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ASSESSMENT OF OXIDATIVE STRESS AND EARLY VASCULAR DAMAGE IN CHILDREN AND YOUNG ADULTS WITH BETA-THALASSEMIA MAJOR

ABSTRACT

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CONTENTS

ABBREVIATIONS

BP	blood pressure
BTM	beta-thalassemia major
DBP	diastolic blood pressure
ECCRA	Expert Center for Coagulopathies and Rare Anemias
BMI	body mass index
MDA	malondialdehyde
OS	oxidative stress
TC	total cholesterol
PP	pulse pressure
SBP	systolic blood pressure
CVD	cardiovascular diseases
MAP	mean arterial pressure
HR	heart rate
TG	triglycerides
TIBC	total iron-binding capacity
TDBT	transfusion-dependent beta-thalassemia
AC	arterial compliance
AtC	atherogenic coefficient
AIx	augmenattion index
AIP	atherogenic index of plasma
BSA	body surface area
CRI-I	Castelli risk index II
CRI-II	Castelli risk index II
Ep	pressure–strain elastic modulus
ET	echo-tracking
PWV	pulse wave velocity
PWVβ	pulse wave velocity β

I. Introduction

Oxidative stress (OS) is defined as a disturbance in the balance between oxidants and antioxidants due to excessive production of prooxidants and free radicals. This imbalance is the cause of molecular and cellular damage and lies in the pathophysiology of many diseases, incl. and in thalassemic syndromes.

Beta-thalassemia is a hereditary hemoglobinopathy with complete or partial damage to the beta-globin gene, resulting in reduced or absent synthesis of β-globin chains. The formed unstable tetrameric aggregates precipitate in erythroid cells, oxidatively damage their membranes and cause their premature hemolysis, manifested by ineffective erythropoiesis, chronic anemia and iron overload. The oxidative effect of free iron accumulated in the body as a result of regular hemotransfusions is also important for the reduced survival of damaged erythroid cells.

The most preferred target of free-radical damage is lipids, and lipid peroxidation is a major molecular mechanism of free-radical toxicity. An end-compound of lipid peroxidation is malondialdehyde (MDA), used as an index of oxidative status and a preferred marker for OS assessment. There is evidence that the processes of its formation play a major role in the pathogenesis of premature atherosclerosis. Numerous studies have demonstrated elevated MDA levels in transfusion-dependent patients with beta-thalassemia major (BTM). Endothelial dysfunction, altered vascular elasticity with intimal thickening, and arterial stiffness have also been demonstrated, correlating with a higher incidence of vascular complications, premature arterial aging, and early atherosclerosis.

The vascular endothelium is described as a dynamic endocrine structure with an active role in maintaining vascular homeostasis. Endothelial dysfunction associated with proinflammatory and prothrombotic activity and with increased OS is considered an important precursor of atherosclerosis. Endothelial dysfunction and increased arterial stiffness have been demonstrated in BTM patients, and therefore the assessment of early vascular damage and OS is a current scientific problem. The condition of the arteries is highly correlated with the biological age of the individual, and changes usually begin in childhood. Timely and adequate care for patients with BTM reliably extends life expectancy, demonstrating a trend toward equalization with that of the general population. A change in the spectrum of concomitant diseases is also expected.

On the other hand, the aging of BTM patients and the prevalence of cardiovascular risk factors related to iron overload, diabetes and smoking confer an increased risk of atherosclerosis and cardiovascular disease (CVD). The onset and progression of vascular damage can be most readily followed by ultrasonography of the carotid arteries, measuring vascular intima-media thickness and arterial elasticity. Routine Doppler ultrasound examination of carotid arteries is recommended for early identification of subclinical atherosclerosis and for timely drug and non-drug prevention.

In summary, the control of vascular health in patients with BTM is an up-to-date scientific and clinical problem and so far studies in this field have not been conducted in Bulgaria. The study of the vascular status in Bulgarian populations with BTM would provide an opportunity for comparative analyzes with foreign publications, for the formulation of some clinical specificities in the early vascular changes and for the definition of possibilities for their therapeutic influence.

II. Aim and objectives

Aim of the study

To identify the presence of early vascular damage in children and young adults with beta-thalassemia major, by examining arterial stiffness of peripheral vessels and to study its correlations with some markers of oxidative stress, lipid profile indicators and lipid indices.

Задачи

1. To carry out a comparative assessment of some initial hemodynamic indicators - heart rate (HR), arterial pressure (BP) and pulse pressure (PP), in children and young adults with BTM and healthy controls.
2. To perform a comparative assessment of some hematological parameters (Hb, Ery and Hct) and indicators of iron overload (serum ferritin) in children and young adults with BTM and healthy controls.
3. To analyze indices of lipid metabolism (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides) and some atherogenic indices as markers of cardiovascular risk in BTM patients and compare them with those in healthy controls.
4. To determine serum MDA concentrations in BTM patients and compare them with those of healthy controls.
5. To measure and compare the local arterial stiffness of the two carotid arteries by means of echo-tracking (ET) methodology in patients with BTM and healthy controls.
6. To investigate the correlations between serum ferritin levels and indices of lipid metabolism and some atherogenic lipid indices in patients with BTM.
7. To investigate correlations between hemoglobin values and lipid profile indicators and lipid indices.
8. To investigate the correlations of the ET parameters of the two carotid arteries with sex, age, indicators of lipid exchange and atherogenic lipid indices in patients with BTM.
9. To investigate the correlations of the ET parameters of the two carotid arteries with the levels of serum ferritin and MDA in patients with BTM.
10. To investigate the correlations between splenectomy and ET parameters of both carotid arteries, indicators of lipid exchange and atherogenic lipid indices in patients with BTM.

III. Materials and methods

1. Material basis for the realization of the dissertation work

The study was conducted in the following structures of the UMBAL "St. Marina" - Varna and MU-Varna:

• Expert Center for Coagulopathies and Rare Anemias (ECCRA) at UMBAL "St. Marina" - Varna
• Clinic for pediatric clinical hematology and oncology, UMBAL "St. Marina" - Varna
• Internal Medicine Clinic, UMBAL "St. Marina" - Varna
• Clinical laboratory, UMBAL "St. Marina" - Varna
• Department of Social Medicine and Health Care Organization, MU-Varna

2. Patients population

In the period December 2021 - March 2023, a total of 78 children and young adults were examined, of which 38 patients (18 males and 20 females) with beta-thalassemia major and 40 (20 males and 20 female) healthy controls. The patients were selected among those undergoing treatment and follow-up at the ECCRA. The study design is case-control in a sought-after 1:1 ratio. Controls were age- and gender-matched to the maximum extent possible.

3. Inclusion and exclusion criteria

Inclusion criteria

• Patients with a diagnosis of BTM confirmed by hemoglobin electrophoresis and/or genetic testing, aged 5 to 44 years
• Age- and sex-matched healthy individuals without underlying heart disease or established anemic syndrome to participate in the control group
• Confirmed and signed informed consent from the patient/control (or parents in the case of minor participants) to participate in the study.

Exclusion criteria

• Refusal of participation in the study by the patient/control (or parents in the case of minor participants)
• Age below 5 and above 44 years
• Presence of documented heart disease or anemic syndrome in the subjects of the control group.

4. Planned visits

For the purpose of the study and for the convenience of patients and persons from the control group, the laboratory and imaging studies included in the study (see item 6. Research methods) were combined and conducted during pre-

arranged visits. For BTM patients, imaging studies were performed during a scheduled visit for hemotransfusion. Laboratory tests were performed immediately *before hemotransfusion*.

5. Information sources

All patients receive regular hemotransfusions in the ECCRA at an interval of two to five weeks and undergo iron chelation therapy according to the type and severity of the iron product. Information on the main application, accompanying complications and/or other diseases, carried out at the time of transfusion and chelation treatment, as well as on the results of laboratory and imaging studies, incl. documenting the iron staff were extracted from users' medical records. Diagnostic procedures, treatment and follow-up of patients with BTM are carried out by an interdisciplinary team with experience in the diagnosis and treatment of the disease and its complications. The teams include a hematologist/pediatric oncohematologist, cardiologist/pediatric cardiologist, endocrinologist/pediatric endocrinologist, gastroenterologist/pediatric gastroenterologist (hepatologist), geneticist, psychologist and social worker. The tracking algorithm was developed by the Thalassemia Working Group of the Bulgarian Medical Society of Hematology (BMSH).

The control group included persons who, at the time of the study, had no laboratory evidence of anemic syndrome, no anamnestic evidence of anemia necessitating regular blood transfusions, and no evidence of existing cardiovascular disease. The control group was recruited from among medical students and doctors, children and acquaintances of the medical staff at the Internal Medicine Clinic and the Children's Clinical Hematology and Oncology Clinic of UMBAL "St. Marina", Varna, friends and colleagues of the doctoral student, as well as on the basis of disseminated information about the study.

Through prior telephone contact or in a personal conversation with the participants and/or the parents of the minors, comprehensive information related to the study design, the type of upcoming procedures, the expected results and individual benefits, as well as the necessary preliminary preparation, was provided. Before signing the informed consent and starting the diagnostic procedures, each participant/parent of a minor participant was again personally provided with detailed information by the researcher about both the overall study and the technical performance of each of the intended studies.

Sociodemographic, anthropometric and health data of the study participants are reflected in individual cards.

The scientific concept of the study was examined and approved by the Committee on Ethics of Scientific Research (KENI) at the University of Medicine - Varna with protocol №. 101/24.03.2021.

Research on the study was funded with the help of a winning project under the Science Fund № 20020/2020: *Influence of oxidative stress on early vascular damage in children and young adults with beta-thalassemia major*.

6. Methods

Survey. All participants were asked to answer the following questions: age, harmful habits, duration of transfusion treatment and type of chelation treatment, antihypertensive therapy and symptoms of cardiovascular disease.

Additionally, information on smoking was collected, incl. statute of limitations, number of cigarettes smoked per day and smoking cessation.

History, physical examination and anthropometric studies. In addition to general somatic status, height (cm), weight (kg) were measured for all participants, and body mass index (BMI) was calculated using the formula BMI = weight (kg) / height (m)^2. Body surface area index (BSA) was also calculated using the Mosteller formula: BSA (m^2) = (height (cm) x weight (kg) / 3600)½. Height and weight were measured with a SECA model electronic height meter, and body weight with an electronic scale. In all participants, arterial pressure was measured during rest and in a sitting position, lasting several minutes before the start of the procedure. A standard manual sphygmomanometer was used. 3 measurements were taken on each arm with a 1-2 minute interval between them. The higher average value was taken as reference. Pulse pressure was calculated using the formula PP (mmHg) = systolic arterial pressure (SBP) (mmHg) – diastolic arterial pressure (DBP) (mmHg). Mean arterial pressure MAP) was also calculated using the formula MAP (mmHg) = DBP + (SBP – DBP)/3.

Laboratory investigations. Venous blood obtained by venipuncture of the cubital or other peripheral vein after a minimum of 12 hours of overnight fasting was used to conduct the laboratory tests. In order to simultaneously carry out the laboratory analyses, serum was separated from the blood sample, which was stored in a freezer at -20°C. All blood parameters were examined in the Central Clinical Laboratory of UMBAL "St. Marina", Varna and include: blood count with differential count (CBC); lipid profile - total cholesterol (TC), LDL-cholesterol, HDL-cholesterol, triglycerides (TG); CRP; serum ferritin, serum iron, total iron-binding capacity (TIBC); malondialdehyde (MDA).

Within the framework of the present study, the following laboratory and

biochemical markers were investigated and the following analytical methods were applied:

- *Blood count with differential count (*5-diff hematology analyzer Sysmex XN 1000): fluorescence flow cytometry with semiconductor laser and hydrodynamic focusing. The hematological analyzer applies impedance and optical method when counting blood cells, fluorescent flow cytometry with side fluorescent light, forward and side scattered light for differential leukocyte enumeration and cyanide-free colorimetric method with *sodium lauryl sulphate when measuring hemoglobin*
- *Total cholesterol* (ADVIA 1800 biochemical analyzer): three-step enzymatic reaction with Trindler termination
- *LDL-cholesterol* (biochemical analyzer ADVIA 1800): calculated according to the Friedewald's formula - LDL-cholesterol = OX - (HDL-cholesterol + TG/2.2), with a value of triglycerides < 4.5 mmol/l. Measured by direct enzymatic method with elimination/catalase, at a triglyceride value > 4.5 mmol/l
- *HDL-cholesterol* (biochemical analyzer ADVIA 1800): direct enzymatic method with elimination/catalase
- *Triglycerides* (biochemical analyzer ADVIA 1800): three-step enzymatic reaction with Trindler termination
- *Serum ferritin* (Analyzer – Roche Cobas 6000): electrochemiluminescent immunoassay
- *Serum iron* (Biochemical analyzer ADVIA 1800): colorimetric method with Ferrozine
- *TIBC* (Biochemical Analyzer ADVIA 1800): direct method with sequential release and uptake of iron
- *MDA* (ELISA): enzyme-linked immunosorbent assay (ELISA) with a ready-made test set from Bio-Techne-Novusbio. The test is a competitive immunoassay with the following analytical characteristics: range of quantification (linearity) – 31.25–2000 ng/mL, sensitivity (Lower Limit of Detection) – 18.75 ng/mL, irreproducibility within a series – CV <5.59% and irreproducibility between series – CV <5.19%, analytical detectability in serum – % recovery – 92-110%.

The following indices and ratios were calculated:

- *Castelli risk index* I (Castelli risk index I, CRI-I) = TC/HDL-cholesterol
- *Castelli risk index II* (Castelli risk index II, CRI-II) = LDL-cholesterol/HDL-cholesterol

• *plasma atherogenic index (Atherogenic index of plasma, AIP)* = {(logTG)/HDL-cholesterol}

• *atherogenic coefficient (Atherogenic coefficient, AtC)* = (TC – HDL-cholesterol)/HDL-cholesterol)

• *non-HDL-cholesterol (non-HDL)* = TC – HDL-cholesterol

AIP calculation was performed using a Czech online calculator for atherogenic risk (Dobiasova, Czech. Calculator of atherogenic risk. Available from: http://www.biomed.cas.cz/fgu/aip/calculator.php).

Imaging studies

All study participants underwent ultrasound examination of both common carotid arteries with an Aloka Hitachi Prosound α7 ultrasound machine using a high-frequency linear transducer. For the purpose of the examination, the patient is placed in supine position, with neck extension and slight rotation at 30° contralateral to the examined area. The measurements taken were performed 2 cm proximal to the bifurcation of the common carotid artery in order to visualize the artery at its greatest width in a longitudinal section, thus the vessel wall and the ultrasound signal were perpendicular. This provides an opportunity to accurately track the change in vessel diameter. After visualization of the artery, the echo-tracking function is turned on from the control screen, during which two markers appear, with the help of which the borders of the examined arterial vessel are marked. Markers are placed on the near and far wall of the artery at the border between the tunica media and the tunica adventitia. Before the start of the study, peripheral electrodes were placed on the patient, with the help of which a simultaneous ECG recording was observed on the screen and the beginning of the systole and diastole of the heart contraction was accurately recorded. It is necessary to record at least six cardiac cycles, during which, if possible, the patient held his breath, which ensures an improvement in image quality. The recording can then be stopped. A graph of vessel diameter changes during the study is visualized on the screen. The received information is processed for all waves and the program calculates an average value of the arterial stiffness indicators - β-stiffness index, arterial compliance (AC), pulse wave velocity β (PWVβ), augmentation index (AIx) and pressure-strain elastic modulus (Ep). It is also necessary to enter the data from the previously measured systolic and diastolic arterial pressure. Arterial elasticity indicators are calculated automatically by mathematical algorithms.

The ultrasound examination of the carotid arteries was performed by the doctoral student after successfully completing the "Carotid Ultrasound MasterClass" online course in January 2022 with teacher Prof. Dr. Thomas Binder. In 2020, the doctoral student also completed a course on Doppler sonography at UMBAL "St. Marina" headed by Assoc. Prof. Chavdar Bachvarov.

7. Statistical methods

Data were processed using a specialized statistical package for a personal computer SPSS Windows, version 25. Graphical display was performed with Excel.

The following statistical methods were used:

Descriptive analysis: This method includes frequency analysis (count and percentage representation), crosstabulation, mean calculation, and sweep. Through frequency analysis, the frequency of each value of the studied variables is determined, which are represented by number and percentage. Cross-tabulation allowed to examine the relationship between more than two variables that represent the frequency of combined features. Means and ranges were calculated to determine the central tendencies and distribution of the data.

The normality of the data distribution was checked by means of the Kolmogorov-Smirnov (K-S) test.

Graphical analysis: this method is used to visually represent the results through graphs and charts.

Non-parametric methods: $\chi 2$ (chi-square). The test was used to test the relationship between two categorical variables.

Parametric methods

- *Correlation analysis using Spearman and Pearson methods.* This analysis was used to analyze the relationships between the data. Pearson correlation is used to establish a relationship between numerical data and estimate linear relationships between two continuous variables. Spearman correlation analyzes the ranks of variable values and calculates a correlation coefficient that indicates the degree of monotonic relationship between them.

The correlation coefficient r can take values between 0 and -1 for an inverse relationship and between 0 and +1 for a straight relationship.

