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ORIGINAL ARTICLE

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Association of serum bilirubin, selected iron status indicators and body composition in non-obese, normoglycemic subjects

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ABSTRACT

Background: Recently cardiometabolic risk reduction has been observed in patients with slightly elevated bilirubin concentration, as well as increased risk in subjects with excessive iron reserves. The aim of this study was to evaluate the relationship between overweight and/or abdominal obesity, serum bilirubin and selected iron status indicators levels in non-obese subjects.

Methods: The study group consisted of 80 healthy, non-obese subjects aged 25–40 years. In all subjects total and direct bilirubin (T-BIL, D-BIL), iron (Fe), transferrin (TRSF), ferritin (FERR) and hepcidin (HEPC) measurements were performed. Anthropometric parameters (BMI, waist circumference, WHR) were measured and body composition (% of body fat, muscles and level of visceral fat) was evaluated using body segment analyzer.

Results: Men showed significantly higher values of waist circumference, WHR, muscle mass, visceral fat level and FERR and HEPC concentrations, compared to women. Lower concentrations of T-BIL, D-BIL and higher concentration of FERR, HEPC occurred in the overweight group. In all subjects and in the overweight group T-BIL, D-BIL showed negative correlations with BMI, waist circumference, fat mass and visceral fat level, while for FERR, HEPC those correlations were positive. Overweight subjects had an approximately 4-fold higher incidence of low T-BIL, D-BIL levels ($p < 0,001$), as well as nearly 2-fold higher incidence of high transferrin level ($p = 0,02$).

Conclusions: Overweight subjects have lower bilirubin levels and higher levels of factors potentially contributing to increased oxidative stress, for example ferritin and hepcidin. Serum bilirubin, ferritin and hepcidin concentration are related to body composition indicators, particullary fat mass and visceral fat level.

Key words: bilirubin, antioxidant, iron metabolism, overweight, obesity

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INTRODUCTION

The incidence of overweight and obesity has increased threefold in the last twenty years, which is a serious health and socio-economic problem. Obesity is involved in the pathogenesis of numerous diseases such as: type 2 diabetes, metabolic syndrome, hypertension, myocardial infarction, ischemic stroke and cancer. In the course of those diseases, inflammatory processes and increased production of free radicals are of great importance. Research over the past several years indicate the protective effect of bilirubin, as a low-molecular antioxidant, on reducing the risk of cardiovascular disease [1]. In its pathogenesis attention was also paid to the important role of iron oxidation processes, leading to increased synthesis of free radicals.

Bilirubin is a product of oxidative heme metabolism in mammals [2]. In this process heme is obtained mainly from hemoglobin, in smaller quantities from other proteins containing heme, i.e. myoglobin and cytochrome P-450 [3]. During its metabolism, bilirubin is modified by esterification with glucuronic acid. The consequence of this process is the formation of ionic derivatives, mono- and diglucuronides, which are excreted together with the bile. It is one of the most important biochemical parameters used to diagnose liver and bile ducts function [4].

Iron is a very important micronutrient for living organisms. It is a cofactor for many proteins of various biological significance. It plays a key role in the formation of hemoglobin, myoglobin and many important biochemical pathways, including energy metabolism, the production of neurotransmitters and the function of the immune system. As the transition metal, iron has useful binding properties to redox ligands [5]. Physiologically, the total iron content in the body of an adult male is about 4 g, in women about 3.5 g. The metabolically active iron constitutes 80% of the total pot, and in this 70% is contained in hemoglobin. Iron stored in ferritin accounts for approx. 20% Only a small percentage of iron is associated with transferrin (transport protein) [6].

Studies show that low bilirubin concentration in vivo eliminates free oxygen radicals and thus leads to alleviation of oxidative stress. The unconjugated bilirubin has the property to „sweep" singlet oxygen. In the presence of hydrogen peroxide or organic hydroxides, bilirubin behaves like a reducing agent for some peroxidases, thus minimizing the amount of potential oxidants [7]. In vitro studies have shown that bilirubin combined with albumin removes superoxide radicals from lysosomal systems and homogeneous solutions [8]. The reduction of inflammatory response in the vascular wall, resulting from the elimination of oxidative stress and the inhibition of endothelial cell activation, is associated with both bilirubin and its derivative, biliverdin [7]. Overexpression of heme oxygenase-1 (HO-1), an enzyme that catalyzes the breakdown of heme, reduces the production of inflammatory mediators, as well as indirectly affects vasodilation, stimulating the expression and production of nitric oxide [9]. Using its antioxidant activity, bilirubin can reduce lipid oxidation by eliminating free radicals and inactivating oxidized lipoproteins (especially LDL) and lipids, resulting in the inhibition of atherosclerotic plaque production [10]. In addition, the plasma concentration of bilirubin correlates inversely with many risk factors for coronary heart disease, including smoking, increased LDL-cholesterol, diabetes, and obesity, while it is positively correlated with HDL cholesterol, which has cardioprotective properties [11].

Excess iron level is considered to be harmful, because it promotes the formation of free radicals, which leads to tissue damage or oxidative stress [12]. Free radicals cause damage to molecules such as proteins, lipids and DNA. Such damaged biomolecules may be involved in the pathogenesis of numerous diseases, for example through co-participation in the process of atherosclerotic plaque formation. [13]. Epidemiological studies have also shown that high levels of iron in the body are associated with increased risk of coronary artery disease [12]. The largest source of iron is the breakdown of heme. During normal metabolism, iron is bound by ferritin to protect cells. In plasma, it is bound and transported by transferrin [7].

Iron as a free active metal occurs in very small amounts. Free iron along with hydrogen peroxide and superoxide anion radical in the Haber-Weiss reaction produce a very toxic hydroxyl radical (OH). The OH radical may be the initiator of lipid peroxidation, by separating the hydrogen atom from the polyunsaturated fatty acid molecule. The lipid peroxides formed in this process with the participation of iron may initiate additional lipid peroxidation [13]. OH also leads to LDL peroxidation, low density lipoprotein molecules. This process leads to the formation of oxidized LDL (ox-LDL) with strong atherogenic properties [6].

Several studies suggest that in overweight/obese subjects iron deficiency is more common, as well as higher ferritin levels. Therefore it seems that the pro-oxidative effects of iron and its relationship to adipose tissue depend not on its serum concentration, but primarily on the iron storage pool [14].

According to recent studies, inflammatory activity of adipose tissue may be strongly associated with disturbances of endogenous antioxidants and potential oxidants, including bilirubin and iron, which is why their determination may be important in assessing the risk of cardiometabolic disorders. The aim of the study was to evaluate the relationship between overweight and/or abdominal obesity, serum bilirubin and selected iron status indicators (iron, transferrin, ferritin, hepcidin) concentration in non-obese, non-smoking subjects with normal fasting glycaemia.

