]

Although these approaches remain largely experimental, targeting microbial metabolites may represent a promising strategy for precision medicine approaches in cardiovascular disease, particularly in conditions characterized by systemic inflammatory and metabolic dysregulation such as atrial fibrillation.

Clinical perspectives

Despite promising experimental findings, microbiome-targeted therapies for atrial fibrillation remain in an early stage of development. Most available evidence derives from preclinical studies or observational clinical data. [3, 5, 18, 20, 21, 23, 36] Large randomized blinded clinical trials are therefore required to determine whether interventions targeting gut microbiota composition or microbial metabolism can effectively reduce AF incidence, progression, or recurrence.

Nevertheless, growing insights into the gut–heart axis suggest that modulation of intestinal microbiota may represent a novel complementary approach in the prevention and management of atrial fibrillation.

Table 2. Potential preventive strategies for atrial fibrillation, as the most extensively studied cardiac arrhythmia linked to the gut microbiota
SCFA — short-chain fatty acids; TMAO — trimethylamine N-oxide; FMT — faecal microbiota transplantation; AF — atrial fibrillation.

Approach / Intervention	Mechanism / Goal	Evidence / Status	Sources
High-fiber / Mediterranean diet	↑ SCFAs, ↓ TMAO, anti-inflammatory effect	Reduction of AF risk factors (hypertension, dyslipidemia, inflammation); animal and human observational data	[33, 43]
Prebiotics	↑ SCFA-producing bacteria	Moderate effects on blood pressure and insulin; limited AF-specific data	[3, 34]
Probiotics	Microbiota modulation, ↓ inflammation	Effective in reducing risk factors; preclinical data for AF	[1]
FMT (Fecal Microbiota Transplantation)	Restoration of healthy microbiota	Animal models: AF reduction; human data are only beginning to emerge	[34, 36, 37]
TMAO Inhibitors (DMB, IMC)	↓ TMAO, ↓ inflammation, ↓ fibrosis	Animal models: AF reduction; lack of large-scale clinical trials	[29, 40]
SCFA Supplementation	Anti-inflammatory and anti-fibrotic effects	AF reduction in animal models; limited human data	[33]

Limitations and future directions

Despite the growing knowledge linking gut microbiota dysbiosis with atrial fibrillation, several important limitations remain. First, the majority of currently available data originate from observational clinical studies and experimental animal models, which limits the ability to establish a clear causal relationship between gut microbiota alterations and atrial fibrillation development. [3, 6, 9]

Although associations between microbial composition, microbiota-derived metabolites, and AF have been consistently reported, the directionality and clinical significance of these interactions remain incompletely understood.

Another important limitation relates to the complexity and variability of the human gut microbiome. Microbiota composition is influenced by numerous factors, including age, diet, geographical location, medication use, and comorbidities such as obesity, diabetes, and cardiovascular disease. These confounding variables may significantly affect microbial profiles and complicate the interpretation of microbiome studies. [3, 6]

Furthermore, many studies investigating gut microbiota in AF involve relatively small sample sizes and heterogeneous patient populations, which may limit the generalizability of the findings. Differences in microbiome sequencing methods, bioinformatic analyses, and study designs may also contribute to variability across studies.

Future research should focus on large-scale longitudinal studies that can better define the temporal relationship between gut microbiota alterations and atrial fibrillation onset. In addition, randomized clinical trials are required to evaluate whether interventions targeting gut microbiota composition or microbial metabolic pathways can effectively prevent AF development or reduce arrhythmia recurrence after therapeutic interventions such as catheter ablation.

Advances in multi-omics approaches, including metagenomics, metabolomics, and transcriptomics, may further improve our understanding of the complex interactions between the gut microbiome and cardiovascular physiology. Such integrative strategies may facilitate the identification of novel biomarkers and therapeutic targets within the gut–heart axis.

Conclusions

Accumulating evidence indicates that gut microbiota dysbiosis plays an important role in the pathophysiology of atrial fibrillation through complex metabolic, inflammatory, and neurohumoral mechanisms. Microbiota-derived metabolites such as TMAO, LPS, and PAGln may promote atrial tissue inflammation, fibrosis, and electrophysiological remodeling, thereby contributing to the development of an arrhythmogenic substrate. In contrast, beneficial metabolites including SCFAs may exert protective effects by reducing systemic inflammation and stabilizing cardiac electrophysiology.