The assessment of the strength of the relationship between two features by the correlation coefficient r e:

- If r is below 0.30 – weak correlation

- If r is between 0.30 and 0.50 – moderate correlation
- If r is between 0.50 and 0.70 – significant correlation
- If r is between 0.70 and 0.90 – strong correlation
- If r is above 0.90 – very strong correlation

- *ANOVA (Analysis of Variance):* The Analysis of Variance (ANOVA) method was applied to compare the mean values of operative time and operative wound closure in patients with different diseases. This analysis made it possible to determine which groups had statistically significant differences.
- *Independent t-test was used for comparison.* This statistical method allowed the researcher to determine whether the mean scores compared were statistically significantly different.

All tests were interpreted as statistically significant at a significance level of $p \leq 0.05$.

IV. Results and discussion

1. General characteristics of the study participants

The present study included a total of 78 participants, of which 38 children and young adults with BTM and 40 healthy controls matched for sex and age.

Before performing the targeted analyses, patients and healthy controls were matched on their demographic and anthropometric parameters, smoking status, type of chelation therapy, and splenectomy performed.

1.1. Demographic and anthropometric characteristics of the participants

Tabl. 1 presents the demographic and anthropometric data of the patients and healthy controls in the study. The Chi-square test was used to compare the gender distribution, and the independent samples t-test was used for the other parameters.

Tabl. 1. *Demographic and anthropometric characteristics of the participants*

Parameter	Patients BTM N=38	Healthy controls N=40	p-value
Age (years)	25.0±10.8	23.1±11.0	p=0.449
Gender -female	53%	49%	
Hight (cm)	160.1±16.1	164.84±17.8	p=0.230
Weight (κg)	54.2±16.4	55.8±17.4	p=0.683
BSA (m^2)	1.54±0.29	1.59±0.35	p=0.509
BMI	20.6±3.55	19.9±3.23	p=0.383

Analysis of the results showed that no significant difference was found between patients and healthy controls in terms of age (p=0.449), sex, height (p=0.230), weight (p=0.683), BSA (p=0.509) and BMI (p=0.383).

Discussion:

In studies conducted by Kremastinos et al. in 2006 and Kostopoulou et al. in 2014 among BTM patients of a similar age to that of our study population, a significantly lower BSA was found among patients compared to healthy controls. The authors explain the growth retardation and smaller body surface with chronic anemia. In the past, short stature in children with BTM varied between 30% and 60%, and the reason for this was not only the anemic syndrome, but also iron overload and subsequent damage to the endocrine glands (*DeSanctis, 2013; Farmakis, 2022*). Hypothyroidism, hypogonadism, growth hormone deficiency, zinc deficiency, chronic liver disease, psychosocial

stress, etc. are also added as contributing factors to short stature in BTM patients. (*Skordis, 2011*). Nowadays, adherence to protocols for hemotransfusion and optimal chelation treatment has significantly reduced the risk of short stature, resp. of smaller body surface area among BTM patients. The improved endocrine status in children compared to previous years is also due to them (*Farmakis, 2022*). In our study, there was no statistically significant difference between BSA in patients and healthy controls (p=0.509). We assume that this is due to the discovery of ECCRA in our hospital, providing the opportunity to apply both optimal transfusion and chelation treatment and a multidisciplinary approach for early diagnosis and follow-up of expected complications of BTM.

1.2. Participant smoking characteristics

When examining the smoking status, it was found that there were 7 (18%) smokers in the patient group and 6 (15%) in the control group. The relative share of smokers in the respective groups is presented in Fig. 1 and Fig. 2. We evaluated the significance of the differences with the non-parametric cross-tabulation and chi-square analysis, which showed a lack of significance (X^2= 0.164; p= 0.685).

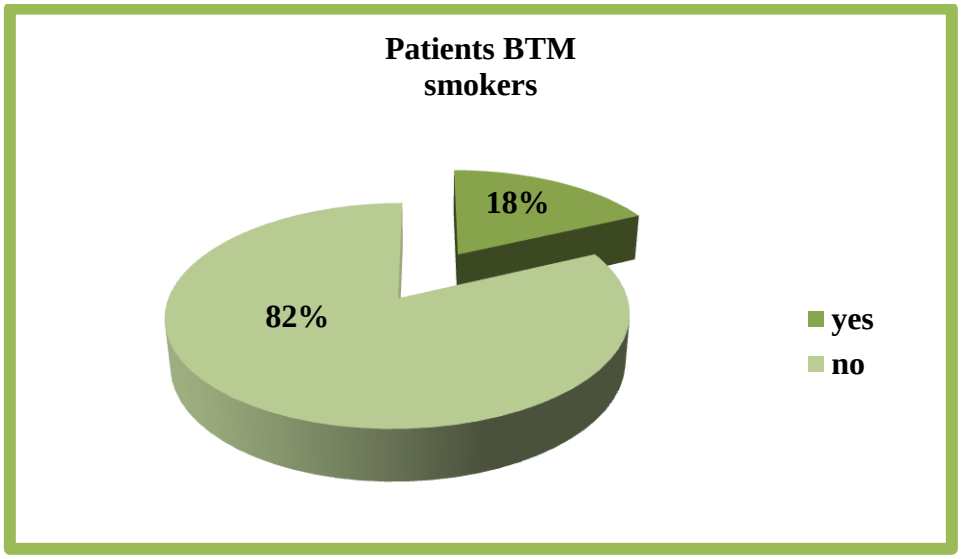

Fig. 1. *Relative proportion of smokers among BTM patients*

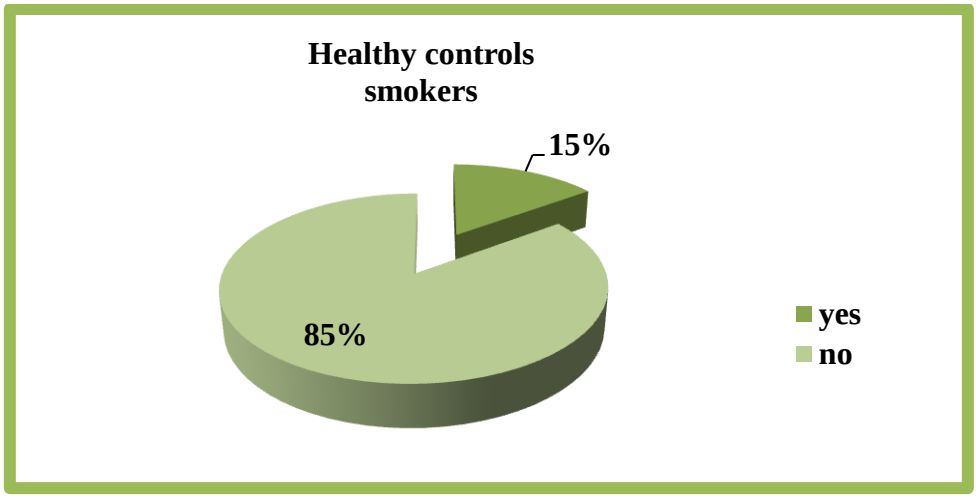

Fig. 2. *Relative proportion of smokers among healthy controls*

1.3. Characteristics of patients by type of chelation therapy

During the study, all patients received chelation therapy, respectively deferasirox – 28 patients (74%), deferiprone – 7 patients (18%) and combined therapy deferasirox and deferiprone – 3 patients (8%). *Fig. 3* shows the relative share of patients according to the type of chelation therapy.

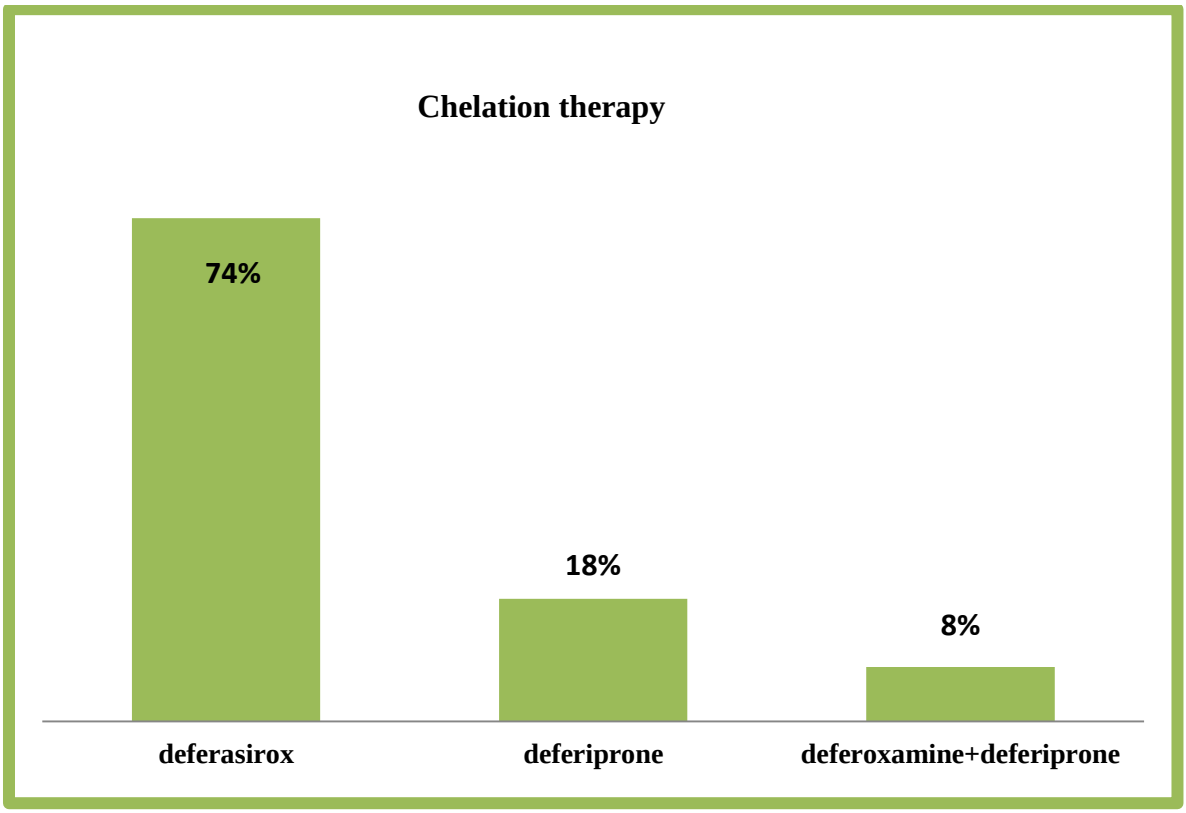

Fig. 3. *Proportion of patients by type of chelation therapy*

1.4. Characteristics of patients with splenectomy performed

In our study group, 13 (34%) of the patients underwent splenectomy for indications related to the underlying disease. *Fig. 4* shows the relative share of splenectomized patients.

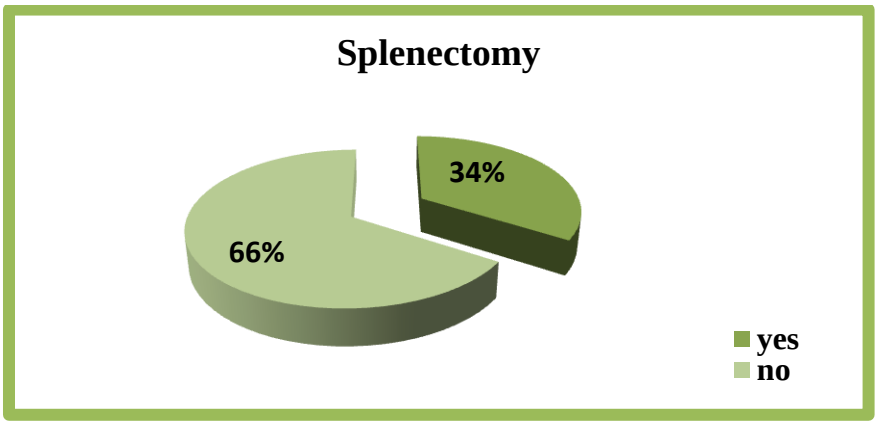

Fig. 4. *Proportion of splenectomized patients*

2. Comparative analysis of the results obtained in patients with BTM and the control group

2.1. Comparative analysis and discussion of hemodynamic parameters

Results for task 1: *To perform a comparative assessment of some baseline hemodynamic indicators - heart rate (HR), arterial pressure (BP) and pulse pressure (PP) in children and young adults with BTM and healthy controls.*

Tabl. 2 presents the hemodynamic parameters of the patients and healthy controls in the study.

Tabl. 2. *Hemodynamic paramtetes of the patients and controls*

Paramter	Patients BTM	Healthy controls	p-value
SBP (mmHg)	106.18±10.4	102.56±11.17	p=0.147
DBP (mmHg)	69.0±7.9	65.5±7.23	**p=0.044**
MAP (mmHg)	81.3±8.26	77.7±7.96	**p=0.05**
HR (beats/min)	87.4±16.9	70.2±11.8	**p=0.0001**
PP (mmHg)	37,9±6,38	37,0±7,49	p=0.586

When examining hemodynamic indicators in patients with BTM and healthy controls, the following values were established:

- **SBP:** the mean value of systolic BP in BTM patients was 106.18±10.4 mmHg, while in healthy controls it was 102.56±11.17 mmHg, the difference not being statistically significant, p=0.147

- **DBP:** mean diastolic BP in BTM patients was 69.0±7.9 mmHg, while in healthy controls it was 65.5±7.23 mmHg, the difference being significant, **p=0.044**
- **MAP:** in the group of patients with BTM, the mean BP was 81.3±8.26 mmHg, while in the group of healthy controls it was 77.7±7.96 mmHg, the difference being statistically significant, **p=0.05**
- **HR:** mean heart rate in BTM patients was 87.4±16.9 beats/min, while in healthy controls it was 70.2±11.8 beats/min, the difference being significant, **p=0.0001**
- **PP:** in BTM patients the mean pulse pressure was 37.9±6.38 mmHg and in healthy controls 37.0±7.49 mmHg, the difference not being significant, p=0.586**.**

The analysis of the results showed that no statistically significant difference was found between the patients and the healthy controls regarding SBP (p=0.147) and PP (p=0.586). A statistically significant difference was found in terms of DBP (p=0.044), MAP (p=0.05) and HR (p=0.0001).

Discussion:

The higher heart rate in patients compared to healthy controls was an expected result. Chronic anemia and reduced tissue oxygen delivery in BTM is compensated for by increased cardiac output through increased heart rate and increased stroke volume. This defines BTM as a high-output state, and most authors find a higher heart rate in patients compared to healthy controls (*Metivier, 2000*). Variations in heart rate values were observed in different studies, which could be explained by a number of factors influencing it, such as the level of hemoglobin, the age of the patients and the emotional state at the time of the examination. *Aessoposs et al.* in 2004 studied a group of 202 patients aged 27.3±6.3 years and compared their heart rate to healthy controls aged 26.0±5.2 years. Found a significantly higher HR in patients 84±12 bpm compared to healthy 77±12 bpm (p=0.001).

Compared with healthy controls, imaging studies in BTM demonstrate larger heart sizes and stroke volumes, as well as higher HF, which is associated with greater metabolic cost. The elevated resting metabolism in these patients is also a source of oxidative stress independent of the free radicals generated by iron overload (*Wood, 2009*).

Two Bulgarian studies among BTM patients, the first by *M. Dimova* from 2017 among adult patients and the second *by K. Ganeva* from 2023 among children, also

found no statistically significant difference between SAN in patients and healthy controls.

Regarding SBP, our results are similar to those of *M. Dimova*, who reported SBP in patients 96.07±11.62 mmHg and SBP in healthy controls – 86.29±9.77 mmHg (p= 0.01). The mean age of her study group was similar to our patient group. With regard to DAN (p=0.04) and MAP (p=0.05), we found a statistically significant difference between the two groups. Our obtained results are similar to those published by *Kostapoulou et al.* in 2014 and *Bosi et al.* in 2003.

2.2. Comparative analysis and discussion of laboratory hematological indicators

Results for task 2: *To perform a comparative assessment of some hematological parameters (Hb, Ery and Hct) and indicators of iron overload (serum ferritin) in children and young adults with BTM and healthy controls.*

Tabl. 3 presents the hematological parameters of the patients and healthy controls in the study.

Tabl. 3. *Laboratory parameters of the participants*

Parameter	Patients	Controls	p-value
Hb (g/L)	92,4±10,6	136,1±11,3	**p=0.0001**
Ery (10^{12}/L)	3,44±0,45	4,81±0,52	**p=0.0001**
Hct	0,28±0,03	0,41±0,03	**p=0.0001**
Serum iron (umol/L)	38,20±10,71	15,56±5,35	**p=0.0001**
TIBC (umol/L)	64,95±30,83	55,55±8,08	**p=0.009**
Serum ferritin (ng/ml)	2695,72±2303	55,76±50,22	**p=0.0001**

The analysis of the hematological indicators showed that a statistically significant difference was found between the patients and the healthy controls in terms of all the studied parameters. As expected, the mean values of Hb, Ery and Hct were lower (p=0.0001) and those of serum iron and serum ferritin were higher (p=0.0001) in BTM patients.

Discussion:

In the group of BTM patients, the mean values of serum ferritin were 2695.72±2303 ng/ml, and in the healthy controls – 55.76±50.22 ng/ml, the difference being significant (p=0.0001) (*Fig. 5*).