Subjects, materials and methods

Study consisted of 80 non-obese (BMI 18.5–27.0 kg/m^2), non-smoking and normoglycemic (fasting glucose 60–99 mg/dL) subjects aged 25–40 years (40 women, 40 men). Basic anthropometric measurements (body weight, waist circumference, BMI, WHR), blood pressure measurements with an automatic blood pressure monitor Omron M6 Comfort (Omron Healthcare, Kyoto, Japan) and medical history of chronic diseases were performed.

Serum and fluoride plasma were collected in the morning (7.00–9.00 am) after 12 hours of fasting. Samples were centrifuged in low temperature (4°C). Laboratory tests: plasma glucose and total and direct bilirubin (T-BIL, D-BIL), iron (Fe), transferrin (TRSF) and ferritin (FERR) in serum were performed on ABX Pentra 400 autoanalyzer (Horiba Ltd., Kyoto, Japan). Serum samples were divided into small aliquots and were frozen in -70°C to avoid peptides degradation for further assays. Concentration of serum hepcidin (HEPC) was determined using Hepcidin 25 (Bioactive) HS enzyme-linked immunosorbent assay (ELISA) kit (DRG Diagnostics GmbH, Marburg, Germany). The limit of detection was 0.30 ng/mL.

All laboratory measurements were performed in the Department of Laboratory Medicine, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Poland.

Body composition (percentage of body fat, muscles and level of visceral fat) was evaluated using body segment analyzer based on electrical bioimpedance (BIA) technology (InnerScan V BC-545N, Tanita). Measurements were performed fasting, in the morning, directly before blood samples collection and in accordance with the manufacturer's instructions. The reference values of these parameters depend on age and for 25–40 year olds the following values were adopted: fat mass women 21–33%, men 8–20%; level of visceral fat: 1–12; muscle mass women > 24%, men > 33% (according to manufacturer's manual).

Statistical analysis was performed using STATISTICA 12.0 PL software (StatSoft Inc. 2014). Data were presented as mean±standard deviation (normal distribution) or median and 25^{th}–75^{th} percentile (non-Gaussian distribution). Differences between study groups were calculated by t-Student, U-Mann-Whitney and ANOVA Kruskal-Wallis tests. P value < 0.05 was considered statistically significant.

The study was approved by the Bioethics Committee at the Nicolaus Copernicus Univeristy Collegium Medicum in Bydgoszcz, Poland (No. KB 627/2010) and complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. From all participants involved in this study an informed written consent was obtained.

Results

In the study group, total bilirubin concentrations ranged 10.9–17.8 μmol/L. Higher, but statistically insignificant, medians of total and direct bilirubin and iron were observed in men, compared to women (Table 1). In women statistically significantly lower values of the following parameters were found: waist circumference, WHR, muscle mass, level of visceral tissue, ferritin and hepcidin. Table 2 shows the comparison of the measured parameters in subjects with normal body weight and overweight. Statistically significant higher concentrations of total and direct bilirubin were observed in normal weight subjects. In contrast, overweight subjects have significantly higher concentrations of ferritin and hepcidin.

In the analysis of the relationship between body composition and biochemical parameters (Table 3), positive correlations between ferritin and hepcidin with waist circumference, WHR, fat mass and level of visceral fat were observed. However, a negative correlation was found between total and direct bilirubin with BMI, waist circumference, fat mass and level of visceral fat. In addition, total bilirubin and hepcidin showed significant relationships with muscle mass. Iron correlated weakly only with fat mass. The correlation was analyzed also in the group of subjects with normal weight and overweight. In subjects with normal body weight, only negative correlations of total and direct bilirubin with BMI (R = -0.48 and -0.40; p < 0,001, respectively) and waist circumference were observed (R = -0.32 and -0.35; p < 0.05, respectively) and positive weak correlation of ferritin and hepcidin with waist circumference

Table 1. Clinical and biochemical characteristics of study group

Variables	Study group (n = 80)	Women (n = 40)	Men (n = 40)	p
Age (years)	31 (27–35)	32 (26–37)	30 (27–33)	ns
BMI [kg/m$^{2]}$	24.3 (± 3.04)	24,33 (± 3.53)	24,28 (± 2,51)	ns
Waist [cm]	87 (76–91.5)	**78.5 (70–89)**	**88,5 (84–93)**	**0,001**
WHR	0,83 (± 0.07)	**0,78 (± 0,06)**	**0,87 (± 0,05)**	**< 0,001**
Fat mass [%]	24.4 ± 6.6	**28.2 ± 5.4**	**20.6 ± 5.4**	**< 0,001**
Muscle mass [%]	46 ± 12.5	**40 ± 3.3**	**51.9 ± 7.7**	**< 0,001**
Visceral fat level	4.0 (2.3–6.0)	**2.8 (1.5–4.0)**	**5,75 (4,0- 8,0)**	**< 0,001**
T-BIL [μmol/L]	13.2 (10.9–17.8)	12,3 (8,7-17,9)	15,4 (12,3-17,8)	ns
D-BIL (μmol/L)	2.7 (2.2–3.6)	2.6 (2.1–3.4)	3.1 (2.4–3.6)	ns
Fe (μmol/L)	17.8 (± 5.9)	16.8 (± 5.7)	18.8 (± 6.1)	ns
TRSF (μmol/L)	32.2 (29.1–36.2)	33.3 (28.3–39.3)	30.5 (29.3–33.9)	ns
FERR (nmol/L)	1,31 (0.64–2.31)	**0,70 (0,44–1,21)**	**1,84 (1.35–2.65)**	**< 0,001**
HEPC (ng/mL)	19.4 (8.4–33.2)	**12.9 (4.9–24.0)**	**27,6 (16.6–35.4)**	**0,002**

Data presented as mean ± SD or median (25–75%)
ns — statistically insignificant (p > 0,05)

Table 2. Comparison of variables in normal weight and overweight subjects

Variables	Normal body weight BMI 18.5–24.9 kg/m^2 (n = 44)	Overweight BMI 25.0–27.0 kg/m^2 (n = 36)	p
Age (years)	28 (26–32)	33 (27–37)	0,048
BMI [kg/m$^{2]}$	21,88 (± 1,86)	26,73 ± 1,76	< 0,001
Waist [cm]	77.5 (68–84)	91 (87–94)	< 0,001
WHR	0.8 ± 0.07	0,85 (± 0,06)	0,002
Fat mass [%]	20.5 (16.2–26.5)	24.4 (21.1–29.0)	0,03
Muscle mass [%]	49.0 (39.2–57.6)	40,2 (32.2–51.5)	0,02
Visceral fat level	3.0 (2.0–5.0)	7.5 (2.5-11.0)	< 0,001
T-BIL [μmol/L]	16.6 (12.5–19.5)	12.1 (8.4–14.4)	< 0,001
D-BIL [μmol/L]	3.2 (2.7–3.9)	2.4 (1.8–3.1)	< 0,001
Fe [μmol/L]	19.1 ± 6.3	16.6 (± 5.3)	ns
TRSF (μmol/L)	31.4 (28.3–33.5)	33.4 (29.8–37.4)	ns
FERR (nmol/L)	1,14 (0,59–2,18)	1,33 (1.15–2.33)	0,03
HEPC (ng/mL)	14.3 (7.2–25.1)	23.2 (13.8–32.2)	0,02

ns — not significant differences (p > 0,05)