The concept of the gut–heart axis highlights the potential importance of intestinal microbiota as a novel factor influencing cardiovascular health and arrhythmia susceptibility. Although current findings strongly suggest a link between gut dysbiosis and atrial fibrillation, most available data are derived from observational studies and experimental models. Further large-scale longitudinal studies and blinded randomized clinical trials are required to clarify causal relationships and determine whether microbiome-targeted interventions may become effective strategies for the prevention and management of atrial fibrillation.

Authors' contributions

Conceptualization, M.S.; Methodology, M.S., K.A.S.; Validation, K.B., J.S.; Investigation, M.S., K.A.S., K.B. and J.S.; Writing — original draft, M.S., K.A.S, Writing — review & editing, M.S., K.A.S., K.B. and J.S.; Visualization, M.S. and K.A.S.; Supervision, K.B. and J.S.

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The original contributions presented in this manuscript are included in the references section. Further inquiries can be directed to the corresponding author.

Conflict of interest

The authors declare no conflict of interest.

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The role of the dentist in the diagnosis and early detection of neoplastic lesions based on the example of Proliferative verrucous leukoplakia, classified as an OPMD

Magdalena Stawarz-Janeczek[1], Katarzyna Łazarz-Bartyzel[2], Andrzej Kiencało[3], Jolanta Pytko-Polończyk[1]

[1] Department of Integrated Dentistry, Jagiellonian University Medical College, Krakow, Poland
[2] Department of Periodontology, Prophylaxis and Oral Medicine, Jagiellonian University, Krakow, Poland
[3] Central Dental Outpatient Clinic, University Dental Clinic in Krakow, Poland

Corresponding author: Prof. Jolanta Pytko-Polończyk, D.M.D., Ph.D.
Department of Integrated Dentistry, Jagiellonian University Medical College
ul. Montelupich 4, 31-155 Kraków, Poland
Phone: +48 12 424 54 65; E-mail: jolanta.pytko-polonczyk@uj.edu.pl

Abstract: Proliferative verrucous leukoplakia (PVL) was first described by Hansen, Olson, and Silverman in 1985 as a form of hyperkeratosis, remained resistant to treatment and was further distinguished by the aggressive nature of the lesions and a high rate of malignant transformation (MT). The OPMDs classification developed in 2020 recognized PVL as a distinct form of multifocal oral leukoplakia, characterized by a progressive clinical course and with changing clinical and histopathological features. According to WHO, the risk of malignant transformation of PVL among OPMDs is approximately 50%. This frequent MT of PVL involves transformation into VC or OSCC, which, according to various sources in the literature, occurs in between 43.87% and 65.8% of cases, and according to some in up to 70% of cases. Some reports even suggest a possible malignant transformation rate of around 100%.
PVL usually occurs in multiple areas of the oral cavity simultaneously and is characterized by slow but continuous progression. The aetiology of PVL and the factors influencing its progression to cancer are unknown. Close monitoring of patients is recommended and, if necessary, biopsies should be performed to assess changes in the appearance, colour, and size of observed lesions, as well as the appearance of subsequent lesions. Early diagnosis, surgical removal, and patient observation are crucial in the treatment of PVL.

Keywords: oral potentially malignant disorders (OPMDs), proliferative verrucous leukoplakia (PVL), malignant transformation (MT), squamous cell carcinoma (OSCC), verrucous carcinoma (VC).

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Introduction

Head and neck cancers (HNCs) are the seventh most common cancer globally. [1] They are diagnosed more often in men than in women, usually around the age of 60, with the incidence of the disease increasing markedly after the age of 45. [2] Almost half of all HNCs are oral cancers, [3] the most frequent form of which is oral squamous cell carcinoma [4] (OSCC), which is the 11th most widespread cancer worldwide, with a 5-year survival rate of 50% to 60%. [5] According to data presented by the Global Cancer Observatory (GCO), in 2022 the global incidence of OSCC exceeded 389,000 cases. [6] It is estimated that in more than half of all cases, the development of OSCC is preceded by oral potentially malignant disorders [5] (OPMDs), [7] which include, among others, proliferative verrucous leukoplakia (PVL). [8, 9]