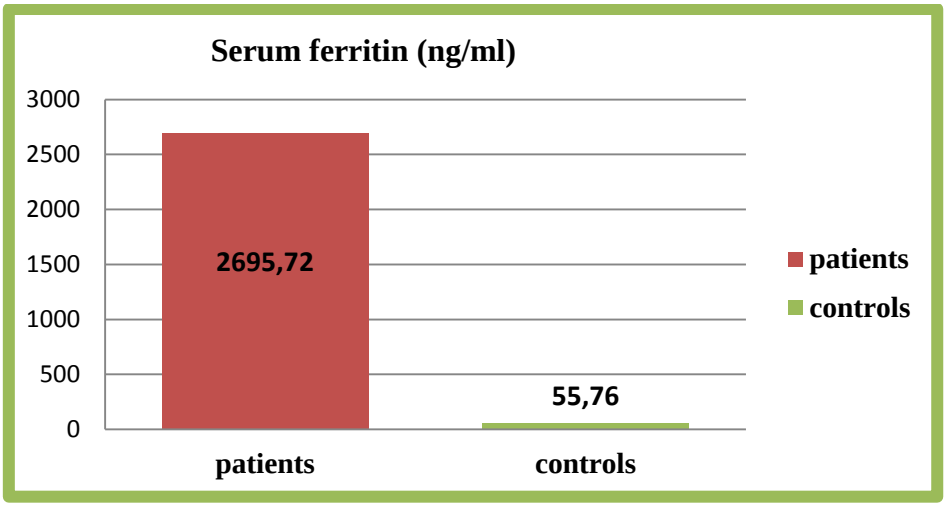

Fig. 5. *Mean values of serum ferritin in both groups*

The results obtained by us are compatible with those of other studies, in which the authors evaluated these values as risky for the development of cardiovascular complications (*Mishra, 2013; Bhalodiya, 2023; Krittayaphong, 2018; Forni, 2023*).

Olivieri et al. in 1994 demonstrated in their study that ferritin values below 2500 ng/ml suggest an excellent prognosis and survival in patients with BTM who receive regular adequate iron chelation therapy, have no evidence of cardiac disease, and adhere to a regular hemotransfusion regimen.*Telfer et al.* in 2000 published a study in which they followed patients for a period of 13.6 years and showed that ferritin values below 1500 ng/ml were associated with fewer long-term complications. *Hahalis et al.* in 2009 studied 36 patients with BTM and found that after a follow-up period of 12 years, ferritin levels above 2800 ng/ml and LVEF <60% were independently associated with CVD mortality. Ten years later, the collective of *Derchi et al.* determined that ferritin values above 3000 ng/ml were associated with a higher risk of heart disease. In 2021, *Kampridis et al.* conducted a study in BTM patients evaluating ferritin values and echocardiographic and MRI findings in relation to survival over a 10-year period. The researchers found that patients with MRI T2* <20 ms, ferritin greater than 2000 ng/ml, and TR Vmax >2.8 m/s had worse long-term survival. The authors conclude that in order to improve overall survival and reduce hospitalizations and mortality from CVD in patients with BTM, it is necessary to maintain lower limits of ferritin below 1700 ng/ml and of MRI T2* above 34 ms.

Considering the main factors determining the higher ferritin levels in patients with TBT (frequency of hemotransfusions, type of chelating medication and adherence to chelation treatment) (*Farmakis, 2022*), we assume that in our

patients the main reason for the higher ferritin is the low rate of adherence to prescribed chelation therapy. Poor medication compliance in patients with chronic diseases, incl. with BTM, is a well-known fact and has been presented in many publications. Some of them emphasize that poor compliance is observed more often in teenage and young patients, which coincides with the average age in our patient group (25.0±10.8 years). (*Mohamed, 2022; Калева, 2015; WHO, 2003;Eziefula, 2022*).

2.3. Comparative analysis and discussion of lipid profile in BTM patients and controls

Outcomes for Task 3: *To analyze parameters of lipid metabolism (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides) and some atherogenic indices as markers of cardiovascular risk in patients with BTM and compare them with those in healthy controls.*

Tabl. 4 shows the lipid profile indicators in the patients and healthy controls in the study.

Table. 4. *Lipid profile of study participants*

Parameter	Reference values	Patients	Controls	p-value
Total choclesterol (mmol/l)	<5,2 mmol/l	3,164±0,83	3,94±0,77	**p=0.0001**
LDL-cholesterol (mmol/l)	<3,36 mmol/l	1,60±0,70	2,11±0,69	**p=0.0002**
HDL – cholesterol (mmol/l)	М > 1,02 mmol/l Ж > 1,29 mmol/l	0,93±0,35	1,50±0,37	**p=0.0001**
Triglycerides (mmol/l)	<1,7 mmol/l	1,36±0,66	0,68±0,22	**p=0.0001**

When examining the lipid profile, we obtained the following results:

- **Total cholesterol:** in the group of patients with BTM, the mean value of total cholesterol was 3.16±0.83 versus 3.94±0.77 in the group of healthy controls, the difference is significant, **p=0.0001**
- **LDL-cholesterol:** in the group of patients with BTM, the mean value of LDL-cholesterol was 1.60±0.70 against 2.11±0.69 in the group of healthy controls, the difference is significant, **p=0.0002**
- **HDL-cholesterol:** in the group of patients with BTM, the mean value of HDL-cholesterol was 0.93±0.35 versus 1.50±0.37 in the group of healthy controls, the difference is significant, **p=0.0001**
- **Triglycerides:** in the BTM patient group, the mean value of triglycerides was 1.36±0.66 versus 0.68±0.22, the difference is significant, p=0.0001**.**

The analysis of the results obtained by us shows that in BTM patients the mean values of total cholesterol, LDL-cholesterol and triglycerides are within reference limits, while the mean value of HDL-cholesterol (0.93±0.35) is lower and for both sexes (>1.02 in men and >1.29 in women). In the group of healthy controls, all the investigated indicators were within the normal range. However, when comparing the mean values between the patient and control groups, statistically significant differences were found in all the studied parameters. In patients with BTM, the values of total cholesterol, LDL-cholesterol and HDL-cholesterol were lower, and the values of triglycerides were higher.

Discussion:

Our results are compatible with the results obtained by many other authors worldwide and confirm their conclusions about lower lipid levels in BTM patients.

Maioli et al. from Italy were one of the first groups to study the lipid profile of 70 BTM patients and, like us, reported lower total cholesterol, LDL-cholesterol and HDL-cholesterol and higher plasma triglyceride concentrations in BTM patients in compared with healthy controls. They published their data in 1997 and hypothesized that these differences could be due to both high iron overload and possibly concomitant hepatic and hormonal damage in BTM patients. A few years later, *Chrysohoou et al.* conducted a lipid profile study in 192 BTM patients undergoing treatment at one of the largest thalassemia centers in Athens. Eighty-eight of the patients were male with a mean age of 25 ± 6 years, and the remaining 104 were female with a mean age of 26 ± 6 years. The results of the ATTICA study conducted in the same year, assessing the lipid profile of age-matched healthy men and women in the Attica region, Greece, were used for comparative analysis (*Panagiotakos, 2004*). The researchers found that in the majority of patients with BTM, the levels of the studied lipids and lipoproteins were within normal limits and significantly lower compared to the general population of the same age. The lowest levels were found when examining HDL-cholesterol, reaching 42% of men and 29% of women with BTM below 0.77 mmol/l (30 md/dl). Based on the rule that the risk of myocardial infarction is higher in individuals with low HDL-cholesterol levels, the authors recommend that the total-to-HDL-cholesterol ratio be used as a prognostic factor for future cardiovascular events in patients with BTM. In support of the recommendation made, the authors also cite the statement of other authors that even individuals with normal total cholesterol levels are at high risk

of developing a myocardial infarction if HDL-cholesterol levels are low (*Franceschini, 2001*; *Panagiotakos, 2004*).

In 2010, *Haghpanah et al.* investigated the lipid profile of BTM and BTI patients in southern Iran and compared them with healthy controls. Mean serum triglyceride concentrations were higher in the thalassemia group than in the healthy controls, but the difference was not significant (p=0.091). A significant difference between the two studied groups was found when comparing the serum concentrations of total and LDL-cholesterol (p<0.001) and non-significant – when comparing the serum concentrations of HDL-cholesterol.

Two years later, *Vefic Arica et al.* conducted a similar design study among 62 Turkish BTM patients aged 5–15 years and found lower levels of total cholesterol, HDL-cholesterol and LDL-cholesterol and higher triglycerides in patients compared to healthy controls. These results are completely analogous to those obtained in our study, as well as to the results of *Sherief et al.* from Egypt (2017) and *Mashaali et al.* (2014) in Iraq.

Higher triglyceride levels in BTM patients were reported by Hartman et al. of Israel for Children and Adolescents (2002) and Richi et al. from Italy for adults (2009). In contrast, in a study by Amendola et al. in Italy (2007) the triglyceride levels in the two study groups were almost the same.

In 2020, *Setoodeh et al.* conducted a study on the influence of oxidative stress, iron overload and insulin resistance on the lipid profile among 48 Iranian patients with BTM aged 21.8 ± 6.4 years, comparing them with healthy controls aged 24.1 ± 5.0 years. Similar to our results , the researchers found significantly higher levels of triglycerides and significantly lower total cholesterol, HDL- and LDL-cholesterol in BTM patients compared to healthy controls (p<0.001). These results are in agreement with many other studies from the Mediterranean region incl. those of *Ragab et al.* (2014) and *Ibrahim et al.* (2020) from Egypt and *Boudrahem-Addour et al.* from Algeria (2014). In the study by Setoodeh et al. 49% of patients had HDL-cholesterol values below 30 md/dl (=0.77 mmol/l) and none of the participants in the healthy control group.

A study by *AlSaadi et al.* from 2022 studied the lipid profile of 62 patients aged between 6 months. and 15 years with BTM from Iraq, comparing with 65 age-matched healthy controls. Similar to our study, in a greater proportion of patients they recorded lower HDL-cholesterol values compared to controls and explained it by macrophage-activated excessive clearance. Regarding the other lipid indicators, they found slightly increased levels of total and LDL-cholesterol in the patient group, which is not consistent with our data.

Lower values of HDL-cholesterol were also published by *Daswanii et al.* in 2021, examining the lipid profile of 100 BTM patients in India aged 1-18 years. They also reported significantly lower total cholesterol and higher triglyceride values in patients compared to healthy controls, which also is in agreement with the results obtained in our study. However, regarding LDL-cholesterol, which in our study had significantly lower levels in BTM patients, they did not find a significant difference when comparing the two studied groups.

From our study, we can conclude that when examining the lipid profile in children and young adults with BTM, dyslipidemia is detected, manifesting in most cases as triglyceridemia and hypocholesterolemia. The obtained results do not differ from those reported in the literature, and considering their proven association with the development of cardiovascular diseases, we accept that our patients have an increased risk of premature onset of cardiac and vascular complications. We support the conclusion that the established very low levels of HDL-cholesterol are an independent prognostic factor for cardiovascular risk. Considering the above facts and with the aim of early detection and prevention of atherogenic cardiovascular complications, we recommend that patients with transfusion-dependent beta-thalassemia (TBT) undergo a lipid profile screening after the age of 5 years.

2.4. Comparative analysis and discussion of lipid indices in BTM patients and controls

Outcomes for Task 3*: To analyze parameters of lipid metabolism (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides) and some atherogenic indices as markers of cardiovascular risk in patients with BTM and compare them with those in healthy controls .*

On *Tabl. 5* are presented the lipid indices of the patients and healthy controls in the study.

Tabl. 5 *Lipid indices of the participants*

Parameter	Reference values	Patients	Controls	p-value
Castelli Risk Index I (CRI-I)	<3,0	3.64±1.19	2,75±0.74	**p=0.0001**
Castelli Risk Index II (CRI-II)	<3,3	1.85±0,91	1.51±0.66	p=0.072
Atherogenic index of plasma (AIP)*	(-0,3 до 0.1)	0.17±0.30	-0,34±0.19	**p=0.0001**
Atherogenic coefficient (AtC)	<3.0	2.62±1.20	2.45±0.73	p=0.43
non-HDL (mmol/l)	<3,37	2.23±0.78	2.41±0.69	p=0.313

**AIP > 0,24 – high risk; 0,1-0,24 – intermediate risk; -0,3–0,1 – low risk*

When studying the lipid indices, we obtained the following results:

- **Castelli risk index I (CRI-I):** when comparing the mean values of CRI-I in BTM patients (3.64±1.19) and healthy controls (2.75±0.74), a statistically significant difference was found, **p=0.0001**
- **Castelli risk index II (CRI-II):** when comparing the mean values of CRI-II in BTM patients (1.85±0.91) and healthy controls (1.51±0.66), no statistically significant difference was found, p=0.072
- **Atherogenic index of plasma (AIP):** when comparing the mean values of AIP in BTM patients (0.17±0.30) and healthy controls (-0.34±0.19), a statistically significant difference was found, **p=0.0001**
- **Atherogenic coefficient (AtC):** when comparing the mean values of AtC in BTM patients (2.62±1.20) and healthy controls (2.45±0.73), no statistically significant difference was found, p=0.43
- **non-HDL-cholesterol:** when comparing the mean values of non-HDL-cholesterol in patients with BTM (2.32±0.78) and healthy controls (2.41±0.69), no statistically significant difference was found, p=0.31.

Analysis of our results showed that the mean values of CRI-I, CRI-II, AIP and AtC were higher than those of healthy controls, but only CRI-I and AIP found significance, p=0.0001 . Our data do not differ from the results published by other studies of children and young adults with BTM, which are still very small in number.

Discussion:

In 2004, Chrysohoou et al. from Greece published the results of a multi-year study on the lipid profile in adult patients with BTM. They observed that 39% of men and 30% of women with BMD had levels of total cholesterol below 5.17 mmol/l and HDL-cholesterol below 0.90 mmol/l for men and below 1.16 mmol/l for women, had a TC/HDL ratio (CRI-I) higher than the normal value of 3.5. Comparing these results with results obtained from the ATTICA study in healthy adults, it was found that only 19% of men and 12% of women with normal total cholesterol levels also had low HDL-cholesterol levels. (*Panagiotakos, 2004*). Considering the much lower proportion of healthy controls with low HDL-cholesterol, the authors conclude that BTM patients have a much higher risk of developing coronary disease. On the other hand, in relation to the ATTICA study, they also emphasize the importance of TC/HDL (CRI-I) in the assessment of lipid status and in the prevention of atherosclerotic disease at the population level.

In 2015, *Ashar et al.* studied the lipid profile in 36 patients with homozygous BTM with a mean age of 12 years (ranging from 5 to 24 years of age), of whom 17 were male and 19 were female. In 36.1% of patients, high values of triglycerides and significantly lower values of HDL- (0.98±0.51) and LDL-cholesterol (2.35±1.22 mmol/L) were registered. Mean TC/HDL-C ratio (CRI-I) values were 5.7 with a norm of <3.5. Our results are similar to those reported by *Ashar et al.*, with the mean CRI-I not being as high and almost approaching the norm.

Three years later, *Nasir et al*. (2018) conducted a study of lipid profile and lipid indices in children with BTM in Indonesia, looking for correlations between vitamin D levels and indices of lipid metabolism. Compared to healthy controls, BTM patients had lower mean HDL-cholesterol values and higher values of TC/HDL-C ratio (CRI-I) = 4.4.

In a study by *Ray et al*. (2022) compared the lipid profile and atherogenic lipid indices in children with transfusion-dependent thalassemia and healthy controls. The study group included 72 patients with BTM aged between 3 and 14 years and 83 healthy controls matched for sex and age. The lipid profile was examined and the following lipid indices were calculated: AIP, CRI-I, CRI-II and AtC. The researchers found that compared to the control group, the mean levels of LDL-, HDL- and total cholesterol were significantly lower in the group of children with BTM ($p < 0.001$), and the levels of triglycerides and lipid indices significantly more high ($p<0.001$). Their published results completely match the results obtained in our study. The mean AIP value in the study by Ray et al. patient group was 0.57 ±0.25, which is a rather high value and corresponds to a high risk according to *Dobiasova et al.* (2011) and the accepted reference values for adult patients (AIP >0.24). The authors report that they were unable to find reference values for the pediatric population, but nevertheless conclude that the high AIP values they obtained may be associated with a higher risk of atherosclerosis in this group. In earlier studies, different research groups also found higher AIP values in children with TBM and, comparing them with values in healthy children, highlighted their high risk potential for the development of cardiovascular diseases (*Sherief, 2017*; *Nasir, 2018*).

According to *Bersot et al*. in patients with BMI and low HDL-cholesterol levels, who are defined as being at risk for cardiovascular disease, the assessment of whether they are subject to drug treatment or lifestyle modification should be made not on the basis of individual lipid parameters, but on base values of TC/HDL ratio (CRI-I). According to the authors, this ratio is a

better predictor of coronary disease risk than the absolute values of HDL- and LDL-cholesterol, and it can identify many more at-risk patients.

In our study, the mean AIP values in BTM patients were 0.17±0.30, which were significantly higher than those obtained in healthy controls (p=0.0001) and corresponded to an average risk of developing CVD. Mean CRI-I values were also significantly higher compared to healthy controls (p=0.0001). These results confirm the conclusion made in the previous section that patients with BTM have an increased risk of coronary events and give us reason to propose their inclusion in the panel of lipid indicators screening the risk of CVD after reaching the age of 5 years.

2.5. Comparative analysis and discussion of malondialdehyde values in BTM patients and controls

Outcomes for Task 4: *To determine serum MDA concentrations in patients with BTM and compare them with those of healthy controls.*

On *Fig. 6* are presented the mean MDA values of the patients and healthy controls in the study.