Table 3. Selected correlation coefficient values in study group (log_{10} — transformed variables)

Variables	T-BIL	D-BIL	Fe	TRSF	FERR	HEPC
BMI	-0,48**	-0,4*	ns	ns	ns	ns
Waist	-0,32*	-0,35*	ns	ns	0,29*	0,37**
WHR	ns	ns	ns	ns	0,32*	0,33*
Fat mass [%]	-0,36*	-0,31*	-0,26*	ns	0,32*	0,44**
Muscle mass [%]	0,31*	ns	ns	ns	ns	-0,30*
Visceral fat level	-0,42**	-0,29*	ns	-0,28*	0,31	0,38**

*p < 0,05; **p < 0,001

Table 4. Correlation between body composition and selected biochemicals parameters in overweight subjects

Variables	T-BIL	D-BIL	FERR	HEPC
BMI	-0,48**	-0,40**	ns	ns
Waist	-0,32*	-0,35*	0,29*	0,37*
WHR	ns	ns	0,32*	0,33*
Fat mass [%]	-0,45**	-0,42**	0,35*	0,46**
Muscle mass [%]	0,42**	ns	ns	-0,42**
Visceral fat level	-0,38*	-0,26*	0,36*	0,40**

*p < 0,05; **p < 0,001

(data not presented). In the overweight group (Table 4) both total and direct bilirubin were correlated with BMI, waist circumference, fat mass and level of visceral fat. However, ferritin and hepcidin showed opposite relationships with body composition. In addition, a significant relationship was found between total bilirubin and hepcidin with muscle mass (R = 0.42 and -0.41; p < 0,001, respectively).

The frequency of selected biochemical changes in individuals with normal weight and overweight was compared. Low values, corresponding to first tertile, were used for total and direct bilirubin and iron. A higher

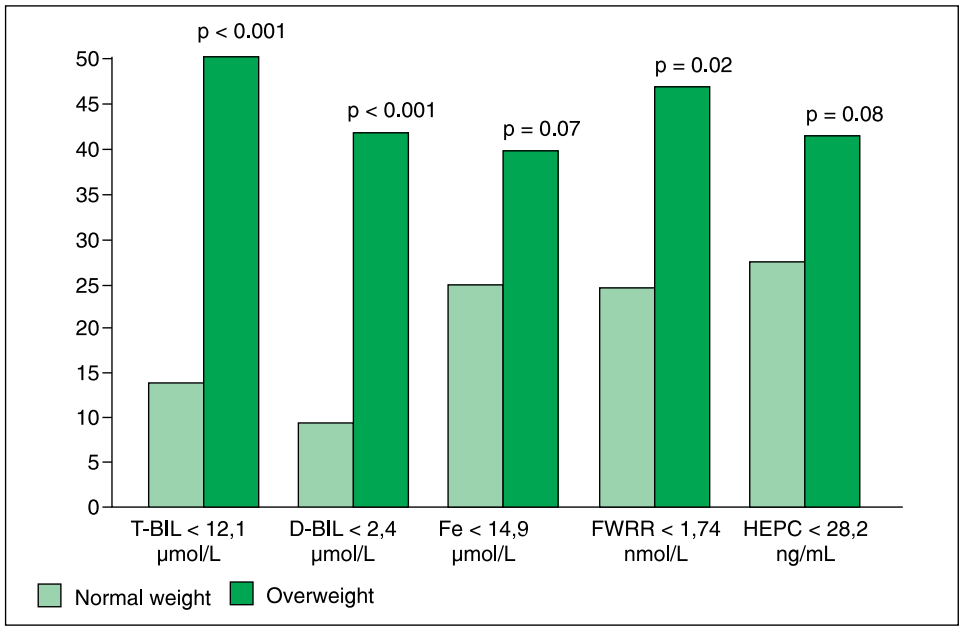

Figure 1. Prevalence of low T-BIL, D-BIL, Fe and high FERR and HEPC levels in normal weight and overweight subjects

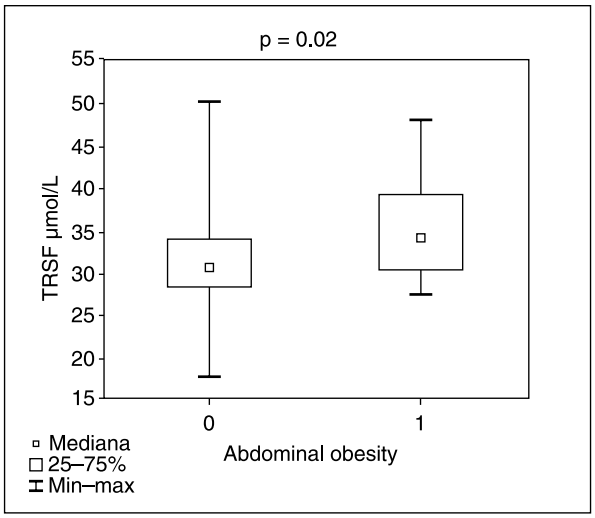

Figure 2. Difference in transferrin levels in subject with (1) and without (0) abdominal obesity

percentage of subjects with low total (< 12,1 µmol/L) and direct (< 2.4 µmol/L) bilirubin was observed in the overweight group, as well as tendency for low levels of iron (< 14.9 µmol/L). Hepcidin and ferritin concentration values corresponding to their third tertile (> 28.18 ng/mL; >1.74 nmol/L) were found more often in overweight subjects (41.6% and 47.2%, respectively), compared to normal weight group. In addition, statistically significant higher concentration of transferrin (Figure 2) was observed in patients with abdominal obesity, defined by waist circumference (≥ 80 cm in women and ≥ 94 cm in men).

Discussion

Bilirubin, the final product of heme metabolism, for many years was considered a toxic, side product. However, studies from the last years suggest that bilirubin is a strong endogenous antioxidant [15]. It belongs to the low molecular weight antioxidants, which low concentrations protect against oxidation or delays this process. This group also includes compounds such as: glutathione, uric acid, carnitine, flavonoids and vitamins A, C and E.

Obesity, especially of the abdominal type, is associated with low-grade inflammation in adipocytes. Inflammation leads to the development of insulin resistance and type 2 diabetes. Adipocytes in obese subjects produce large amounts of proinflammatory cytokines: TNF-α (tumor necrosis factor α) and interleukin 6, whose concentration is under physiological conditions decreased by HO-1, involved in the synthesis of bilirubin. It can be assumed that in obese subjects, the concentration of bilirubin in the blood may be lower than in people with normal body weight [16]. Oxidative stress initiated by the iron oxidation activity is involved in the pathogenesis of many diseases, i.e. diabetes or atherosclerosis [13]. A common risk factor in these diseases is also overweight or obesity, which also affect iron metabolism. In obese subjects hepcidin concentration, produced by adipocytes, is significantly increased. It inhibits the intestinal absorption of iron and its release from macrophages and liver, leading to its accumulation in the body [17]. Therefore, it is suggested that overweight or obese individuals may have higher values of iron status indicators, which may reflect an increased risk of cardiometabolic disorders.