In their practice, dentists encounter lesions classified as OPMDs. The most common of these is leukoplakia, [8] while PVL is characterized by a high rate of recurrence and malignant transformation [5] it also includes the presence of multiple leukoplakias with variable clinical features. [10]

One important issue that should be considered in connection with PVL is possibility of diagnostic error. [8] In view of this fact, it is worth discussing this topic, reminding dentists of this problem and raising awareness of the challenges related to OPMDs, and PVL in particular, that dentists may encounter in their daily work. This condition can progress unnoticed by the patient, and routine dental check-ups can often detect any disturbing changes. [11] Furthermore, due to the high risk of malignant transformation, early diagnosis is crucial. [12]

Definition and place among OPMDs

PVL was first described by Hansen, Olson, and Silverman in 1985 [13, 14, 15, 16] as a form of hyperkeratosis based on an analysis of 30 cases of patients affected by this condition. A follow-up of patients showed that initially simple hyperkeratosis progressed to multifocal lesions, with erythematous components often observed in the examined tissues. Over time, the lesions became exophytic and verrucous. In some cases, the development of verrucous carcinoma (VC) or OSCC is observed. Hyperkeratosis, described by Hansen *et al.* as PVL, remained resistant to treatment [14] and was further distinguished by the aggressive nature of the lesions [17] and a high rate of malignant transformation (MT). [13, 14]

In 2005, the World Health Organization (WHO) Collaborating Centre for Oral Cancer organized a workshop in the UK. [18] It was during this meeting that a working group of experts first introduced the term potentially malignant disorders (PMDs), [18] nowadays referred to as OPMDs, [8] to define lesions and conditions in the oral cavity with a risk of malignant transformation into cancer. [18] This does not mean that all such lesions will develop into cancer, only that they have an elevated risk of MT. [5] Among other things, the working group came up with a revised definition of oral leukoplakia (OL). Henceforth, WHO describes leukoplakia as any white plaque of questionable risk after first excluding (other) known diseases or disorders characterised by no increased risk of cancer. [5, 18] The working group also introduced the term erythroleukoplakia, which is described as a lesion with a mixed white and red surface, [8, 18, 19] and divided leukoplakias into homogeneous and non-homogeneous forms. [8, 18] PVL was (according to the 2005 WHO report) [8, 18, 19] classified as a subtype of non-homogeneous leukoplakia, characterized by the simultaneous occurrence of multiple multifocal leukoplakias. [8, 18] Warnakulasuriya emphasizes that PVL-type lesions fit perfectly into the terminology of PMDs, [18] currently termed OPMDs. [8]

The above-mentioned classification of OPMDs, developed in 2005, was published two years later, [9] and the nomenclature and classification were updated during subsequent workshops held in 2020. [20] Most of the above-described conditions retained their current status, [8] including leukoplakia, which is the most common condition among OPMDs. [8] PVL was recognized as a distinct form of multifocal oral leukoplakia, characterized by a progressive clinical course and with changing clinical and histopathological features. [8, 20]

PVL had also previously been described as a form of oral papillomatosis, although it does not always have a verrucous appearance. [21] In 2011, Jose M. Aguirre-Urizar proposed the term [22] proliferative multifocal leukoplakia [21, 22] for PVL, emphasizing the hyperplastic and multifocal nature of this condition. [22] Researchers based their theory on the belief that multifocality is a more important factor than the verrucous appearance of a lesion. [11] In a 2018 publication, Villa *et al.* described a study of 42 patients diagnosed with PVL, in whom clinical differentiation of the examined lesions had been confirmed. Villa *et al.* [13] proposed the name "proliferative leukoplakia" due to the fact that the condition does not always have a verrucous or nodular form. [13, 23]