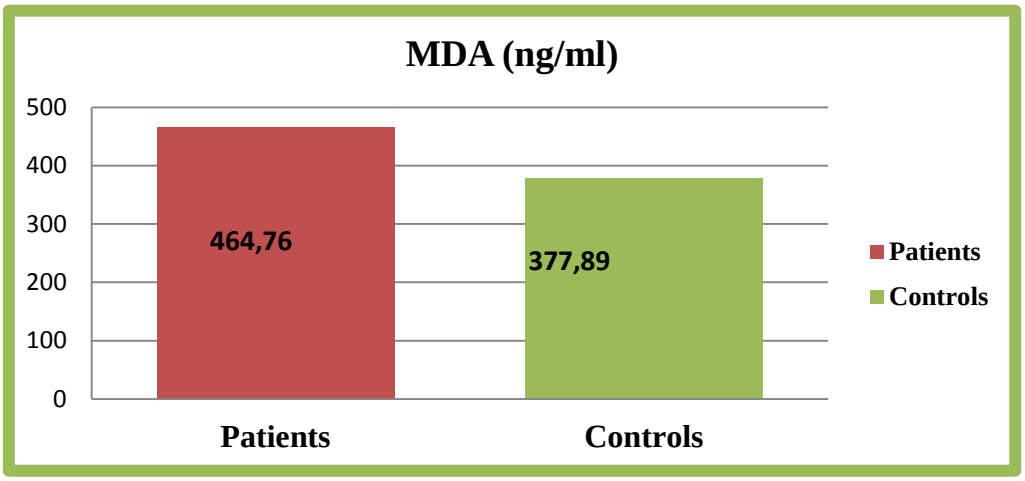

Fig. 6. *Mean MDA values of the participants*

The analysis of the results we obtained showed that the mean value of MDA in the patient group was 464.76±395.56 ng/ml, while in the healthy controls the mean value was 377.89±342.15 ng/ml, the difference being not significant, p=0.302.

Discussion:

The mean value of MDA in patients with BTM in our study was higher compared to healthy controls, but unlike the majority of studies by other authors,

been known since the 1990s (*Livrea, 1996; Suttnar, 1997*) and continues to be the subject of many studies from around the world (*Boudrahem-Addour, 2014*).

In a study conducted by *Caroline et al.* in 2021, the mean MDA level in BTM patients was 0.43 μmol/L and that of healthy controls was 0.14 μmol/L. These statistically significant results are consistent with those obtained by *Sengsuk et al.* and *Elham Abed Mahdi*, who reported significantly higher MDA levels in BTM patients on a regular hemotransfusion regimen compared to healthy controls. Jabbar et al. also found elevated serum MDA in BTM patients compared to healthy controls. A similar result was obtained by Patne et al. in India and *Gunarish et al*. in Indonesia. In 2021, Basu et al. published a meta-analysis of all case-control studies examining and comparing MDA and serum ferritin values in BTM patients and healthy controls. The results of the meta-analysis showed that serum levels of MDA and serum ferritin were higher in BTM patients ($p<0.001$), regardless of disease severity, age, sex, and frequency of hemotransfusions. *Atmakusuma et al.* (2021) initiated a study that aimed to compare MDA levels as a marker of OS in patients with TBT and non-transfusion-dependent beta-thalassemia before and after transfusion. This is also the first study to look for a correlation between serum ferritin and transferrin levels with plasma MDA before and after transfusion. The authors found that in both groups of patients no significant difference was found in both MDA levels and correlations of MDA with levels of serum HF markers before and after hemotransfusion. They hypothesized that the lack of plasma MDA increase after blood transfusion in some subjects may be related to routine antioxidant supplementation.

The lack of a significant difference in MDA values in our studied BTM patients and healthy controls could be explained by the intake of a larger amount of antioxidants by the patients in the study period, which coincides with the COVID-19 pandemic. In accordance with the recommendations for nutrition during infections for the additional import of micronutrients (*Goldberg, 2018; Semova, 2015; D'Arqom, 2020*) and with the opinion of TIF and other experts that patients with hemoglobinopathies are exposed to an increased risk of developing of severe complications from COVID-19 (*D'Arqom, 2020; Chowdhury, 2020; Taher, 2020; De Sanctis, 2020*; *Farmakis, 2020*; *Ghatreh-Samani, 2016*), all BTM patients from our center have been taking multivitamins and nutritional supplements for a long time, including the antioxidants vit. E, vit. C and vit. D. In support of our assumption about the relationship of exogenous antioxidants with oxidative stress is the study of

Trangsuwan et al., in which the authors prove that low levels of vit. E can be increased by prolonged intake of medicinal vit. E.

Discussing the possible reasons for the lack of significance when comparing the MDA values in the two studied groups, we consider as a weakness of our study the missed opportunity to compare the serum levels of antioxidant vitamins and their comparison with the MDA values. These studies were not included in the study design due to the fact that the scientific project on the topic was approved by the Committee on Ethics of Scientific Research (KENI) at the University of Medicine - Varna before the onset of the epidemic of COVID-19. To confirm and explain our suspected reason for non-significance of MDA values in BTM patients and healthy controls, we recommend conducting a further study.

2.6. Comparative analysis and discussion of echo-tracking parameters of the carotid arteries in patients with BTM and the control group

Results for task 5: *To measure and compare the local arterial stiffness of the two carotid arteries by means of echo-tracking (ET) methodology in patients with BTM and healthy controls***.**

On *Tabl. 6* are presented ET parameters of the right common carotid artery of the patients and healthy controls in the study.

Tabl. 6. *ET parameters of the right common carotid artery of the participants*

Parameter	Patients	Controls	p-value
β-stiffness index (R)	4,63±2,38	4,46±1,53	p=0.716
PWVβ (R) (*m/s*)	4.35±1,07	4.24±0,75	p=0.795
Ep (R) (*kPa*)	53,10±26,6	49,42±18,6	p=0.864
AIx (R) (*%*)	15,29±21,29	21,10±29,80	p=0.096
AC (R) (*mm^2/kPa*)	1,54±0,75	1,23±0,41	**p=0.05**

(R): right common carotid artery

When examining the ET indicators of the right common carotid artery, we obtained the following results:

- β-stiffness index (R): the mean values of β-stiffness index (R) in the patient group was 4.63±2.38 and in the healthy controls it was 4.46±1.53. The difference is not significant, p=0.716
- PWVβ (R): the mean PWVβ (R) in the patient group was 4.35±1.07 and in the healthy controls it was 4.24±0.75. No significant difference was found, p=0.795

- Ep (R): the mean values of Ep (R) in the patient group was 53.10±26.6 and in the healthy controls it was 49.42±18.6. No significant difference was found, p=0.864
- AIx (R): In patients with BTM, the measured mean values of AIx (R) were 15.29±21.29, and in healthy controls, respectively, 21.10±29.80. The difference is not significant, p=0.096
- AC (R): the mean values of AC (R) in the patient group was 1.54±0.75 and in the healthy controls it was 1.23±0.41, with a borderline significance level, p=0.05.

Analysis of ET indicators of the right common carotid artery found a borderline level of significance only in AC (R), p=0.05

Tabl. 7 presents ET parameters of the left common carotid artery of the patients and healthy controls in the study.

Tabl. 7. *ET parameters of the left common carotid artery of the patients and healthy controls in the study.*

Parameter	Patients	Controls	p-value
β-stiffness index (L)	4,51±2,46	4,26±1,24	p=0.582
PWVβ (L) (*m/s*)	4,29±1,13	4,16±0,75	p=0.975
Ep (L) (*kPa*)	51,89±28,90	47,15±15,54	p=0.996
AIx (L) (*%*)	14,13±22,51	11,77±21,83	p=0.720
AC (L) (*mm^2/kPa*)	1,56±0,79	1,26±0,41	p=0.107

(L): left common carotid artery

When examining the ET indicators of the left common carotid artery, we obtained the following results:

- β-stiffness index (L): the mean values of β-stiffness index (L) in the patient group was 4.51±2.46 and in the healthy controls it was 4.26±1.24. The difference is not significant, p=0582
- PWVβ (L): mean PWVβ (L) in the patient group was 4.29±1.13 and in healthy controls it was 4.24±0.75. No significant difference was found, p=0.975
- Ep (L): the mean values of Ep (L) in the patient group was 51.89±28.90 and in the healthy controls it was 47.15±15.54. No significant difference was found, p=0.996

- AIx (L): In patients with BTM, the measured average values of AIx (L) were 14.13±22.51, and in healthy controls, respectively, 11.77±21.83. The difference is not significant, p=0.720
- AC (L): mean AC (L) values in the patient group was 1.56±0.79 and in the healthy controls it was 1.26±0.41, no significant difference, p=0.107.

The analysis of the ET indicators of the left common carotid artery did not establish a statistically significant difference in the compared indicators.

Discussion:

In the analysis of the results of the echographic examination of the carotid arteries, we did not find a significant difference between the two studied groups regarding the mean values of the β-stiffness index and the values of PWVβ in both carotid arteries, but the values in the patients were higher. No statistically significant difference was found regarding the Er and Alx parameters either. A difference was found only when comparing the mean AC values of the right carotid artery – 1.54±0.75 in patients with BTM versus 1.23±0.41 in controls (p=0.05). A possible explanation could be the younger age of our patients.

Cheung et al. in 2002 conducted a study in which 30 patients with BTM aged 22.2 ± 7.4 years without overt heart disease and a corresponding number of healthy controls were included. The aim of the research team is to look for echocardiographic changes in the myocardium and to assess the endothelial function of the participants through blood flow-mediated vasodilation of the carotid and brachial arteries. *Cheung et al.* reported that significantly greater left ventricular muscle mass, increased carotid artery stiffness and decreased brachial artery pulse velocity and impaired blood flow-mediated dilation were measured in BTM patients. The authors attributed the results to the high iron overload, tissue damage from the accumulation of free oxygen radicals, and low levels of nitric oxide in the patient group. The aforementioned factors cause increased vascular stiffness, endothelial vascular dysfunction with impaired ventricular-vascular coupling, and left ventricular muscle hypertrophy, which lead to left ventricular diastolic dysfunction and subsequently systolic thalassemic cardiomyopathy. In the study by *Cheung et al.* the stiffness index of the carotid artery was significantly higher in patients (8.1±3.5) compared to controls (5.5±1.6), p=0.001. Also, brachioradial PWV was higher in patients (8.9±2.4 m/s) versus controls (7.9±1.7 m/s), p=0.03.

Our results are similar to those reported by *Cusmà Piccione et al*. in 2013, in 32 patients (23 women, mean age 35 ±8 years) with BTM and 33 healthy controls (20 women, mean age 35 ±6 years). The purpose of the study was to evaluate the left ventricular myocardial deformation and the stiffness of the carotid arteries using

two-dimensional strain and echo-tracking studies in asymptomatic patients with BTM and to look for subclinical cardiovascular changes. The thickness of the carotid intima-media complex was measured in both groups, with no significant difference in mean values (0.67±0.20 mm vs. 0.66±0.15 mm). After applying the echo-tracking methodology, the researchers found that the β-stiffness index of the patients was 6.16±1.31, and that of the healthy controls was 4.65±0.82, the difference being significant ($p<0.001$). Similar to our results, the difference in PWV values between the two groups was not significant, however, the values in patients were higher than controls ($p=0.07$). The researchers' conclusion is that two-dimensional strain and echo-tracking techniques are more accurate than standard echocardiography and carotid Doppler for identifying early signs of cardiovascular involvement. The data they obtained are in agreement with previous findings and confirm that even well-chelated patients develop early vascular damage, presenting as increased arterial stiffness. The authors also demonstrate that measuring the thickness of the intima-media complex of carotid arteries very often cannot reflect the presence of the earliest vascular changes and recommend that other parameters, such as arterial stiffness, be investigated to more precisely assess vascular involvement in patients with BTM.

3. Correlatios in the group of patients with BTM

3.1 Correlations between serum ferritin levels and lipid profile indicators and lipid indices

Outcomes for task 6: *To examine the correlations between serum ferritin levels and indices of lipid metabolism and some atherogenic lipid indices in patients with BTM.*

Tabl. 8 presents the correlation dependences between serum ferritin values and lipid profile indicators in patients with BTM.

Tabl. 8. *Correlations between serum ferritin values and lipid profile indicators in patients with BTM.*

		TC	LDL	HDL	TG
Serum ferritin	*Pearson* correlation **(r)**	.602**	.656**	-.222	.401*
	p	**0.0001**	**0.0001**	0.187	**0.014**

When conducting a correlation analysis using the Pearson method, we obtained the following results:

- Strong positive correlation between serum ferritin values and total cholesterol values ($r=0.602$, $p=0.0001$)

• Strong positive correlation between serum ferritin and LDL-cholesterol values (r=0.656, p=0.0001)

• Lack of significant correlation between serum ferritin and HDL-cholesterol values (r=-0.222, p=0.187)

• Moderate positive correlation between ferritin values and triglycerides (r=0.401, p=0.014).

Tabl. 9 presents the correlation dependences between serum ferritin values and lipid indices in patients with BTM.

Tabl. 9. *Correlation dependences between serum ferritin values and lipid indices in patients with BTM.*

		CRI-I	**CRI-II**	**AIP**	**AtC**	**non-HDL**
Serum ferritin	*Pearson* Correlation **(r)**	.702**	.722**	.282	.709**	.746**
	p	**0.0001**	**0.0001**	0.091	**0.0001**	**0.0001**

When conducting a correlation analysis using the Pearson method, we obtained the following results:

- Strong positive correlation between serum ferritin values and CRI-I (r=0.702, p=0.0001) and CRI-II (r=0.722, p=0.0001)
- Lack of significant correlation between serum ferritin values and AIP (r=0.282, p=0.091)
- Strong positive correlation between serum ferritin values and AtC (r=0.709, p=0.0001)
- Strong positive correlation between serum ferritin and non-HDL-cholesterol values (r=0.746, p=0.0001).

The analysis of the results obtained by us proves that the higher levels of serum ferritin in children and young adults with BTM have a strong positive correlation with a large number of parameters of lipid metabolism and lipid indices such as total cholesterol, LDL-cholesterol, triglycerides, CRI- I, CRI-II and AtC.

The statistically significant correlations are also presented in a graphical version (*Fig 7* to *Fig. 13*):

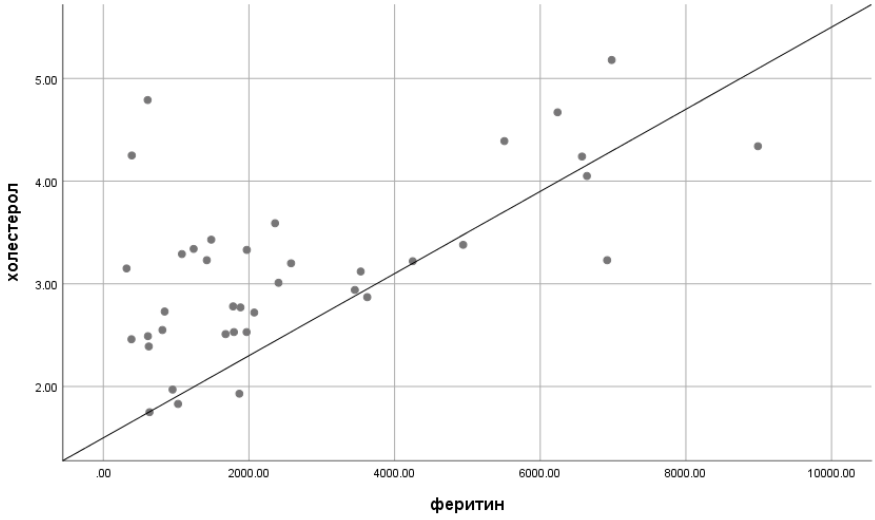

Fig. 7. *Correlation between serum ferritin values and total cholesterol values* **(r=0,602, p=0.0001)**

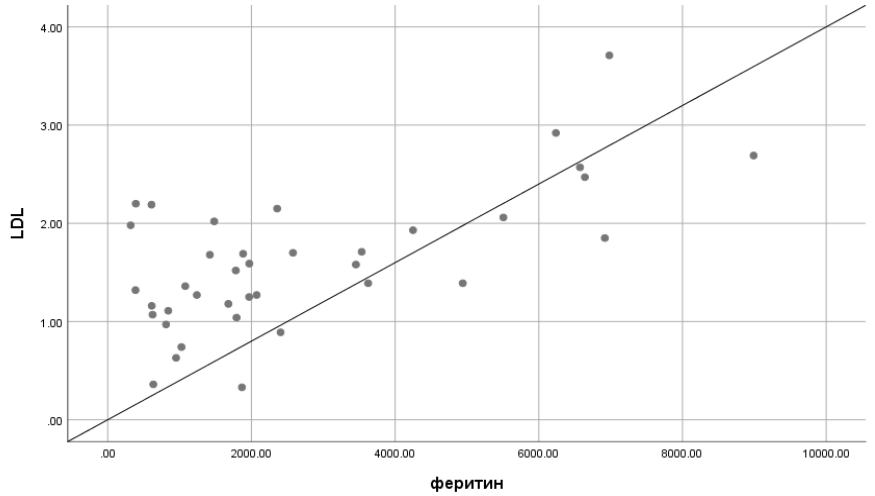

Fig. 8. *Correlation between serum ferritin values and LDL-cholesterol values* **(r=0,656, p=0.0001)**