In this study, overweight subjects had statistically significantly lower total and direct bilirubin concentrations. Andersson et al. stated that the lower body fat content and weight loss are associated with increased bilirubin [18]. Similarly, Swift et al. observed that aerobic physical training has a beneficial effect on body weight, and thus may increase the concentration of bilirubin [19]. In the last years, attention has been paid to the relationship between Gilbert's syndrome and the risk of ischemic heart disease. Maruhasi et al. in their study showed that patients with Gilbert's syndrome have low oxidative stress, resulting from genetically conditioned hyperbilirubinemia, which protects endothelium. This beneficial effect on blood vessels may contribute to the reduction of vascular complications in atherosclerosis in patients with Gilbert's syndrome, compared to patients without the syndrome [20]. There is increasing evidence that moderately elevated concentrations of iron stores, below the values found in genetic hemochromatosis, may adversely affect the cardiovascular system as well as lipid and carbohydrate metabolism. In population studies, elevated levels of ferritin have been shown to be associated with hypertension, dyslipidemia, elevated insulin and glucose, and abdominal obesity. In several studies, the relation between elevated iron concentration and the occurrence of metabolic syndrome was also observed [21].

Serum ferritin concentration is a good indicator of iron stores in the body. Epidemiological studies have shown that increased ferritin levels are associated with an increased cardiometabolic risk [22]. In this study, significant differences in the concentration of ferritin were observed, depending on the sex and BMI values. De Goda et al. reported a relationship between high ferritin concentration and the degree of coronary stenosis in women with obstructive coronary artery disease. In the case of men, its concentration was not a prognostic element of the degree of vessel obstruction [23]. Escobar-Morreale et al. also observed higher concentration of ferritin in the group of women with polycystic ovary syndrome and overweight than in women with normal body weight [24]. The relationship between ferritin concentration and obesity was also noted in the study by Williams et al. The study group consisted of 815 young men and women under 26 years of age. In women, ferritin correlated with waist circumference, BMI, triglycerides and C-reactive protein (CRP) concentration [25]. It is worth noting that ferritin may be treated as an indicator of inflammation, which is increased in abdominal obesity and results from the pro-inflammatory activity of visceral adipose tissue.

Several studies suggest a connection between iron deficiency and overweight/obesity. In a study conducted by Nead et al. on the overweight children and adolescents group, iron deficiency was observed frequently in this group. The incidence of iron deficiency increased with the increase in BMI values [26]. The higher incidence of iron deficiency in overweight children and adolescents compared to the normal body group was also found in the study by Pinhas-Hamiel et al. In the study, higher iron concentrations were also observed in the normal body group [27]. The association of iron deficiency with obesity is explained in several ways. Due to the overexpression of hepcidin in obese people, intestinal iron absorption is inhibited. Moreover, on account of low-grade inflammation, the iron does not fulfill its physiological role [28].

Hepcidin is recently consider as one of the most important regulators of iron metabolism. Cheng et al. showed in their study the relationship between obesity and minor disturbances of iron metabolism. In the group of healthy young women with overweight and obesity, the median concentration of hepcidin was 6.4 ng/mL. The lowest concentrations of hepcidin were observed in subjects with lower iron concentrations [29]. However, in a population study conducted on a group of 1391 individuals, Martinelli et al. noted an elevated concentration of hepcidin in patients with metabolic syndrome. For both women and men, the hepcidin concentration increased with increasing concentration of classic risk factors, as well as of ferritin. In addition, it was concluded that in the metabolic syndrome, the hepcidin concentration gradually increases in response to a moderate increase in iron stores in the body [30].

The results of own research in the group of healthy, young people allow to determine the relationship between the weight, body composition and the concentration of both total and direct bilirubin, as well as selected iron status indicators, particularly the concentration of ferritin and hepcidin. The interaction between oxidative stress, inflammation and iron metabolism has not been fully understood, which makes it difficult to determine the exact role of iron as a cardiometabolic risk factor. The results obtained in this study may suggest an important role of the analyzed parameters in the diagnosis of cardiometabolic disorders. However, despite the promising results in this study, attention should be paid to its limitations, especially a small number of respondents. Therefore, the results would require verification in a large population-based study.

Disclosure of interest: The authors state that there are no conflicts of interestregarding the publication of this article.

List of abbreviations

BIA — bioelectrical impedance analysis
BMI — body mass index
CRP — C-reactive protein

D-BIL – direct bilirubin
ELISA — enzyme-linked immunosorbent assay
Fe — iron
FERR — ferritin
HDL — high-density lipoprotein
HEPC — hepcidin
HO-1 — heme oxygenase-1
LDL — low-density lipoprotein
OH — hydroxyl radical
Ox-LDL — oxidized low-density lipoprotein
T-BIL — total bilirubin
TNF-a — tumor necrosis factor α
TRSF — transferrin
WHR — waist-to-hip ratio

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VIA MEDICA

ORIGINAL ARTICLE

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Selected nutritional risk parameters in patients with laryngeal cancer — a comparison with other patients hospitalized in a Department of Laryngology and patients with colorectal cancer

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ABSTRACT

Background: It is assumed that neoplasm greater in size may affect a patients' nutritional status and prognosis stronger than smaller one. The aim of this study was to compare the nutritional status and prognosis of patients with laryngeal cancer (LC), recognized as tumour smaller in size, and patients with colorectal cancer (CRC) who were hospitalized in our hospital during the one year period.

Methods: The retrospective review of medical documentation of all 1,134 patients hospitalized in a Department of Otolaryngology.

Results: The laryngeal tumour was smaller than colorectal. Nutritional risk concerned 9% of patients with LC, was greater than in patients with other laryngeal disorders (1.4%), and lower than in patients with CRC (37%). A Nutritional Risk Screening (NRS) 2002 score ≥ 3 was the only significant factor influencing the risk of in-hospital all-cause mortality, 14- and 30-day readmissions in patients with LC, and the risk of 14-day rehospitalization in patients with CRC.

Conclusions: Risk of malnutrition in patients with LC was lower than in counterparts with CRC, and concern 9% and 37% of patients, respectively. Nutritional risk diagnosed in patients with LC had a stronger association with the prevalence of the measured outcomes (in-hospital death, the risk of 14-day and 30-day readmission, length of hospitalization) than in individuals with CRC.