Risk of malignant transformation

The risk of malignant transformation among OPMDs is estimated at 7.9%, [12, 18, 24] and PVL [8, 25, 26, 27] not only has the highest degree of malignant transformation, [5, 8, 9, 10, 14, 19, 26, 27] but also by the highest potential for recurrence [8, 9, 19, 26, 27] as a markedly aggressive form of the disease. [10, 23] According to WHO, the risk of malignant transformation of PVL among OPMDs is approximately 50%. [20] This frequent MT of PVL involves transformation into VC or OSCC, [16, 28] which, according to various sources in the literature, occurs in between 43.87% and 65.8% [27] of cases, and according to some in up to 70% of cases. [8, 28] Some reports even suggest a possible malignant transformation rate of around 100%. [10]

In their 2021 meta-analysis, González-Moles *et al.* showed that the majority of patients, i.e. 72.21%, developed OSCC, while 33.66% of patients with PVL later went on to be diagnosed with VC. [20]

In their retrospective clinicopathological analysis of PVL, non-dysplasia leukoplakia (LK), oral lichen planus (OLP)/oral lichenoid lesions (OLL), and VC, Alkan *et al.* (2022) demonstrated that in all the studied cases, malignancy developed on the basis of PVL, most frequently in the form of squamous cell carcinoma. In addition to a high rate of malignant transformation, the authors also noted the aggressive nature of the studied lesions. [10] In a systematic review conducted by Ramos-Garcia *et al.* in 2020, the rate of MT in cases of PVL was estimated at 43.87%. [9] On the other hand, based on a systematic review from 2021 Palaya *et al.* demonstrated that the risk of malignant transformation developing from PVL, most often in the form of OSCC, is approximately 46.5% [19, 21] and thus in almost half of all cases. [19] Previous publications had reported malignant transformation in over 70% of all cases of PVL. [17] MT rates of as high as 100% have even been claimed (Zakrzewska *et al.* 1996). [29, 30] A 2024 review by Vergier *et al.* focusing on the gingival form of PVL, reported that the malignant transformation rate of the disease was 47.75%, with a patient mortality rate of 5.84%. The mean follow-up time before malignant transformation was 3 years. [31] A 2025 review by Mohideen *et al.* demonstrated a 48.4% malignant transformation rate. The probability of progression of PVL lesions to malignancy was statistically higher in women than in men, i.e. 1.96 times higher. [21] Similar conclusions were reached by Palaya *et al.* in 2021, confirming that exposure to MT in women with PVL was 1.7 times higher compared to men. [19]

As Mohideen *et al.* reported in their 2025 publication, gingival lesions of PVL have a tendency to transform into OSCC in over 50% of cases. [21] The same researchers demonstrated that patients with PVL may develop OSCC more frequently than VC. [21] Similar conclusions were drawn by Gonzales and Moles based on a meta-analysis conducted in 2021, in which the majority of patients, i.e. 72.21%, went on to develop OSCC, while VC affected a smaller number, i.e. 33.66%. [20]

The risk of malignant transformation among OPMDs is also most often associated with the development of OSCC. [8, 12, 18, 24] Patients diagnosed with OSCC additionally face a greater risk of developing another cancer of this kind. [16] This was confirmed by Faustino *et al*. in their 2023 review, in which they emphasized that OSCC arising from PVL often results not only in the recurrence of this cancer, but also in the possibility of new, primary tumours developing. [32] In their 2021 systematic review, Proaño-Haro *et al.* showed a recurrence rate of 67.27% in patients treated for PVL. [23] In contrast, in 2025 Mohideen *et al.* noted a 55.56% recurrence rate in patients diagnosed with cancer arising from PVL. [21]

Clinical picture, diagnostic difficulties

In the initial clinical picture, flat [13, 33] single lesions or several white lesions [13] can be observed. [13, 33] Over time, as the lesion spreads or several adjacent lesions merge, lesions can appear in other locations. [8] As a consequence, over time, the initial lesion may not only enlarge, but new lesions may develop. [28] This type of disease is characterized not only by a tendency for new lesions to develop but also for them to grow and recur after treatment (proliferative). In addition, the surface of the lesion can transform from flat to rough and warty. [8, 10, 16] Additionally, the appearance of red spots, warty surface, erosions, paresthesia, or pain are features suggesting possible malignancy of the lesion. [10, 28] The initial clinical picture may correspond to lichen planus or leukoplakia. [26] PVL is characterized not only by the progressive spread of lesions, [5, 10] but also by subsequent foci appearing over time, [10] and thus by the multifocal nature of this disease. [5, 10, 11]