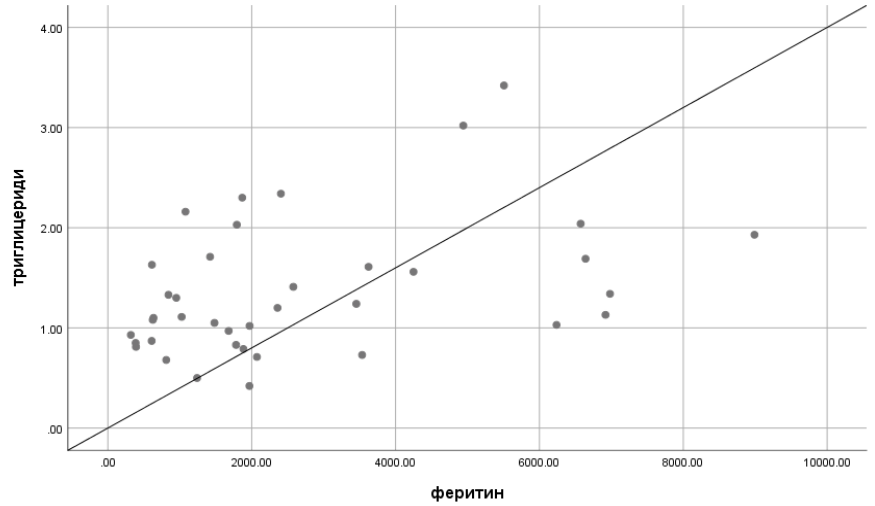

Fig. 9. *Correlation between serum ferritin values and triglyceride values* **(r=0,401, p=0.014)**

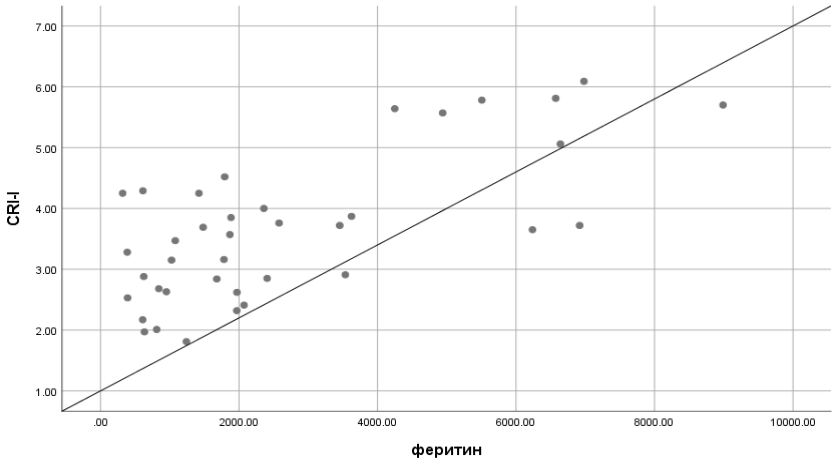

Fig. 10. *Correlation between serum ferritin values and CRI-I values* **(r=0,702, p=0.0001)**

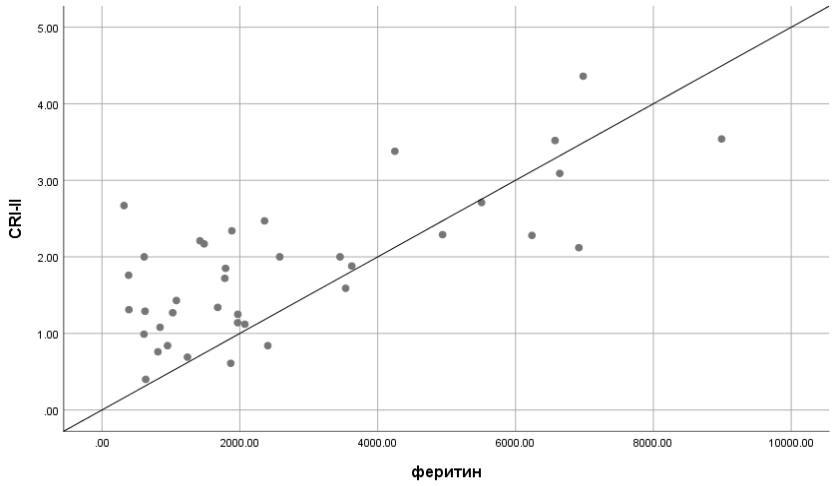

Fig. 11. *Correlation between serum ferritin values and CRI-II values* **(r=0,722, p=0.0001)**

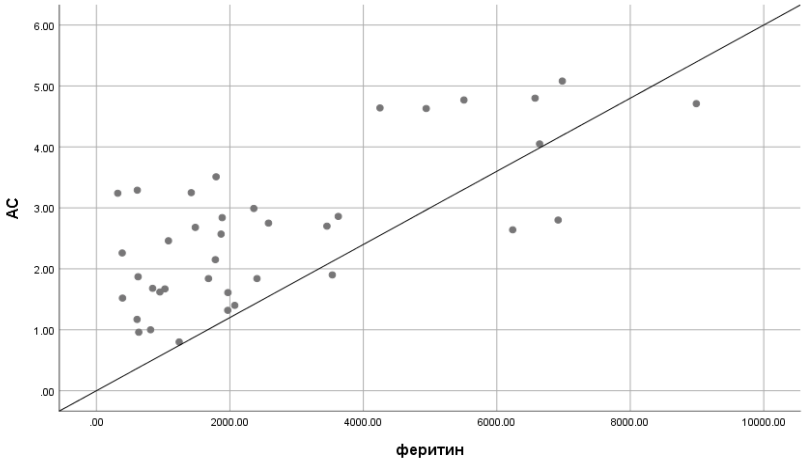

Fig. 12. *Correlation between serum ferritin values and AtC values* **(r=0,709, p=0.0001)**

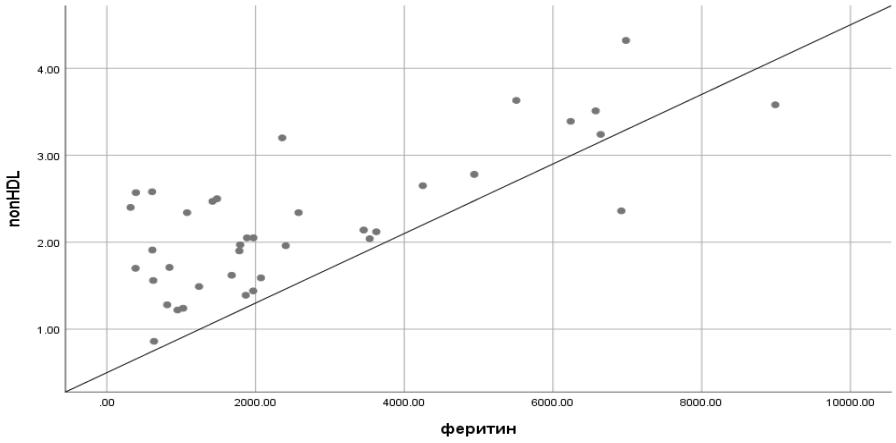

Fig 13. *Correlation between serum ferritin values and non-HDL-cholesterol values* **(r=0,746, p=0.0001)**

Discussion:

A similar dependence in patients with BTM has been established by many other authors. In 2008 in Jordan, *Mansi et al*. have described a positive correlation between serum ferritin and triglyceride levels, characterizing this as an "important predictor of atherosclerosis". In later studies, *Ragab et al.* from Iraq (2014), *Sherief et al.* from Egypt (2017) and *Suman et al.* from India (2017) also demonstrate a positive correlation between ferritin and triglycerides and support the hypothesis of their involvement in the pathogenesis of LDL-cholesterol oxidation. When comparing these indicators in our study, we obtained similar correlations (r=0.401, p=0.014), which do not differ from those published in 2012 by *Arica et al.* Their study was conducted in 85 children with BTM and 55 healthy controls in Turkey, where they found a significant correlation between triglyceride levels and serum ferritin.

Shams et al. in 2009 in Iran studied 78 children with homozygous BTM aged 16±6 years who were treated in their center and received regular iron chelation therapy. The study also included 122 healthy children aged 14±4 years, who had no evidence of hematological or other chronic inflammatory disease. Lipid profile, serum ferritin, liver enzymes, insulin and fasting glucose were examined on both groups of participants. Total cholesterol values in the patient group were significantly lower than controls (3.4±1.2 vs. 4.1±0.7 mmol/L, p=0.0001). The published data is in agreement with the results obtained by us, incl. and for the significance between the mean values of total cholesterol in the two studied groups – 3.16±0.83 mmol/L versus 3.94±0.77 mmol/L (p=0.0001). Unlike us, the authors did not find a statistically significant

correlation between total cholesterol and ferritin values. In our study, this correlation was strongly positive (r=0.602, p=0.0001).

In 2014, *El Gindi et al.* from Egypt, when looking for correlations between serum ferritin values and other lipid parameters in children with BTM, found a strong positive correlation with LDL-cholesterol (r=0.301, p=0.003), a strong negative correlation with HDL-cholesterol (r= -0.326 , p=0.001) and no correlation with serum triglycerides. When comparing their results with those of our study, we found a match only with regard to correlations with LDL-cholesterol, which were strongly positive in both studies. Regarding HDL-cholesterol, the correlations in both studies were negative, but lacked statistical significance in our patients.

A strong positive correlation between serum ferritin and LDL-cholesterol in patients with BTM was also reported by *Hartman et al.* from Israel (2002). Analyzing the obtained results, the authors conclude that this strong dependence is due to the high sensitivity of the lipid fraction LDL-cholesterol to the effects of free iron and oxidative stress.

In 2014, *Boudrahem-Addour et al*. reported a positive association between elevated TC/HDL-c ratio (CRI-I) values with serum ferritin and triglyceride levels, especially in young BTM patients. By associating higher CRI-I values with increased coronary risk, the authors suggest that CRI-I should be included in the follow-up parameters of BTM patients, especially for splenectomy survivors. In our study, we found a strong positive correlation between both serum ferritin and CRI-I values (r=0.702, p=0.0001) and between serum ferritin and CRI-II. (r=0.722, p=0.0001)

Ray et al. in 2023 in India reported a positive correlation of CRI-I (r=0.286, p=0.015), CRI-II (r=0.273, p=0.020) and AtC (r=0.286, p=0.015) with levels of serum ferritin. These results are consistent with the strong positive correlations we obtained between ferritin values and CRI-I (r=0.702, p=0.0001), CRI-II (r=0.722, p=0.0001) and AtC (r=0.709, p =0.0001).

In conclusion, considering the atherogenic potential of lipid parameters and the positive correlations found by us and other authors with serum ferritin, we suggest that serum ferritin should be included as an additional marker for CVD risk assessment in BTM patients. On the other hand, we assume that the disturbed lipid profile in patients with BTM may serve as an indirect evidence of the presence of iron overload and an indirect criterion for evaluating the effect of chelation treatment.

3.2 Correlation relationships between hemoglobin values and indicators of lipid profile and lipid indices

Outcomes for Task 7: *To examine correlations between hemoglobin values and lipid profile indicators and lipid indices.*

On *Tabl. 10* are presented the correlation dependences between hemoglobin values and lipid profile indicators in patients with BTM.

Tabl. 10. *Correlations between hemoglobin values and lipid profile indicators in patients with BTM*

		TC	LDL	HDL	TG
Hb	*Pearson* Correlation	0.532**	0.517**	0.489**	-0.295
	p	**0.001**	**0.001**	**0.002**	0.072

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Strong positive correlation between hemoglobin values and total cholesterol (r=0.532, p=0.001)

• Strong positive correlation between hemoglobin values and LDL-cholesterol (r=0.517, p=0.001)

• Moderate positive correlation between hemoglobin values and HDL-cholesterol (r=0.489, p=0.002)

• Lack of significant correlation between hemoglobin values and triglycerides (r= -0.295, p=0.072).

Tabl. 11 presents the correlation dependences between hemoglobin values and lipid indices in patients with BTM.

Tabl. 11. *Correlations dependences between hemoglobin values and lipid indices in patients with BTM are presented*

		CRI-I	CRI-II	AIP	AtC	non-HDL
Hb	*Pearson* Correlation	-0.046	0.196	-0.466**	-0.033	0.354*
	p	0.786	0.239	**0.003**	0.843	**0.029**

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Lack of significant correlation between hemoglobin values and CRI-I (r= -0.046, p=0.786)

• Lack of significant correlation between hemoglobin values and CRI-II (r=0.196, p=0.239)

• Moderate negative correlation between hemoglobin values and AIP (r= -0.466, p=0.003)
• Lack of significant correlation between hemoglobin values and AtC (r= -0.033, p=0.843)
• Moderate positive correlation between hemoglobin values and non-HDL-cholesterol (r= 0.354, p=0.029).

Correlation analysis of hemoglobin values and lipid profile indicators revealed strong positive significance with total cholesterol and LDL-cholesterol values, moderate positive with HDL-cholesterol values and moderate negative with AIP values. A similar relationship has been found in other studies of correlational relationships between these indicators in BTM patients worldwide.

Discussion:

In 2022 in India, *Jabbar et al.* found a significant negative correlation of triglycerides and hemoglobin among children with BTM. In our group of patients, this correlation was also negative, but not statistically significant. According to Jabbar et al. prolonged persistence of low hemoglobin levels places patients at increased risk of reduced extrahepatic lipolytic activity leading to high serum triglyceride levels. Regarding the correlations between hemoglobin values and total cholesterol, our results of a strong positive relationship (r=0.532, p=0.001) fully coincide with the results of the aforementioned study (r=0.041, p<0.001). They are analogous to the results obtained by *Daswani et al.* from India (2021), who explain them to the increased consumption of cholesterol to build the erythroid membrane and the presence of a hyperplastic and hyperactivated reticuloendothelial system in BTM patients. *Daswani et al.* also reported a significant positive correlation of hemoglobin with LDL-cholesterol (p=0.033, β=1.594), which was also confirmed in our study (r=0.517, p=0.001). Unlike them, however, who did not find a correlation between hemoglobin and HDL-cholesterol, our results prove it in a moderate degree of positive dependence (r=0.489, p=0.002).

In the cited study by *Ray et al.* from 2023, the authors also reported a significant negative correlation of hemoglobin with CRI-I (r=-0.320, p=0.001) and CRI-II (r=-0.266, p <0.001), AIP (r=-0.647, p <0.001) and AtC (r=-0.320, p <0.001) in children with BTM. In contrast, in our patients only a moderate negative correlation was found between hemoglobin values and AIP (r=-0.466,

p=0.003), thus proving that as hemoglobin values decrease, so does the atherogenic potential.

Conclusion:

In BTM patients, lipid indices (CRI-I, CRI-II, AtC) were significantly correlated with pretransfusion hemoglobin levels and serum ferritin levels. Lower pre-transfusion hemoglobin levels are associated with more severe dyslipidemia, which means that ineffective erythropoiesis is not suppressed and this leads to higher levels of oxidative stress and hence exacerbates the degree of dyslipidemia. These results and findings confirm the need to maintain pretransfusion hemoglobin levels above 95 g/L as recommended by the International Thalassemia Federation. One of the discussed reasons for this laboratory constellation is the presence of increased consumption of cholesterol, necessary for the construction of the cell membrane and the presence of a hyperplastic and overactive reticuloendothelial system. (*Ricchi, 2009*).

3.3 Correlation dependences between ET parameters of both carotid arteries with sex, age, indicators of lipid exchange and atherogenic lipid indices in patients with BTM

Outcomes for task 8: *To investigate the correlations of ET parameters of both carotid arteries with gender, age, lipid metabolism indices and atherogenic lipid indices in patients with BTM.*

3.3.1 Correlation dependences between ET parameters of both carotid arteries and age in patients with BTM

Tabl. 12 presents the correlation dependences between age and ET indicators of the right carotid artery in patients with BTM, and *Tabl. 13* – the correlation dependences between age and ET indicators of the left carotid artery.

Tabl. 12. *Correlation dependences between age and ET indicators of the right carotid artery in patients with BTM*

		β-stiffness (R)	**Ep (R)**	**AIx (R)**	**AC (R)**	**PWVβ (R)**
Age	*Pearson* correlation	.491**	.486**	.141	-.320	.486**
	p	**0.002**	**0.002**	0.399	**0.050**	**0.002**

(R): right common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Moderate positive correlation between age and β-stiffness index (R) values of patients (r= 0.491, p=0.002)

• Moderate positive relationship between age and Ep values (R) (r=0.486, p=0.002)
• Lack of significant correlation between age and AIx values (R) (r=0.141, p=0.399)
• Moderate negative correlation of age of patients with AC (R) (r= -0.320, p=0.050)
• Moderate positive correlation of patients' age and PWVβ values (R) (r=0.486, p=0.002).

Tabl. 13. *Correlation dependences between age and ET indicators of the left carotid artery in patients with BTM*

		β-stiffness (L)	**Ep (L)**	**AIx (L)**	**AC (L)**	**PWVβ (L)**
Age	*Pearson* correlation	.609**	.572**	.107	-.274	.615**
	p	**0.0001**	**0.0001**	0.522	0.096	**0.0001**

(L): left common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

- Strong positive correlation between age and β-stiffness index (L) values (r= 0.609, p=0.0001)
- Strong positive correlation between age and Ep (L) values (r= 0.572, p=0.0001)
- Lack of significant correlation between age and AIx values (L) (r=0.107 p=0.522)
- Lack of significant correlation between age and AC values (L), (r=-0.274 p=0.096)
- Strong positive correlation of age and PWVβ (L) values (r= 0.615, p=0.0001).