Key words: nutritional status; laryngeal cancer; colorectal cancer

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Introduction

Malnutrition and cachexia are common disorders in patients with cancer. Among individuals with neoplasms of the head or larynx, these disorders affect about 35–50% of patients [1]. Malnutrition is an important risk factor for in-hospital mortality, rehospitalization, prolonged general inpatient hospital stays [2], as well as the incidence of complications [1, 3, 4]. In patients treated for laryngeal cancer (LC), these complications include immunodeficiencies, poor wound healing, wound infection, anastomotic leakage, fistula, respiratory insufficiency, and sepsis. The many nutritional risk parameters (e.g. blood concentrations of albumin, prealbumin, transferrin, as well as lymphocyte count) [5, 6], have been analyzed as factors affecting the outcomes of LC treatment, but even low body mass index (BMI) before surgery was related to poor prognosis in patients with squamous laryngeal cancer [7] and individuals with colorectal cancer (CRC) [8]. The causes of malnutrition in these patients were not only associated with cancer-related cachexia, but also to local tumour effects (e.g. pharyngeal dysphagia, odynophagia and ileus), anorexia, and alcoholism [1]. These factors may be responsible for greater nutritional risk among patients with head and neck cancers compared to neo-

plasms in other localizations. To check this hypothesis, we analyzed the values of some nutritional parameters over a one-year period in patients with LC and CRC, hospitalized, respectively, in the Departments of Otolaryngology and Surgery at our hospital, and compared the nutritional risk.

Patients and methods

We performed an analysis of the medical documentation of 1,134 patients treated in the Department of Otolaryngology and 92 patients with CRC treated in the Department of Surgery between July 1st, 2014 and June 30th, 2015 in a university hospital. The premise for choosing CRC as the counterpart for patients with LC was that both neoplasms can affect patients' nutritional status through changes in the functioning of the alimentary tract.

The following clinical data, nutritional screening scores and nutritional assessment parameters were evaluated: age, gender, number of days hospitalized, hospitalization mode (whether urgent or scheduled), in-hospital all-cause mortality, non-elective readmission, Nutritional Risk Screening (NRS) 2002 score (a score of at least 3 points for the questionnaire indicates a risk of malnutrition), body mass, height, BMI, blood concentration of hemoglobin, total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, glucose, albumin, C-reactive protein (CRP), and absolute lymphocyte count. All the biochemical parameters included in the analysis were the first determinations during the respective hospitalizations. The following secondary parameters were also calculated:

- an "ideal weight" was calculated according to the Lorentz formula: for female patients, ideal weight = [height (cm) — 100] - {[height (cm) —150]/2}; and for male patients, ideal weight = [height (cm) - 100] — {[height (cm) - 150]/4} [9];
- an "absolute difference between the actual and ideal body weight" was calculated according to the following formula: actual body mass — ideal weight;
- a "relative difference between actual and ideal body weight" was calculated according to the following formula: 100 x (actual body mass — ideal weight)/ actual body mass;
- body mass deficit was defined as a negative value of the "absolute difference between the actual and ideal body weight";
- the Nutritional Risk Index (NRI) was calculated according to the following formula: NRI = 1.519 x blood albumin concentration (g/l) + 41.7 x actual body mass/ideal body mass [9,10];
- Onodera's (Preoperative) Prognostic Nutritional Index (OPNI) was calculated as 10 × serum albumin concentration (g/dl) + 0.005 × lymphocyte count (per mm^3) [11–15];
- blood CRP/albumin ratio.

The above acted as nutritional screening scores and nutritional assessment parameters [2].

The diagnosis of cancerous and non-cancerous disease in the respective patients was determined according to the ICD-10 Classification of Diseases. The LC and CRC staging was assessed according to the 7th edition of the Cancer Staging Manual of the American Joint Committee on Cancer [16].

Measured outcomes

The following outcomes were measured: hospital length of stay (LOS; the number of days hospitalized), in-hospital all-cause mortality, and non-elective readmission (the second and subsequent hospitalizations during the period analyzed) in the 14-day and 30-day periods following discharge.

Bioethics

The investigation was conducted in compliance with the Declaration of Helsinki for medical research.

Statistics

Statistical analysis was conducted using licensed versions of STATISTICA statistical software (a data analysis software system), StatSoft, Inc. (2017), version 13.1. The normal distribution of the study variables was checked using the Shapiro-Wilk test. The results were mainly presented as the mean ± standard deviation (SD), or n, %. The statistical significance of differences between groups was verified using the Student's t-test and Chi^2 test. Spearman rank correlation was also used. The statistical significance level was set at a p-value < 0.05. The odds ratio (OR) was defined as the odds that an outcome will occur with the association of some value of an estimated variable (a clinical or biochemical parameter), compared to the odds of the outcome occurring in the absence of that association. The OR was calculated according to the following formula: the product of the number of subjects with the measured outcome and the presence of the variables analyzed (exposed cases) and the number of subjects without the presence of the variables analyzed (unexposed non-cases) divided by the product of the numbers of exposed non-cases and unexposed cases. For this purpose, we used free statistical calculators (e.g. https://www.medcalc.org/calc/odds_ratio.php). These analyses were performed both per hospitalization and per patient.

Table 1. Demographic and clinical data of the patients analyzed

Feature	Patients with laryngeal cancer (n = 33)	Patients in the Department of Laryngology without a diagnosis of laryngeal cancer (n = 1,101)	Patients with colorectal cancer (n = 92)
Age (years)	62.0 ± 10.9	47.3 ± 18.6 +	69.3 ± 11.3 +
Age ≥ 65 years (n, %)	13 (39.4%)	201 (18.3%) +	82 (89.1%) +
Male gender (n, %)	31 (93.9%)	602 (54.7%) +	65 (70.7%) +
Tumor size (cm)	2.74 ± 1.02		4.52 ± 2.69 +
Neoplastic disease stage			
I (n, %)	9 (27.3%)		28 (30.4%)
II (n, %)	5 (15.1%)		20 (21.7%)
III (n, %)	9 (27.3%)		21 (22.8%)
IV (n, %)	10 (30.3%)		23 (25.0%)
Diabetes (n, %)	3 (9.1%)	37 (3.4%)	20 (21.7%) *
Duration of hospitalization (days)	9.6 ± 6.8	3.1 ± 2.9 +	10.7 ± 9.5
In-hospital death (n, %)	1 (3%)	2 (0.18%) *	9 (9.8%) +
Rehospitalization within 14 days (n, %)	1 (3%)	5 (0.45%)	8 (8.7%) +
Rehospitalization within 30 days (n, %)	1 (3%)	13 (1.2%)	13 (14.1%)
Body mass (kg)	77.7 ± 12.3	75.5 ± 17.4	75.90 ± 17.4
Height (cm)	176.9 ± 8.2	170.2 ± 11.6 *	167.1 ± 8.9 +
BMI (kg/m^2)	24.9 ± 4.1	25.9 ± 4.9	27.1 ± 5.4 *
Ideal body weight (kg)	69.7 ± 6.7	63.71 ± 9.1 +	61.7 ± 7.4 +
Absolute difference between actual and ideal body weight (kg)	8.05 ± 12.3	11.81 ± 13.8	14.3 ± 15.3 *
Relative difference between actual and ideal body weight (%)	10.3 ± 14.8	12.5 ± 17.4	15.8 ± 15.6 *
Body mass deficit (n, %)	8 (24.2%)	192 (17.4%)	17 (18.5%)
NRS-2002 score	1.0 ± 1.2	0.2 ± 0.6 +	2.14 ± 1.5 +
NRS-2002 ≥ 3 score	3 (9.1%)	15 (1.4%) *	34 (37.0%) +
NRI	50.1 ± 9.3	54.9 ± 13.5	54.3 ± 11.8
Albumin (g/l)	3.2 ± 0.6	3.3 ± 0.8	3.1 ± 0.8
CRP (mg/dl)	49.4 ± 53.2	64.2 ± 117.9	78.8 ± 75.4
CRP/albumin ratio	19.84 ± 20.9	54.14 ± 99.5	24.6 ± 31.2
Blood lymphocyte count (G/l)	1.7 ± 0.7	2.0 ± 1.0	1.8 ± 2.9
OPNI	31.8 ± 5.6	31.2 ± 9.3	30.9 ± 7.9
Hemoglobin (g/dl)	13.5 ± 2.2	14.0 ± 1.8	11.3 ± 2.5
Cholesterol (mg/dl)	179.1 ± 49.3	190.9 ± 67.0	150.0 ± 60.9
LDL cholesterol (mg/dl)	76.7 ± 10.0	128.3 ± 59.8	105.7 ± 43.7
Triglycerides (mg/dl)	120.7 ± 40.7	126.2 ± 74.5	123.4 ± 67.5
Glucose (mg/dl)	118.2 ± 36.4	108.6 ± 44.7	131.0 ± 50.1