Over time, the above-described flat lesion gradually spreads, becoming diffuse and exophytic. [5] However, in its early stages it does not always have a verrucous appearance, [11, 33] which obviously complicates the diagnosis. [11] Diagnostic difficulties are likewise encountered when the lesions take on a lichenoid-like form. In 2020, Biancardi *et al.* described the case of three patients treated for lichenoid lesions in the oral cavity confirmed by a microscopic diagnosis of lichen planus. A final diagnosis of PVL was only made several years later. As a consequence, problems may arise when the clinical symptoms are similar to lichen planus, resulting in an incorrect diagnosis and, consequently, ineffective treatment. [34] PVL is characterized by its progression and irreversibility. [8]

Various clinical stages in the course of PVL can be distinguished. These include the initial occurrence of one or more white patches on the oral mucosa, which subsequently grow and spread to other sites. This in turn is followed by a change in the surface of some or all leukoplakia, assuming a more verrucous appearance. Finally, malignant transformation occurs, either in the form of VC or OSCC. [14, 35] The aggressive clinical course of PVL had already been pointed out by Hansen in 1985. [14, 17]

Not only may the physician examining the patient have problems diagnosing PVL, but so too can the pathologist. [8] Aguirre-Urizar *et al.* (2011) also noted the frequent underdiagnosis of PVL. [22] The condition may progress unnoticed by the patient, as a consequence of which frequent dental check-ups are necessary to detect alarming changes. [11]

PVL is characterized by the presence of multiple leukoplakia with variable clinical and histopathological features. [8] Microscopic findings in PVL may include hyperplasia, [10] hyperkeratosis, verrucous hyperplasia, verrucous carcinoma, or squamous cell carcinoma. [7, 10]

Occurrence and location

PVL usually occurs in multiple areas of the oral cavity simultaneously and is characterized by slow but continuous progression. [18, 36] It most often affects the gingiva, [8, 11, 37] the alveolar mucosa, [11, 31] as well as the tongue and buccal mucosa. [11] The most common location for PVL is within the gingiva, as was confirmed by a meta-analysis performed by Torrejon-Moya *et al.* from 2020, which showed that 50.9% of all PVL cases were identified in this area. [37] Similarly, in their 2021 review Ramos-García *et al.* confirmed the occurrence of PVL within the gingiva in 39.6% of all cases and within the buccal mucosa in another 21.6%. [9] The gingiva is also the most common location of MT, accounting for 39.9% of all incidences of the disease. [9] In 2025 Mohideen *et al.* showed that PVL changes, in the MT group most frequently affected the gums (in approximately 20% of all cases), followed by the gums and buccal mucosa (17.4%), the tongue (9%), and the buccal mucosa (8.4%). [21]

Prevalence, epidemiology, etiology, risk factors

PVL usually affects older people. [19] In their systematic review Torrejon *et al*. found the mean age of patients to be over 60 years. [37] According Palaia *et al.* in 2021, PVL affects older women to a greater extent. [19] This was confirmed by, among others, Ramos-Garcia *et al.* in their 2021 systematic review, in which 64.02% of all cases of PVL were diagnosed in women and 35.98% in men. [9] In 2022 de Pauli Paglioni *et al.* reported a higher risk of MT associated with PVL in women. [5] However, in 2025 Mohideen *et al.* identified a higher incidence of PVL in women than in men, with women accounting for 66.22% of all cases and men for 33.78%. Similarly, malignant transformation mainly affected women, who accounted for 74.3% of all cases, compared with 25.7% for men. [21]

PVL is a rare oral disease, [10, 38] an uncommon form of leukoplakia (OL), [28, 39] which, unlike PVL, is considered one of the most frequently occurring OPMDs, [5] with a prevalence of approximately 1–3%. [24, 40] OPMDs affect 2% of the world's population, [12, 18, 24] and the incidence of malignant transformation among them is 7.9%. [12] The aetiology of PVL is unknown, and it has no prognostic biomarkers in clinical practice. [38] This has been confirmed by a study conducted in 2025 (Vergier *et al.*), which showed no risk of increased MT gingival PVL in smokers compared to non-smokers. [31] In their 2021 review Ramos-Garcia *et al.* were unable to clearly identify the relationship between MT and gender, age or the use of stimulants such as smoking or alcohol. [9] This is in contrast to OL, which occurs more often in men and is associated with smoking (as it is 6 times more common in people who smoke tobacco), and also includes additional risk factors such as alcohol consumption and human papillomavirus. [11] In the case of PVL, on the other hand, tobacco and alcohol are not aetiological factors. [19, 35]