The statistically significant correlations are also presented in a graphical version (*Fig. 14* to *Fig. 20*):

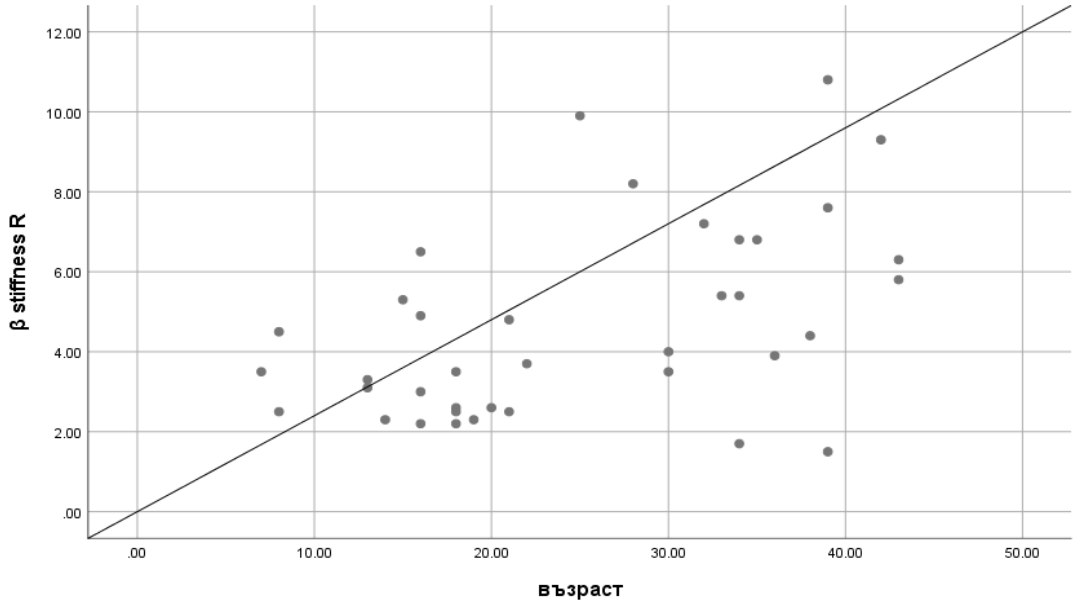

Fig. 14. *Correlation between age and values of β-stiffness index (R)* **(r=0,491, p=0.002)**

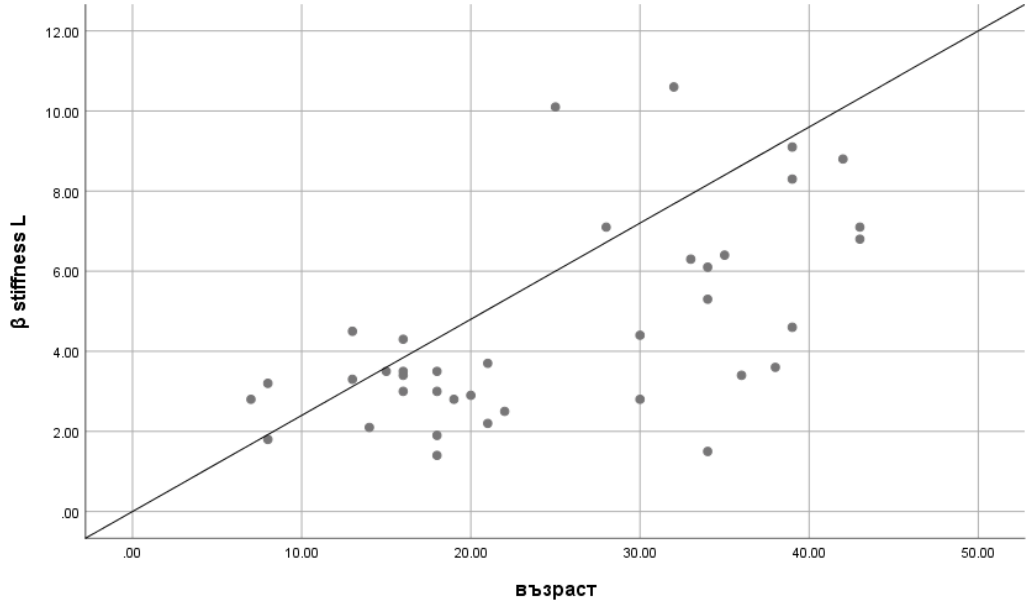

Fig. 15. *Correlation between age and values of β-stiffness index (L)* **(r=0,609, p=0.0001)**

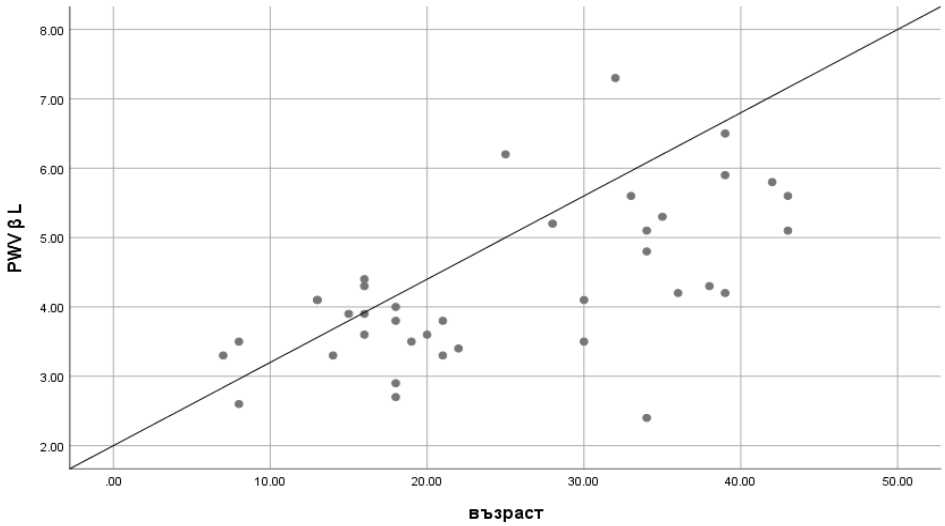

Fig. 16. *Correlation between age and values of PWV*β (L) **(r=0,615, p=0.0001)**

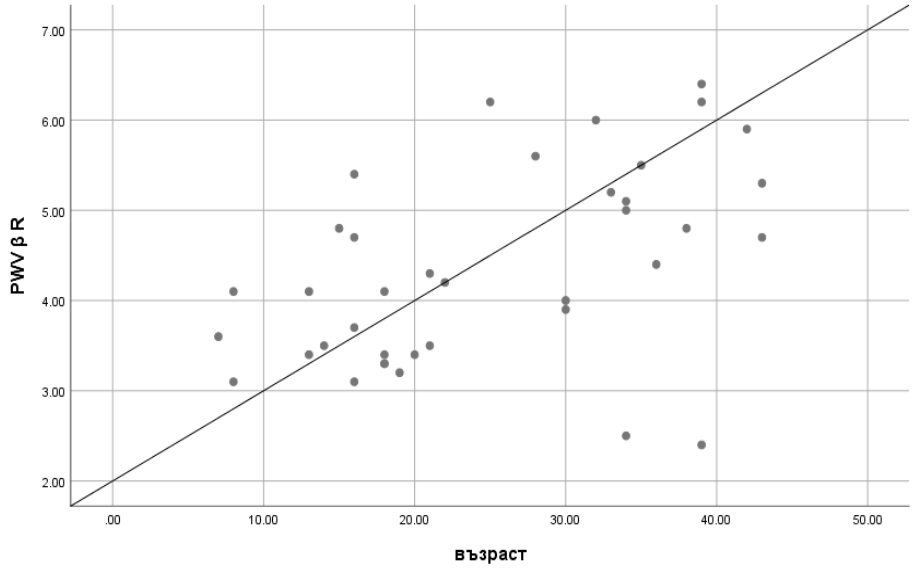

Fig. 17. *Correlation between age and values of PWV*β (R) **(r=0,486, p=0.002)**

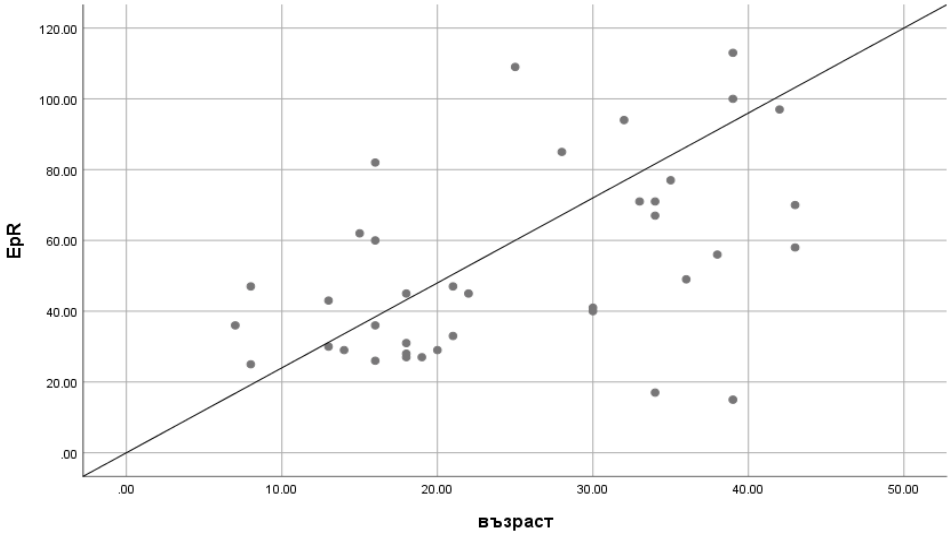

Fig. 18. *Correlation between age and values of Ep* (R) **(r=0,486, p=0.002)**

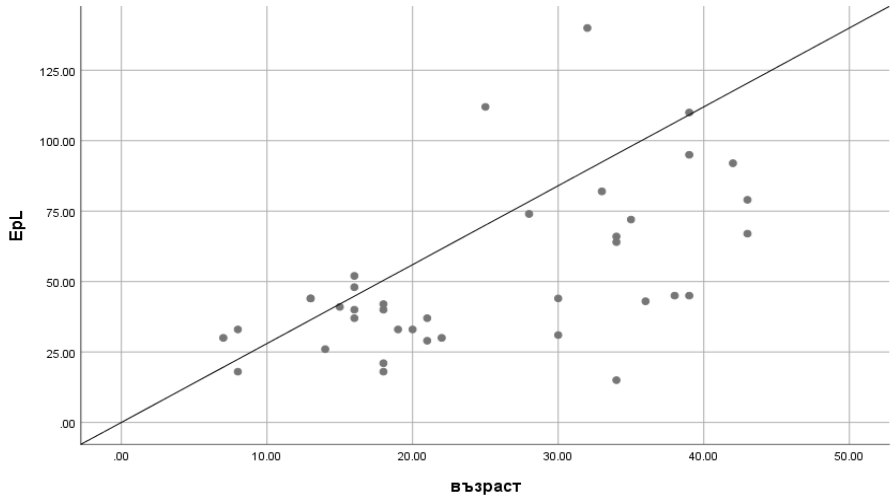

Fig. 19. *Correlation between age and values of Ep* (L) **(r=0,572, p=0.0001)**

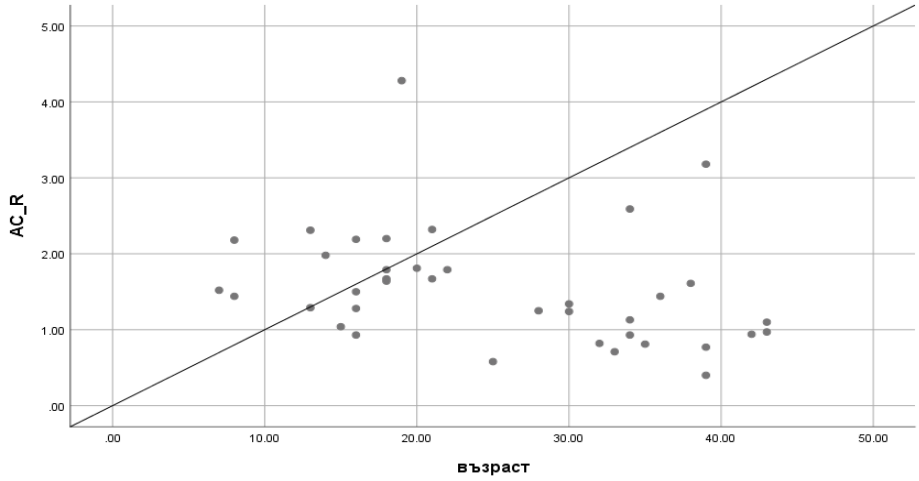

Fig. 20. *Correlation between age and values of AC* (R) **(r= -320, p=0.050)**

Discussion:

In our group of BTM patients, there was a moderate positive correlation between age and PWVβ (R) values (r=0.486, p=0.002) and a strong positive correlation between age and PWVβ (L) values (r=0.615, p=0.0001). In our group of patients with BTM, there was a moderate positive correlation between age and β-stiffness (R) values (r=0.491, p=0.002) and a strong positive correlation between age and β-stiffness (L) values (r=0.609 , p=0.0001). When comparing these indicators in patients under and over 26 years of age, higher values were also found in the older age group. Our results confirm the data published in the literature that changes in arterial elasticity begin at a young age and increase with increasing age (see *Table 14*) (*Cheung, 2002*).

Табл. 14. *Comparative analysis of ET indicators of the right and left common carotid artery of patients with BTM under and over 26 years.*

Patients	Age	N	Mean values± SD	Independent t test/p-value	Confidence interval
β-stiffness (R)	<26 г	21	3.7±1.84	t= -2.97, **p=0.005**	[-3.53; -0.66]
	>26 г	17	5.8±2.50		
PWVβ (R)	<26 г	21	3.9±0.80	t= -2.98, **p=0.005**	[-1.59; -0.30]
	>26 г	17	4.87±1.15		
	>26 г	17	0.73±0.06		
β-stiffness (L)	<26 г	21	3.30±1.74	t= -3.87, **p=0.001**	[-1.14; -1.26]
	>26 г	17	6.01±2.43		
PWVβ (L)	<26 г	21	3.72±0.74	t= -4.09, **p=0.0001**	[-1.90; -0.64]
	>26 г	17	4.99±1.15		
	>26 г	17	0.71±0.06		

Many studies demonstrate a non-linear increase in PWV with age, with an acceleration observed after the 5th decade (*Loboz-Rudnicka, 2008*). An increase in PWV also leads to an increase in cardiovascular risk and this is observed in both hypertensive and non-hypertensive individuals (*Hamburg, 2008*). Our data, obtained using the ET methodology, found that in patients with BTM, arterial stiffness increased with advancing age and confirmed the results of other authors obtained using flow-mediated vasodilation (*Cheung, 2002*).

Noori et al. compared aortic stiffness in children and young adults by enrolling in a study 130 participants aged between 6 and 19 years, of whom 65 were BTM patients and 65 sex- and age-matched healthy controls. To determine indices of arterial stiffness, researchers used two-dimensional (2D) and Doppler echocardiography. Similar to our results, the authors reported that aortic arterial stiffness indices increased with age. Therefore, aortic stiffening can be considered a marker reflecting the age of the arteries and can be considered as a major risk factor for cardiovascular events. *Noori et al.* suggest that arterial stiffness parameters are influenced more by age and not so much by the level of BP.

It is well known that the atherosclerotic process causes changes in both the structure and function of the arterial tree. In adults, arterial stiffness is associated with atherosclerotic risk factors and has become an important predictor of cardiovascular events and mortality (*Laurent, 2006; Pereira, 2015*). Data on studies measuring and evaluating arterial stiffness in pediatric populations are scarce (*Riggio, 2010; Urbina, 2009*). There is increasing interest in the effects of age and gender on carotid intima-media thickness and arterial stiffness in childhood (*Doyon, 2013*).

Calabro et al. studied 131 healthy children aged 3–16 years who were without cardiovascular risk factors and without cardiac pathology. The aim of the researchers was to determine the values of the thickness of the intima-media complex of the carotids and to assess the presence of arterial stiffness using the ET method. A relationship was sought between the two indicators and the age, gender and height of the studied cohort of healthy children of Italian origin. A significant positive correlation was found between age and β-stiffness index and between height and β-stiffness index in both sexes, with the correlation coefficient being higher in boys. The researchers observed that the values of the β-stiffness index progressively increased throughout childhood, independent of gender or height, while the thickness of the carotid intima-media complex depended on both indicators.

3.3.2 Correlation dependences between ET parameters of both carotid arteries and gender in patients with BTM

On *Tabl. 15* are presented the correlation dependences between gender and ET indicators of the right carotid artery in patients with BTM and on *Tabl. 16* – correlational dependences between gender and ET parameters of the left carotid artery.

Tabl. 15. *Correlation dependences between gender and ET indicators of the right carotid artery in patients with BTM*

		β-stiffness (R)	**Ep (R)**	**AIx (R)**	**AC (R)**	**PWV β (R)**
Gender	Spearman's rho	.356* women	.320	-.053	-.437** men	.320
	p	**0.028**	**0.050**	0.753	**0.006**	0.050

(R): right common carotid artery

When conducting a Spearman correlation analysis, we obtained the following results:

• Moderate positive relationship between gender and β-stiffness (R), more pronounced in women (rho=0.356, p=0.028)
• Lack of significant correlation between gender and AIx (R) (rho= -.053, p=0.753)
• Moderate negative correlation between gender and AC (R), showing strong dependence at high levels among men (rho= -0.437, p=0.006).