BMI — body mass index; NRS — Nutritional Risk Screening; NRI — Nutritional Risk Index; CRP — C-reactive protein; OPNI — Onodera's Prognostic Nutritional Index; LDL — low-density lipoprotein. Student's t-test in relation to patients with laryngeal cancer: * — $p < 0.05$; + — $p < 0.001$

Results

Patients with LC were predominantly male and younger than patients with CRC but, on average, older than other patients admitted to the Department of Otolaryngology (Tab.1). The tumour size in patients with LC was lower than in individuals with CRC. The percentages of patients with the respective clinical stage of neoplasm were similar (Tab.1). The duration of hospitalization of patients with LC was similar to

that of patients with CRC. In relation to nutritional risk, compared to patients with CRC, patients with LC had a significantly lower BMI value, and both a lower absolute and relative difference between actual and ideal body weight (Tab.1). They also had lower nutritional risk expressed by NRS-2002 score and a lower prevalence of an NRS-2002 score ≥ 3. However, the remaining biochemical parameters of nutritional status did not differ in patients with LC compared with those with CRC.

In patients with LC, the number of hospitalization days correlated significantly and positively with NRS-2002 score (R = 0.47; p = 0.006); however, in patients with CRC, the length of hospital stay was significantly associated with patients' age (R = 0.21; p = 0.022), hyperglycemia (blood concentration of triglycerides greater than 200 mg/dl) on admission (R = 0.34; p = 0.016), NRS-2002 score (R = 0.39; p = 0.0001), and body weight deficit (R = 0.21; p = 0.039). LC stage correlated significantly with an NRS-2002 score ≥ 3. It was also related to the length of patients' in-hospital stay. CRC stage significantly positively correlated with blood lymphocyte count, and negatively with BMI, NRI and relative difference between actual and ideal body weight (Tab.2).

Next, we analyzed factors influencing the occurrence of the measured outcomes (Tab.3). We found that only an NRS-2002 score ≥ 3 had a significant effect on the risk of in-hospital all-cause mortality, 14- and 30-day readmissions in patients with LC, and the risk of 14-day rehospitalization in patients with CRC.

Discussion

The main rationale for this study was to check what is more important for patients nutritional risk, tumour size or its localization. To test this hypothesis we compared nutritional risk and prevalence of measured outcomes, related to nutritional risk among patients with LC and CRC. Moreover, in order to better evaluation of the clinical importance of disease localization, we compared some clinical data between patients with LC and the other laryngeal disorders required hospitalization. This study, to the best of our knowledge, is the first comparison of parameters of nutritional screening scores and nutritional status assessment parameters between patients with LC and CRC, the sixth and the second most common cancers in the world, respectively [17]. We found that patients with LC compared to individuals with CRC had significantly lower BMI values and a non-significantly lower prevalence of body mass deficit (Tab. 1). At the same time, they had a lower nutritional risk, expressed as an NRS-2002 score, and a lower absolute and relative difference between actual and ideal body weight (Tab. 1). Only 9% of our patients with LC had increased nutritional risk expressed as a score of at least

Table 2. Spearman's correlations of laryngeal cancer (LC) and colorectal cancer (CRC) clinical stage with selected parameters of nutritional screening and assessment

Cancer stage correlation with	LC (n = 33)		CRC (n = 92)	
	R	p <	R	p
Age	-0.17	0.33	-0.02	0.84
Gender (female/male)	-0.02	0.95	-0.1	0.36
In-hospital length of stay (days)	0.59	0.001	0.05	0.63
BMI (kg/m^2)	0.10	0.64	-0.23	0.039
NRS-2002 score	0.32	0.08	0.23	0.07
NRS-2002 score ≥ 3	0.40	0.024	0.23	0.07
Relative difference between actual and ideal body weight (%)	0.02	0.91	-0.23	0.044
NRI	0.71	0.02	-0.45	0.035
Hemoglobin (g/dl)	-0.28	0.23	-0.17	0.11
Total blood cholesterol (mg/dl)	-0.04	0.95	0.05	0.88
Blood glucose (mg/dl)	- 0.13	0.65	0.25	0.15
CRP (mg/dl)	0.37	0.47	0.12	0.46
Lymphocyte count (G/l)	0.14	0.77	0.44	0.019
Blood albumin (g/l)	-0.35	0.056	0.10	0.63
CRP/albumin ratio	0.35	0.055	0.22	0.23
OPNI	-0.35	0.056	-0.06	0.79

BMI — body mass index; NRS — Nutritional Risk Screening; NRI — Nutritional Risk Index; CRP — C-reactive protein; OPNI — Onodera's (Preoperative) Prognostic Nutritional Index (OPNI)

3 in the NRS-2002 questionnaire compared to 37% of individuals with CRC (Tab. 1). Other authors have shown that malnutrition prevalence among patients with head and neck cancers was greater than in our investigation and amounted to 30-50% [1, 6]. Kwag et al. reported a prevalence of malnutrition in patients with CRC in the Korean population at a level similar to ours [18]. However, other studies have shown that the prevalence of malnutrition among patients with CRC amounted to between 19.2% [19] and 30–60% [20]. Until now, only a few authors have compared the nutritional status of patients with neoplasms in different localizations. In a study by Du et al. [21], the proportions of patients with low blood levels of albumin, prealbumin, transferrin, red blood cells, hemoglobin and hematocrit were higher for gastric cancer than for colon cancer, which was explained by the greater susceptibility of gastric patients to malnutrition and loss of fatty tissue.