Similarly, a statistical review and meta-analysis from 2025 (Mohideen *et al.*) confirmed the lack of statistically significant differences between a group of smoking patients with cancer progression and a group without progression. Similar results were obtained for alcohol consumption. Regarding HPV, a statistically insignificant difference was noted in both patients with and without

cancer progression. [21] On the other hand, de la Cour *et al.* assessed the prevalence of HPV in leukoplakia, lichen planus, and PVL at 20.2%, 23.0%, and 24.7%, respectively. [24] Unfortunately, the relationship between HPV and the occurrence of cancer in PVL has not been clarified. [21] It is not possible to exclude or confirm the role of HPV in the etiopathogenesis of PVL. [19]

Further research is necessary. Similarly, the possible role of microbial factors in the pathogenesis of PVL remains unclear. Understanding the molecular mechanisms offers hope for solving the mystery of MT in PVL. A pilot study revealed dysregulation of 140 genes related to the immune system. This issue requires clarification. [21] As a consequence, there is a lack of prognostic biomarkers in the clinical diagnosis of PVL. [38]

In summary, both the aetiology of PVL and the factors influencing its progression to cancer are unknown. Close monitoring of patients is recommended and, if necessary, biopsies should be performed to assess changes in the appearance, colour, and size of observed lesions, as well as the appearance of subsequent lesions. [21]

The importance of observation and the role of the dentist

PVL carries a particularly high risk of malignant transformation. [11, 12] Furthermore, due to the frequency of multifocal occurrence, [27, 41] this condition remains difficult not only to treat [16, 42] but also to diagnose. [11, 27] Diagnosis is most often retrospective, based on clinical and histopathological examinations, which change over time. [10, 19] The approach of the dentist should be determined by the non-specific presentation of the lesion and necessitate frequent dental examinations of the patient as well as histopathological tests, sometimes multiple times and from multiple sites within the oral cavity. [9] Multiple biopsies are recommended, [21] especially in the case of newly formed red or nodular areas [19] as well as when changes occur in the size or appearance of existing lesions or new lesions emerge. [28] It is important to note that histopathological features may vary depending on the stage of the disease or the biopsy site. [21] Notice should be taken of any leukoplakia lesion whose surface assumes a papillary form, increases in size, or becomes exophytic. [11] Early diagnosis and early treatment of PVL are crucial due to the aforementioned high risk of MT. [12] Therefore, dental check-ups are of pivotal importance in identifying the disease, [11] as treatment depends on prompt diagnosis combined with periodic biopsies and patient observation. [11, 19]

Mortality associated with the disease can be as high as 30–50%. [28] As a consequence, dentists play a key role in the early detection and monitoring of these types of oral lesions. It is also important to educate patients about oncological vigilance. Often, dentists may be the first to observe and diagnose "disturbing changes" in the oral cavity.

If OPMDs lesions are suspected, the physician should refer the patient to a specialist for immediate treatment. A biopsy of the lesion should be performed, and follow-up appointments should be scheduled based on an individual risk assessment. [5] Therefore, early diagnosis, surgical removal, and patient observation are crucial in the treatment of PVL. [12]

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All tables and figures should be cited in the text. Each figure must have a corresponding legend. Legends to tables and figures should contain sufficient information to be understood without reference to the text. All symbols, arrows, numbers, or letters used in the figure should be explained. For photomicrographs, information on the method of staining or preparation as well as information on scale and/or magnification should be included.

Proofs

Proofs will be available as PDF files. The detailed instructions will be sent via e-mail when the proof is ready for review. Adobe Acrobat Reader is required in order to read this file, which can be downloaded (free of charge) from http://get.adobe.com/reader. Proofs must be returned by e-mail to the Editorial Office within seven days.


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