Tabl. 16. *Correaltions between gender and ET parameters of the left carotid artery.*

		β-stiffness (L)	Ep (L)	AIx(L)	AC (L)	PWVβ (L)
Gender	Spearman's rho	.363* women	.351* women	.043	-.464** man	.339* women
	p	**0.025**	**0.031**	0.797	**0.003**	**0.037**

(L): left common carotid artery

When conducting a Spearman correlation analysis, we obtained the following results:

• Moderate positive relationship between gender and β-stiffness (L), more pronounced in women (rho=0.363, p=0.025)

• Moderate positive relationship between gender and Ep (L) values, more pronounced in women (rho=0.351, p=0.031)

• Lack of significant correlation between gender and AIx (L) (rho= -.043, p=0.797)

• In AC (L), the negative correlation shows a strong dependence of high levels among men (rho= -0.464, p=0.003)

• Moderate positive relationship between gender and PWVβ (L), more pronounced in women (rho=0.339, p=0.037).

Discussion:

In the group of patients studied by us, higher values of arterial stiffness indicators were observed among women. A strong positive correlation between right and left carotid artery β-stiffness and gender was demonstrated, being more pronounced in women. A strong positive relationship was also found regarding PWVβ of the left carotid artery and gender, more pronounced in women.

Similar to other cardiovascular pathologies, there are gender differences in arterial stiffness. Arterial stiffness, assessed by measuring carotid-femoral PWV, increases with age in both men and women (*Mitchell, 2004; DuPont, 2019*). The observed faster increase in arterial stiffness in women after the onset of menopause may be explained by the elimination of the action of estrogen and its cardioprotective action. Female sex hormones (estrogens) are thought to have a protective effect on vascular health. Studies have shown that premenopausal women are protected from developing CVD compared to men of the same age and that the incidence of CVD increases disproportionately after menopause (*Mitchell, 2004*). *Zaydun et al.* in 2006 demonstrated that menopause is a distinct, independent risk factor that complements the age-related development of arterial stiffness, assessed by measuring brachio-ankle PWV and the reduction in AC. A study by *Baker et al.* in 2003 reported that men may develop heart disease 10-15 years earlier than women due to a gradual decline in

estrogen levels after puberty. Conversely, men aged 70 had a lower overall cardiovascular risk than women aged 50 (the average age of menopause in women), suggesting that the decline in estrogen levels had a greater impact on the risk of CVD in women than in men (*Lloyd-Jones, 2006; Rodgers, 2019*).

A possible explanation for the higher values of β-stiffness index and PWVβ among women in our study group, despite the young age, could be found in hypogonadism. Endocrine glands are extremely sensitive to excess iron in the body because they have high levels of transferrin receptors. In developed countries, endocrine complications usually develop after 10 years of age, but with suboptimal chelation and hemotransfusion treatment, they could appear even earlier (*Al-Zukairy, 2020*). In BTM, most patients have hypogonadotropic hypogonadism, with iron overload considered to be a major factor involved in its pathogenesis (*DeSanctis, 2016*). Studies have reported an association between high serum ferritin levels and the presence of hypogonadism, as well as a more rapid evolution of hypogonadism in patients with significant iron overload (*Belhoul, 2013*). A study by *Chatterjee et al.* since 2000 found that in BTM patients who have less severe iron overload and organ damage along the hypothalamic-pituitary axis, hypogonadotropic hypogonadism may be reversible. In contrast, however, hypogonadism is irreversible in patients with severe iron overload.

Other causes are possible to explain hypogonadism in BTM patients. A study conducted by *Belhoul et al.* in 2012, demonstrated that splenectomy was associated with the presence of hypogonadism independent of serum ferritin levels, although the mechanism of this association was unknown (*Belhoul, 2012*). The severity of the underlying beta-globin synthesis gene defect, liver dysfunction, diabetes mellitus, hypothyroidism, and lower hemoglobin levels have also been reported as possible causes of hypogonadism in BTM patients. (*Chern, 2003; Dragos Albu, 2018*).

There is no direct evidence linking OS to gonadal dysfunction in BTM, but it could be a putative and reasonable explanation. A study conducted by *Appasamy et al.* in 2008 among patients undergoing assisted reproduction demonstrated that total antioxidant capacity was positively correlated with follicular fluid estradiol levels, indirectly supporting the thesis that OS may have a negative impact on follicular development. Iron deposition in ovarian stromal tissue has also been shown to be associated with OS-induced ovarian senescence (*Asano, 2012*). According to a study by *Al-Azemi et al.* as of 2009, low levels of antioxidants have been linked to anovulation in women.

3.3.3 Correlation dependences between ET parameters of both carotid arteries and lipid profile in patients with BTM

Tabl. 17 presents the correlation dependences between the values of the β-stiffness index of the right carotid artery and the indicators of the lipid profile in the patients with BTM, and *Table. 18* – the correlation dependences of the β-stiffness index of the left carotid artery and the indicators of the lipid profile.

Tabl. 17. *Correlation dependences between the values of the β-stiffness index of the right carotid artery and the indicators of the lipid profile in the patients with BTM*

		TC	LDL	HDL	TG
β -stiffness (R)	*Pearson* Correlation	.301	.304	-.228	.385*
	p	0.067	0.063	0.169	**0.017**

(R): right common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Absence of significant correlations between β-stiffness (R) and the values of total cholesterol (r=0.301, p=0.067), LDL-cholesterol (r=0.304, p=0.063) and HDL-cholesterol. (r= -0.228, p=0.169)
• Moderate positive correlation between β-stiffness (R) and triglyceride values (r= 0.385, p=0.017).

Tabl. 18. *Correlation dependences of the β-stiffness index of the left carotid artery and the indicators of the lipid profile.*

		TC	LDL	HDL	TG
β-stiffness (L)	*Pearson* Correlation	.363*	.318	-.099	.378*
	p	**0.025**	**0.052**	0.554	**0.019**

(L): left common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:
• Moderate positive correlation between β-stiffness (L) and total cholesterol values (r=0.363, p=0.025)
• Lack of significant correlation between β-stiffness (L) and the values of HDL-cholesterol (r= -0.099, p=0.554) and LDL-cholesterol (r=0.318, p=0.052)
• Moderate positive correlation β-stiffness index (L) and triglyceride values (r= 0.378, p=0.019).

On *Tabl. 19* are presented the correlation dependences between the values of PWVβ of the right carotid artery and the indicators of the lipid profile in the patients with BTM, and on *Tabl. 20* – the correlation dependences between the PWVβ values of the left carotid artery and the indicators of the lipid profile.

Tabl 19. *Correlation relationships between right carotid artery PWVβ values and lipid profile indicators in BTM patients*

		TC	LDL	HDL	TG
PWVβ (R)	*Pearson* Correlation	.221	.224	-.265	.396*
	p	0.182	0.177	0.108	**0.014**

(R): right common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Lack of significant correlation between PWVβ values (R) and total cholesterol (r=0.221, p=0.182), LDL-cholesterol (r=0.224, p=0.177) and HDL-cholesterol (r= -0.265, p =0.108)

• Moderate positive correlation between PWVβ values (R) and triglyceride values (r=0.396, p=0.014).

Tabl. 20. *Correlation relationships between left carotid artery PWVβ values and lipid profile indicators in BTM patients*

		TC	LDL	HDL	TG
PWVβ (L)	*Pearson* Correlation	.296	.248	-.119	.377*
	p	0.072	0.133	0.475	**0.020**

(L): left common cartid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Lack of significant correlation between PWVβ values (L) and total cholesterol (r=0.296, p=0.072), LDL-cholesterol (r=0.248, p=0.133) and HDL-cholesterol (r=-0.119, p =0.475)

• Moderate positive correlation between PWVβ values (L) and triglyceride values (r= 0.377, p=0.020).

3.3.4 Correlation dependences between ET parameters of both carotid arteries and lipid indices in patients with BTM

On *Tabl. 21* are presented the correlation dependences between the values of the β-stiffness index of the right carotid artery and the indicators of the lipid indices

in patients with BTM and on *Table. 22* – the correlation dependences of the β-stiffness index of the left carotid artery and the indicators of the lipid indices.

Tabl. 21. *Correlation relationships between the values of the β-stiffness index of the right carotid artery and the parameters of the lipid indices in patients with BTM*

		CRI-I	**CRI-II**	**AIP**	**AtC**	**non-HDL**
β-stiffness (R)	*Pearson* Correlation	.514**	.463**	.267	.521**	.457
	p	**0.001**	**0.003**	0.105	**0.001**	**0.004**

(R): right common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Strong positive correlation between β-stiffness (R) and CRI-I values (r=0.514, p=0.001)

• Moderate positive correlation between β-stiffness (R) and CRI-II values (r=0.463, p=0.003)

• Lack of significant correlation between β-stiffness (R) and AIP values (r=0.267, p=0.105)

• Strong positive correlation between β-stiffness (R) and AtC values (r=0.521, p=0.001).

• Moderate positive correlation between β-stiffness (R) and non-HDL-cholesterol values (r=0.457, p=0.004)

Tabl. 22. *Correlation relationships between the values of the β-stiffness index of the left carotid artery and the parameters of the lipid indices in patients with BTM*

		CRI-I	**CRI-II**	**AIP**	**AtC**	**non-HDL**
β-stiffness (L)	*Pearson* Correlation	.495**	.442**	.206	.503**	.463
	p	**0.002**	**0.005**	0.215	**0.001**	**0.003**

(L): left common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Moderate positive correlation between β-stiffness (L) and CRI-I (r=0.495, p=0.002) and CRI-II (r= 0.442, p=0.005) values

• Lack of significant correlation between β-stiffness (L) and AIP values, (r= 0.206, p=0.215)

• Strong positive correlation between β-stiffness (L) and AtC values, (r=0.503, p=0.001)

• Moderate positive correlation between β-stiffness (L) and non-HDL-cholesterol values (r=0.463, p=0.003).

On *Tabl. 23* are presented the correlation dependences between the PWVβ values of the right carotid artery and the indicators of the lipid indices in the patients with BTM and on *Table. 24* – the correlation dependences of PWVβ of the left carotid artery and the indicators of the lipid indices.

Tabl. 23. *Correlation relationships between PWVβ values of the right carotid artery and lipid indices in patients with BTM*

		CR-I	**CR-II**	**AIP**	**AtC**	**non-HDL**
PWVβ (R)	*Pearson* Correlation	.493**	.431**	.285	.493**	.379
	p	**0.002**	**0.007**	0.082	**0.002**	**0.019**

(R): right common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Moderate positive correlation PWVβ values (R) and CRI-I (r=0.493, p=0.002), CRI-II (r=0.431, p=0.007) and AtC (r=0.496, p=0.002) values

• No significant correlation between PWVβ values (R) and AIP values (r=0.285, p=0.082)

• Moderate positive correlation between PWVβ values (R) and non-HDL-cholesterol values (r=0.379, p=0.019).

Tabl. 24. *Correlation relationships between PWVβ values of the left carotid artery and lipid indices in patients with BTM*

		CRI-I	**CRI-II**	**AIP**	**AtC**	**non-HDL**
PWVβ (L)	*Pearson* Correlation	.462**	.405	.203	.468**	.393
	p	**0.004**	**0.012**	0.222	**0.012**	**0.015**

(L): left common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Moderate positive correlation between the values of PWVβ (L) and the values of CRI-I (r=0.462, p=0.004), CRI-II (r=0.405, p=0.012) and AtC (r=0.468, p=0.003)

• No significant correlation between PWVβ values (L) and AIP values (r=0.203, p=0.222)

• Moderate positive correlation between the values of PWVβ (L) and the values of non-HDL-cholesterol (r=0.393, p=0.015).

On *Tabl. 25* are presented the correlation dependences between the values of Ep of the right carotid artery and the lipid indices and on *Table. 26* – between the Ep values of the left carotid artery and the lipid indices.

Tabl. 25. *Correlation dependences between Ep values of the right carotid artery and lipid indices in patients with BTM*

		CRI -I	**CRI-II**	**AIP**	**AtC**	**non-HDL**
Ep (R)	*Pearson* Correlation	.501**	.446**	.280	.505**	.397*
	P	**0.001**	**0.005**	0.089	**0.001**	**0.014**

(R): right common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results

• Strong positive correlation between Er values (R) and CRI-I values (r= 0501, p=0.001)

• Moderate positive correlation between Er values (R) and CRI-II values (r= 0.446 p=0.005)

• Lack of significant correlation between Er values (R) and AIP values (r= 0.280 p=0.089)

• Strong positive correlation between Er values (R) and AtC values (r=0.505, p=0.001)

• Moderate positive correlation between the values of Er (R) and the values of non-HDL-cholesterol (r= 0.397 p=0.014).

Tabl. 26. *Correlation relationships between Ep values of the left carotid artery and lipid indices in patients with BTM*

		CRI-I	**CRI-II**	**AIP**	**AtC**	**non-HDL**
Ep (L)	*Pearson* Correlation	.481**	.422**	.231	.486**	.386*
	p	**0.002**	**0.008**	0.163	**0.002**	**0.017**

(L): left common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Moderate positive correlation between Ep (L) values and CRI-I values (r= 0.481, p=0.002)

• Moderate positive correlation between Ep (L) values and CRI-II values (r=0.422, p=0.008)

• Lack of significant correlation between Ep (L) values and AIP values (r=0.231, p=0.163)
• Moderate positive correlation between Ep (L) values and AtC values (r=0.486, p=0.002)
• Moderate positive correlation between Ep (L) values and non-HDL-cholesterol values (r=0.386, p=0.017).

On *Tabl. 27* are presented the correlation dependences between the AC values of the right carotid artery and the lipid indices and on *Table. 28* – between AC values of the left carotid artery and lipid indices.

Tabl. 27. *Correlation relationships between AC values of the right carotid artery and lipid indices in patients with BTM*

		CRI-I	**CRI-II**	**AIP**	**AtC**	**non-HDL**
AC (R)	*Pearson* Correlation	-.414**	-.409*	-.166	-.418**	-.339
	p	**0.010**	**0.011**	0.318	**0.009**	**0.038**

(R): right common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:
• Moderate negative correlation between AC (R) values and CRI-I values (r= -0.414, p=0.010)
• Moderate negative correlation between AC (R) values and CRI-II values (r= -0.409 p=0.011)
• Lack of significant correlation between AC values (R) and AIP values (r= -0.166 p=0.318)
• Moderate negative correlation between AC values (R) and AtC values (r= -0.418, p=0.009)
• Moderate negative correlation between AC values (R) and non-HDL-cholesterol values (r= -0.339 p=0.038).

Tabl. 28. *Correlation relationships between AC values of the left carotid artery and lipid indices in patients with BTM*

		CRI-I	**CRI-II**	**AIP**	**AtC**	**non-HDL**
AC (L)	*Pearson* Correlation	-.344*	-.388*	-.035	-.345*	-.318*
	p	**0.035**	**0.016**	0.834	**0.034**	**0.052**

(L): left common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Moderate negative correlation between AC (L) values and CRI-I values (r= -0.344, p=0.035)

• Moderate negative correlation between AC (L) values and CRI-II values (r= -0.388 p=0.016)

• Lack of significant correlation between AC (L) values and AIP values (r= -0.035 p=0.834)

• Moderate negative correlation between AC (L) values and AtC values (r= -0.345 p=0.034)

• Moderate negative correlation between AC (L) values and non-HDL-cholesterol values (r= -0.318 p=0.052).

Discussion:

In our study, ET methodology was used to assess the arterial stiffness of the common carotid arteries. The obtained results prove a strong statistically significant positive correlation of PWVβ of the right and left carotid arteries and the indicators CRI-I, CRI-II, AtC and triglycerides, as well as a positive correlation of the β-stiffness parameter in the two common carotid arteries and the same lipid indicators. The values of the β-stiffness parameter, measured on the left carotid artery, also correlate positively with the mean values of total cholesterol.

Lipid parameters are those that largely contribute to the development of atherosclerosis, and this makes them associated with the development of arterial stiffness. Various studies have studied the relationship between different lipid parameters and vessel stiffness. The role of an adverse lipid profile in the development of atherosclerosis is well known, but the associations of plasma lipids with measures of arterial stiffness are still under investigation and not fully elucidated. As can be seen from our results, despite the dominant role of LDL-cholesterol in the onset and development of atherosclerosis, its association with PWVβ is rather weak (*Cecelja, 2009*).

Similar results to ours were reported by *Zhan et al.* in 2019. The authors conducted their study among middle-aged and elderly Chinese people known to have a higher prevalence of arterial stiffness and found that those with higher brachio-ankle PWV values have correspondingly higher CRI-II values. A positive linear relationship between the two metrics is described, similar to the one we obtained. *Kilic et al.* in 2020 conducted a study among elderly patients (58.8±14.2 years) and also demonstrated significantly higher CRI-II values in patients with high PWV values.

Vallée et al. included in the study 602 patients, some of whom had previous cardiovascular events. Most of the participants required routine follow-up, and the rest were referred by a general practitioner for a routine cardiovascular examination due to the presence of one or more cardiovascular risk factors. All participants had their lipid profile examined, electrocardiogram (ECG) performed, BP and aortic PWV measured by applanation tonometry. The researchers found that total cholesterol (p=0.03), LDL-cholesterol (p=0.03), non-HDL-cholesterol (p=0.03), CRI-I (p=0.01) and CRI-II (p=0.03) correlated significantly with PWV.

According to other studies, those participants who had increased brachio-ankle PWV also had higher CRI-I values (*Kilic, 2021; Wen, 2017; Baba, 2023; Si, 2019*). In the study by Wen et al. the presence of higher brachio-ankle PWV values was significantly and independently predicted by the CRI-I ratio. The study by Wen et al. from 2019 demonstrated the positive association between CRI-I and arterial stiffness, independent of LDL-cholesterol levels, even when they were below 70 mg/dl.