It is also known that hypoalbuminemia significantly increases the length of hospital stay, rates of surgical site infections, and the risk of enterocutaneous fistula formation and deep vein thrombosis, particularly in patients with CRC [22, 23]. Moreover, serum albumin was superior to prealbumin for predicting short-term recurrence in patients with operable CRC [24], and the morbidity and mortality rates in patients with CRC decreased by 7.3% and 15.6%, respectively, for each 0.1 g/dl increase in preoperative serum albumin level.[23] However, in our study, blood albumin concentration was similar both in patients with LC and CRC, as well as in the patients in the Department of Laryngology without a diagnosis of laryngeal cancer (Tab. 1). In our patients with LC, blood albumin concentration correlated with neoplasm stage only with borderline statistical significance, similarly to derivative composed parameters, such as the CRP/albumin ratio and OPNI (Tab. 2). The last parameter, OPNI, which is the product of blood albumin concentration and lymphocyte count [11–15], linked LC with CRC because, in our patients with CRC, neoplasm advancement was associated with lymphocyte count, NRI and BMI (Tab. 2).

It is known that a patient's nutritional status, particularly malnutrition but also obesity, are common but poor prognosis factors in individuals with cancers in a number of localizations [1, 6, 20]. We also found that nutritional risk assessed using the NRS-2002 survey was related to an increased prevalence of the measured outcomes, such as patients' in-hospital mortality and readmissions (Tab. 3). This demonstrates the necessity for further studies focused on the clinical importance of nutritional status assessment in patients with LC, as well as the need to evaluate the effectiveness of nutritional support and its financial impact on health services [6]. On the other hand, it should be underlined that, other than the NRS-2002, we did not find any single parameter of nutritional status assessment which related both to LC and CRC clinical stage (Tab. 2) and could be used to predict patients' outcomes (Tab. 3). In our review of the literature, we did not find one recommended nutritional screening scores or nutritional assessment parameters dedicated to patients with LC either [5–7]; however, for patients with CRC, the most frequently used instruments for this purpose were as follows: the NRS-2002 [18-19], Malnutrition Universal Screening Tool (MUST), Subjective Global Assessment (SGA) [25, 26], Patient-Generated Subjective Global Assessment (PG-SGA), NRI, and OPNI [11-15, 25, 26]. In the study by Kwag et al. [18], the NRS-2002 was an independent predictor of postoperative complications (OR 3.05; $p = 0.045$), such as anastomotic leakage and wound infection in patients with CRC.

The practical importance of our observations is that both in patients with LC and CRC, nutritional screening and assessment should be performed using more than one diagnostic tool at the same time. This approach to patient management might potentially help identify patients with an increased risk of malnutrition and postoperative complications. An increased NRS-2002 score may also identify those LC and CRC patients who might potentially benefit from nutritional support [27–30].

As with most authors, we could not avoid some methodological shortcomings that could have influenced the strength of the deductions based on our results. The main limitation is a retrospective study design based on documentation analysis, although such a study design was described previously [4]. Moreover, our sample size was small, and we observed a low number of measured outcomes. It should also be taken into consideration that the clinical outcomes analyzed might be influenced by a number of factors other than nutritional status alone, e.g. main disease and comorbidity severity, which may also bias the results obtained. Such an observation is also justified by the analysis of Table 1, which shows an imbalance in potential confounding factors between groups, mainly concerning age, gender, and diabetes prevalence.

Conclusions

The average nutritional risk in patients with laryngeal cancer amounted to 9% and was greater than in individuals with other conditions that required hospitalization in the Department of Otolaryngology but lower than in patients with CRC (37%). Nutritional risk diagnosed in patients with laryngeal cancer had a stronger association with the prevalence of the measured outcomes (in-hospital death, the risk of 14-day and 30-day readmission, length of hospitalization) than in individuals with colorectal cancer. However, due to

Table 3. Risk of the occurrence of measured outcomes in patients with laryngeal and colorectal cancer

Parameter	Patients with laryngeal cancer (n = 33)			Patients with colorectal cancer (n = 92)		
	In-hospital death (n = 1; 3%)	**Readmission during 14 days (n = 1; 3%)**	**Readmission within 30 days (n = 1; 3%)**	**In-hospital death (n = 9; 9.8%)**	**Readmission within 14 days (n = 8; 8.7%)**	**Readmission with 30 days (n = 13; 14%)**
NRS-2002 ≥ 3	33.0% vs 0% p= 0.04 OR 36.6 95% CI 1.2-1153.2	33.0% vs 0% p = 0.04 OR 36.6 95% CI 1.2-1153.2	33.0% vs 0% p = 0.04 OR 36.6 95% CI 1.2-1153.2	14.7% vs 2.2% p = 0.07 OR 7.8 95% CI 12.2-60.0	9.4% vs. 4.4% p < 0.0001 OR 25.0 95% CI 5.6-112.5	15.6% vs. 6.5% p = 0.21 OR 2.7 95% CI 0.6 - 12.0
Body mass deficit	12.5% vs 0% p = 0.21 OR 2.6 95% CI 0.3-234.8	0% vs. 4.8% p = 0.89 OR 0.80 95% CI 0.03-21.8	0% vs. 4.8% p = 0.89 OR 0.80 95% CI 0.03-21.8	11.8% vs 3.7% p = 0.19 OR 3.5 95% CI 0.5-22.8	11.8% vs. 7.4% p = 0.55 OR 1.67 95% CI 0.3-9.1	17.7% vs. 9.9% p = 0.36 OR 1.9 95% CI 0.5-8.3
BMI ≥ 25kg/m^2	0% vs. 7.7% p = 0.41 OR 0.25 95% CI 0.01-6.7	6.3% vs. 0% p = 0.56 OR 2.61 95% CI 0.1-69.6	6.3% vs. 0% p = 0.56 OR 2.61 95% CI 0.1-69.6	4.7% vs. 5.7% p = 0.82 OR 0.81 95% CI 0.13-5.1	7.8% vs.8.8% p = 0.86 OR 0.88 95% CI 0.2-3.9	9.8% vs. 14.7% p = 0.43 OR 0.60 95% CI 0.2-2.1
Diabetes	0% vs 0%	0% vs. 8.3% p = 0.95 OR 1.1 95% CI 0.04-33.4	0% vs. 8.3% p = 0.95 OR 1.1 95% CI 0.04-33.4	10.0% vs 16.7% p = 0.61 OR 0.55 95% CI 0.1-3.2	5.2% vs. 10.0% p = 0.58 OR 0.51 95% CI 0.04-5.2	10.0% vs. 10.5% p = 0.95 OR 1.06 95% CI 0.2-7.0
Albumin ≥ 3.35 g/l	0% vs 0%	0% vs 0%	0% vs 0%	6.7% vs 20.0% p = 0.51 OR 0.29 95% CI 0.03-2.7	6.7% vs. 17.3% p = 0.35 OR 0.33 95% CI 0.03-3.4	6.7% vs. 17.3% p = 0.24 OR 0.26 95% CI 0.03-2.5
Lymphocyte count ≥ 1.55 G/l	4.4% vs 2.9% p = 0.73 OR 1.5 95% CI 0.1-17.7	4.6% vs. 6.1% p = 0.27 OR 0.75 95% CI 0.1-5.7	4.6% vs. 9.1% p = 0.44 OR 0.49 95% CI 0.2-5.7	13.3% vs 16.4% p = 0.81 OR 0.81 95% CI 0.13-5.1	0% vs. 17.4% p = 0.20 OR 0.14 95% CI 0.01-2.8	6.7% vs. 17.4% p = 0.35 OR 0.34 95% CI 0.03-3.4
OPNI ≤ 40	15% vs. 0 p = 0.89 OR 1.3 95% CI 0.03-53.5	0% vs. 3.8% p = 0.14 OR 0.3 95% CI 0.003-2.8	0% vs. 3.8% p = 0.14 OR 0.3 95% CI 0.003-2.8	23.1% vs. 3.2% p = 0.92 OR 4.12 95% CI 0.2-83.5	12.5% vs. 16.5% p = 0.26 OR 0.7 95% CI 0.1-8.4	16.7% vs. 16.7% p = 1.00 OR 1.00 95% CI 0.1-11.1