3.4 Correlation dependences between ET parameters of both carotid arteries with serum ferritin and MDA levels in patients with BTM

Results for task 9: *To investigate the correlations of the ET parameters of the two carotid arteries with the levels of serum ferritin and malondialdehyde (MDA) in patients with BTM.3.4.1 Correlation relationships between ET parameters of both carotid arteries and serum ferritin levels in patients with BTM*

On *Tabl. 29* are presented the correlation dependences between the values of serum ferritin and the ET indicators of the right carotid artery and on *Table. 30* – the correlation between serum ferritin values and ET parameters of the left carotid artery

Tabl. 29. *Correlation between serum ferritin values and ET parameters of the right carotid artery in patients with BTM*

		β-stiffness (R)	Ep (R)	AIx (R)	AC (R)	PWVβ (R)
Serum ferritin	*Pearson* Correlation	.318	.268	.090	-.260	.273
	p	**0.052**	0.104	0.590	0.114	0.098

(R): right common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Moderate positive correlation between ferritin values and β-stiffness (R) values (r=0.318, p=0.052)
• Lack of significant correlation between ferritin values and the other ET parameters of the right carotid artery.

Tabl. 30. *Correlation correlations between serum ferritin values and left carotid artery ET indicators in patients with BTM*

		β-stiffness (L)	**Ep (L)**	**AIx (L)**	**AC (L)**	**PWVβ (L)**
Serum ferritin	*Pearson* Correlation	.257	.208	-.052	-.209	.187
	p	0.120	0.210	0.756	0.209	0.260

(L): left common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Lack of significant correlation between ferritin values and ET indicators of left carotid artery.

3.4.2 Correlation dependences between ET parameters of both carotid arteries and MDA levels in patients with BTM

On *Tabl. 31* are presented the correlation dependences between the values of MDA and ET indicators of the right carotid artery and on *Table. 32* – between the values of MDA and ET indicators of the left carotid artery**.**

Tabl. 31. *Correlation dependences between MDA values and ET indicators of right carotid artery in patients with BTM*

		β-stiffness (R)	**Ep (R)**	**AIx (R)**	**AC (R)**	**PWVβ (R)**
MDA	*Pearson* Correlation	.144	.134	.030	.077	.101
	p	0.387	0.423	0.858	0.644	0.546

(R): right common carotid artery

Tabl. 32. *Correlation dependences between MDA values and ET indicators of left carotid artery in patients with BTM*

		β-stiffness (L)	**Ep (L)**	**AIx (L)**	**AC (L)**	**PWVβ (L)**
MDA	*Pearson* Correlation	.197	.177	-.080	.003	.166
	p	0.236	0.634	0.634	0.986	0.319

(L): left common carotid artery

When conducting a correlation analysis using the Pearson method, we obtained the following results:

• Lack of significant correlation between MDA values and ET indicators of the right carotid artery

• Lack of significant correlation between MDA values and ET indicators of left carotid artery.

Discussion:

The results obtained by us do not prove a statistically significant correlation between ET indicators of arterial stiffness and laboratory values of MDA. Regarding serum ferritin, a statistically significant positive correlation was demonstrated only with the β-stiffness index for the right carotid artery ($p=0.052$). A possible reason for the lack of correlations between these parameters could be related to the small group of patients studied.

In 2016, *Merchant et al*. conducted a study on the carotid intima-media complex and stiffness index in 53 patients with BTM (mean age 21.85±4.58 years) and compared them with 25 age-matched healthy controls. Additionally, correlations were sought between these parameters and the mean values of serum ferritin and indicators of cardiac and hepatic iron overload documented by MRI T2*. The authors found no correlation between the thickness of the intima-media complex of the carotid arteries and MRI T2* indicators of iron overload and explained this by the young age of the participants and the small sample size. In patients, however, they reported higher stiffness index values compared to healthy controls, thus proving their higher risk of developing atherosclerosis and CVD. In patients with BTM, a positive correlation between stiffness index-a and cardiac MRI T2* was also demonstrated. According to the authors, the obtained results provide information not only about the degree of iron overload in the cardiac muscles, but also about the impaired elasticity of the vessels. Similar to our data, their study also found no correlation between serum ferritin values and stiffness index-a, which we take as evidence that serum ferritin alone cannot be a sufficient and reliable marker of vascular health.

In our study, no association was found between arterial stiffness and serum ferritin, confirming the data published in 2002 by *Cheung et al*. for missing correlation between them. However, in another study conducted by *Gedikli et al.* in 2007 among Turkish patients with BTM, demonstrated that aortic stiffness parameters may have a significant relationship with serum ferritin values.

Arterial stiffness is the subject of research and the search for correlational dependencies not only in symptomatic patients with BTM. In a study by *Stakos et al*. found that in asymptomatic thalassemia patients who do not have

significant iron overload, it is associated with increased left ventricular mass and left atrial dilatation. Based on these results, the authors conclude that arterial stiffness can be used as a marker of early cardiovascular disease and vascular involvement in asymptomatic BTM patients without significant iron overload.

3.5 Correlation relationships between splenectomy and ET parameters of both carotid arteries, indices of lipid exchange and atherogenic lipid indices in patients with BTM

Outcomes for task 10: *To investigate the correlations between splenectomy and ET parameters of both carotid arteries, indices of lipid metabolism and atherogenic lipid indices in patients with BTM.3.5.1 Correlation relationships between splenectomy and ET parameters of both carotid arteries in patients with BTM*

Tabl. 33 presents the correlation dependences between splenectomy and ET indicators of the right carotid artery and *Table. 34* – correlational dependences between splenectomy and ET indicators of the left carotid artery**.**

Табл. 33. *Correlation between splenectomy and ET parameters of the right carotid artery in patients with BTM*

		β-stiffness (R)	Ep (R)	AIx (R)	AC (R)	PWVβ (R)
Splenectomy	Spearman's rho	.301	.273	-.003	-.240	.266
	p	0.066	0.097	0.988	0.146	0.107

(R): right common carotid artery

When conducting a Spearman correlation analysis, we obtained the following results:

• Lack of significant correlations between the splenectomy and the indicators from the echo-tracking methodology in the right common carotid artery.

Tabl. 34. *Correlation between splenectomy and ET parameters of the left carotid artery in patients with BTM*

		β-stiffness (L)	Ep (L)	AIx (L)	AC (L)	PWVβ (L)
Splenectomy	Spearman's rho	.407*	.395*	.063	-.266	.382*
	p	**0.011**	**0.014**	0.706	0.107	**0.018**

(L): left common carotid artery

When conducting a Spearman correlation analysis, we obtained the following results:

• Moderate positive correlation between splenectomy and β-stiffness (L) values (rho=0.407, p=0.011)
• Moderate positive correlation between splenectomy and Ep (L) values (rho=0.395, p=0.014)
• Lack of significant correlation between splenectomy and levels of AIx (L) (rho=0.063, p=0.706) and AC (L) (rho= - 0.266, p=0.107)
• Moderate positive association between splenectomy and PWVβ (L) values (rho=0.382, p=0.018).

3.5.2 Correlation relationships between splenectomy and lipid profile parameters and lipid indices in patients with BTM

Tabl. 35 presents the correlation dependences between splenectomy and lipid profile indicators in patients with BTM and in *Tabl. 36* – correlation dependences with lipid indices.

Tabl. 35. *Correlation relationships between splenectomy and lipid profile indices*

		TC	LDL	HDL	TG
Splenectomy	Spearman's rho	.564**	.599**	.073	.422
	P	**0.0001**	**0.0001**	0.661	0.422

When conducting a Spearman correlation analysis, we obtained the following results:
• Strong positive correlation between splenectomy and total cholesterol values (rho=0.564, p=0.0001)
• Strong positive association between splenectomy and LDL-cholesterol levels (rho=0.599, p=0.0001)
• Lack of significant correlation between splenectomy and the values of HDL-cholesterol (rho=0.599, p=0.661) and triglycerides (rho=0.422, p=0.422).

Tabl. 36. *Correlations between splenectomy and lipid indices in BTM patients*

		CRI-I	CRI-II	AIP	AtC	non-HDL
Splenectomy	Spearman's rho	.298	.422**	-.033	.281	0.567**
	p	0.069	**0.008**	0.845	0.088	**0.0001**

When conducting a Spearman correlation analysis, we obtained the following results:

• Moderate positive association between splenectomy and CRI-II levels (**rho=0.422, p=0.008**)
• Strong positive association between splenectomy and non-HDL-cholesterol levels (**rho=0.567, p=0.0001**)
• Lack of significant correlation between splenectomy and the values of CRI-I (rho=0.298 p=0.069), AIP (rho= -0.033, p=0845) and AtC (rho=0.281, p=0.088).

Discussion:

Our results demonstrate a strong positive association between splenectomy and LDL-cholesterol (rho=0.599, p=0.0001) and total cholesterol (rho=0.564, p=0.0001) values. A strong positive relationship of splenectomy was also found with the levels of non-HDL-cholesterol (rho=0.567, p=0.0001), and a moderate positive – with the levels of CRI-II (rho=0.422, p=0.008). Regarding the relationship between splenectomy and arterial stiffness indicators, a moderate positive relationship was found with β-stiffness (L) values (rho=0.407, p=0.011), with Ep (L) (rho=0.395, p=0.014) and with PWVβ (L) (rho=0.382, p=0.018). Our results are consistent with published data from other experimental and clinical studies, and based on their significance, we assume that the risk of CVD is greater in splenectomized compared with nonsplenectomized BTM patients.

Back in the 1970s, *Robinette and Fraumeni Jr.* observed greater changes in lipid profile and higher mortality from cardiovascular events in soldiers who had been splenectomized during World War II (*Dennis Robinette, 1977*). In the following years, numerous animal and human studies were conducted that showed identical results. One reason for this is the reduced activity of the reticuloendothelial system in splenectomized individuals, which impairs LDL-cholesterol catabolism. Our results support this claim, finding higher LDL-cholesterol levels in splenectomy patients (rho=0.599, p=0.0001). *Asai et al.* in 1988 found that splenectomized rabbits developed atherosclerosis more often than non-splenectomized ones due to higher serum levels of triglycerides, total cholesterol, and LDL-cholesterol and lower HDL-cholesterol. *Petroianu et al.* in 2006 also observed lower HDL-cholesterol and higher total cholesterol and LDL-cholesterol in female rats. The authors demonstrate that partial splenectomy does not lead to similar metabolic changes, proving the important role of the spleen in lipid metabolism.

In 2012, *Bordbar et al.* studied the lipid profile of 100 BTM patients aged 21.1 ± 4.6 years in Iran and, similar to our results, reported higher values of total cholesterol and LDL-cholesterol in splenectomized patients. A few years later, Hezaveh et al. conducted a similar study in Iran among 41 splenectomized and 42 nonsplenectomized BTM patients aged between 18 and 30 years. Among the splenectomized patients, higher values of LDL-cholesterol, triglycerides, CRI-I and CRI-II and lower values of HDL-cholesterol were found. Their results are similar to those obtained in our and other studies and confirm the conclusions drawn that splenectomized patients have a higher risk of developing cardiovascular events.

Boudrahem-Addour et al. investigated the lipid profile and OS levels in 46 BTM patients with a mean age of 19 years, comparing them to healthy controls. Similar to our results, CRI-II values were higher among splenectomized patients ($p<0.05$). In contrast to us, the research team also demonstrated higher CRI-I values ($p<0.05$). Our obtained higher values of LDL-cholesterol, non-HDL-cholesterol, CRI-II, PWVβ (L), β-stiffness (L) and Ep (L) in surviving splenectomy patients with BTM compared to non-splenectomized ones gives us reason to conclude that splenectomy is an additional risk factor for cardiovascular complications in BTM. With this in mind, we recommend that in these patients monitoring procedures for the assessment of lipid and cardiovascular status be conducted using an adapted risk-specific algorithm.

V. Conclusions

1. Compared with healthy controls, patients with BTM present with significantly higher values of heart rate (HR), diastolic pressure (DBP) and mean arterial pressure (MAP). There were no significant differences for systolic arterial pressure (SBP) and pulse pressure (PP).
2. Compared with healthy controls, BTM patients present with significantly lower values of hematological parameters Hb, Ery and Hct and significantly higher values of serum iron and serum ferritin.
3. Compared with healthy controls, patients with BTM demonstrate reliable differences in parameters of lipid metabolism – lower values of total cholesterol, LDL-cholesterol and HDL-cholesterol and higher values of triglycerides. There were significantly higher values of the atherogenic lipid indices CRI-I and AIP.
4. Compared with healthy controls, BTM patients demonstrated higher serum concentrations of malondialdehyde (MDA) without statistical significance.
5. Compared with healthy controls, regarding echo-tracking (ET) parameters, BTM patients demonstrate significantly higher values of arterial compliance (AC) (R). There are no significant differences in the local arterial stiffness (AR) parameters of the carotid arteries bilaterally - β-stiffness index, PWVβ, modulus of elasticity (Er) and augmentation index (AIx).
6. In patients with BTM, there is a reliable positive correlation of serum ferritin levels with some indicators of lipid metabolism - total cholesterol, LDL-cholesterol and triglycerides and with the lipid indices CRI-I, CRI-II, AtC and non-HDL-cholesterol.
7. In patients with BTM, there is a reliable positive correlation of hemoglobin values with some indicators of lipid metabolism - total cholesterol, LDL-cholesterol, HDL-cholesterol and the lipid indices AIP and non-HDL-cholesterol.

8. In patients with BTM, there is a reliable correlation between some echo-tracking (ET) parameters for arterial stiffness of the two carotid arteries with age, sex, indicators of lipid exchange and atherogenic lipid indices:

- positive correlation between age and β-stiffness index (R), β-stiffness index (L), PWVβ (R), PWVβ (L), Ep (R), Ep (L), AC (R)
- positive correlation between gender and β-stiffness (R), β-stiffness (L), Ep (L) and PWVβ (L), more pronounced in women
- negative correlation between gender and AC (R) and AC (L), with a strong dependence of high levels among men
- positive correlation between the β-stiffness index of the two carotid arteries and total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, CRI-I, CRI-I and AtC.

9. In patients with BTM, there are no significant relationships between ET indicators of arterial stiffness with the levels of serum ferritin and MDA.

10. In patients with BTM, there is a reliable positive correlation between splenectomy and some ET indicators of arterial stiffness – β-stiffness (R), β-stiffness (L), Ep (L) and PWVβ (L). Significant positive correlations were also present with total cholesterol, LDL-cholesterol, CRI-II and non-HD-cholesterol.

VI. Contributions

With an original character

- For the first time in Bulgaria, lipid profiles and lipid indices are assessed in patients with BTM and their relationship with indicators of iron overload, oxidative stress and arterial stiffness is analysed.
- For the first time in Bulgaria, the stiffness of the carotid arteries was measured in patients with BTM using echo-tracking methodology.

Of a scientific and practical nature

- Calculation of lipid indices is a potential clinical tool for cardiovascular risk assessment in patients with BTM.
- Determining carotid artery stiffness using echo-tracking is an affordable, non-invasive and useful method for assessing vascular health in children and young adults and can be included in the follow-up algorithm for patients with BTM.
- Identification of subclinical vascular damage is a potential approach for screening and prevention of cardiovascular complications in patients with BTM.

Confirmatory

- Changes in the lipid profile and lipid indices are observed in children and young adults with BTM.
- In children and young adults, there is a positive correlation between lipid indices and arterial stiffness indicators.
- Pulse wave velocity and arterial stiffness increase with age.
- Splenectomy correlates with more severe dyslipidemia and arterial stiffness.

VII. Conclusion

Advances in hematological control and increased life expectancy in patients with BTM have resulted in a progressive increase in the risk of accelerated atherosclerosis and cardiovascular disease in recent decades. Based on this problem, we formulated the motives of the dissertation work in the monitoring of vascular health, the clinical specifics of early vascular changes and the prediction of their therapeutic impact.

Our comparative analyzes of BTM patients from a Bulgarian population provide an opportunity for laboratory and clinical parallels with already generally accepted paradigms for vascular damage and risk prediction. Biochemical assessment of lipid profiles/indices and changes in carotid artery elasticity/stiffness have proven to be effective approaches for cardiovascular risk stratification. On the other hand, the specific parameters and correlations we found are a potential substrate for risk-based algorithms for monitoring and selection of drug preventions.

Analyzing the pathophysiological chain – from oxidative stress, endothelial dysfunction, altered vascular elasticity to arterial stiffness, our results and conclusions offer an accessible, multimodal and reproducible clinical perspective to the problem of modern vascular care in BTM patients.

VIII. Scientific publications and announcements related to the dissertation work

Publications:

1. Ganeva K., Shivachev P., **Petrova K**. Changes in cardiac function associated with iron overload in young patients with beta-thalassemia major – what we have learned so far. New methods for early diagnosis. Pediatrics 2021; Supplementum: 22-26

2. **Petrova K.,** Endothelial dysfunction in beta-thalassemia. Varna Medical Forum 2023; item 12, Online fisrt

3. **Petrova K.,** Ganeva K. Oxidative stress in patients with beta-thalassemia major. Pediatrics 2023; 2:13-16

Participations in scientific meeting:

1. **K. Petrova.** Oxidative stress in beta-thalassemia major. First working meeting Rare benign hematological diseases. Sliven, 18-20.03.2022

2. **K. Petrova.** Oxidative stress and endothelial dysfunction in children and young adults with beta-thalassemia major. Scientific conference 60 years "Pediatrics" Department, MU-Varna. Saint Vlas, 6-8.10.2023

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