NRS — Nutritional Risk Screening; OR — odds ratio; CI — confidence interval; BMI — body mass index; OPNI — Onodera's Prognostic Nutritional Index

study limitations, the clinical significance of nutritional risk assessment in patients with LC and its financial impact on health services need further investigation.

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VIA MEDICA

ORIGINAL ARTICLE

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Clinical presentations and hemodynamic parameters in patients hospitalized due to acute heart failure stratified by the left-ventricular ejection fraction

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ABSTRACT

Background: Currently, one of the most common causes of hospitalization, especially in the elderly, is heart failure (HF) exacerbation. In nearly 95% of patients, this is caused by fluid overload. There have been studies comparing the rates of comorbidities and biochemical disturbances in HF patients; however, their hemodynamic parameters have not yet been assessed. Thus, the aim of this study was to compare the clinical presentations and hemodynamic parameters assessed via impedance cardiography (ICG) in patients hospitalized due to acute HF, stratified by the left-ventricular ejection fraction (LVEF).

Methods: This study enrolled 102 patients, aged > 18 years, hospitalized due to decompensated HF. Ninety-seven patients (74 men, 23 women) underwent echocardiographic examination. Biochemical and hemodynamic parameters were assessed on the day of admission and, subsequently, every other day during hospitalization. Based on echocardiographic findings and the ESC guidelines the study group was divided into the following subgroups: HFrEF (EF < 40%), HFpEF (EF > 50%), and HFmrEF (EF 40–49%).

Results: The HFrEF group, which constituted 60.8% of patients (n = 58), was predominantly male (P = 0.0005); and most had elevated N-terminal pro-brain natriuretic peptide levels (P = 0.0008). The HFpEF and HFmrEF subgroups, jointly (n = 38), were characterized by higher systolic blood pressure (P = 0.0001), and lower hemoglobin levels (P = 0.003). The hemodynamic assessment showed that HFrEF patients had higher total fluid content (P = 0.005) and lower systolic time ratio (P = 0.0002).

Conclusions: Despite similar clinical presentation, patients with HF exhibited different values of hemodynamic and biochemical parameters depending on their LVEF; this indicates non-homogeneity of pathomechanisms and causes of HF decompensation.

Key words: heart failure, acute heart failure, hemodynamic parameters, impedance cardiography, left-ventricular ejection fraction

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Introduction

Diagnostics and treatment of acute heart failure (AHF) are one of the key problems in intensive cardiac care [1]. The prognosis remains poor, with in-hospital mortality of 4.1–13.9% [2–6]. The current European Society of Cardiology (ESC) guidelines emphasize the need for urgent AHF management [2, 7]. In order to be effective, management should be based on detailed clinical assessment, aiming to identify the key mechanism of cardiovascular decompensation [8]. Whereas most patients with heart failure (HF) and left-ventricular ejection fraction (LVEF) < 40% (i.e. HF with reduced ejection fraction, HFrEF) exhibit evidence of fluid accumulation and fluid redistribution to the lungs, which leads to pulmonary congestion, those with HF with mid-range (mildly reduced) ejection fraction (HFmrEF) and HF with preserved ejection fraction (HFpEF) typically

show more diverse pathomechanisms [9–13]. The latter two subgroups (HFmrEF and HFpEF) constitute an increasing proportion of patients with AHF [14,15,16]. These patients are typically elderly, often with concomitant diabetes mellitus, hypertension, atrial fibrillation, and/or obesity [17, 18]. Their treatment may, therefore, present more challenges, as recommendations for their management are based mainly on expert opinions.

Thus, it is useful to search for diagnostic methods that would provide additional data compared to that obtained from routine assessments, while at the same time being simple enough to be used in intensive-care settings. These conditions seem to be met by impedance cardiography (ICG), a simple, non-invasive method of assessing the hemodynamic parameters that reflect the cardiac function as a pump (including cardiac index (CI), stroke index (SI), systemic vascular resistance index (SVRI)) and thoracic fluid content (TFC) [19].

Therefore, the aim of this study was to compare clinical presentations between subgroups of patients hospitalized for AHF stratified by LVEF, with a particular emphasis on their hemodynamic profiles.

Methods

This prospective, observational study enrolled patients of both sexes, aged > 18 years, who were admitted to the Department of Cardiology and Internal Diseases due to decompensated HF (defined based on ESC guidelines) in the period between November 2014 and March 2017 and required intravenous diuretic treatment.

Exclusion criteria were: 1) unstable angina; 2) history of acute coronary syndrome (ACS) within the last 12 weeks and/or coronary artery bypass grafting (CABG) surgery within the last 12 weeks; 3) cardiac resynchronization therapy (CRT) introduced within the last year (or planned CRT implantation within the next 24 months); 4) non-cardiogenic shock; 5) valvular disease or other acquired heart defects requiring surgical intervention; 6) hypertrophic cardiomyopathy; 7) severe pulmonary hypertension or other severe lung condition (severe form of chronic obstructive pulmonary disease (COPD) or bronchial asthma); 8) poorly controlled hypertension; 9) anaemia (haemoglobin < 10.0 g/dL); 10) acute and/or decompensated non-cardiovascular disease; 11) end-stage CKD and/or ongoing hemodialysis therapy; 12) severe or chronic inflammatory disease, severe infection (including febrile conditions, radiologically-confirmed pneumonia, suspected septic shock); 13) neoplastic disease; 14) severe psychiatric disorder; 15) the lack of informed consent.

The study protocol was approved by the Military Institute of Medicine Institutional Review Board (approval No. 14/WIM/2012), and all study participants provided their written informed consent. This study was registered at ClinicalTrials.gov (NCT 02355769).

Clinical examinations were conducted with a particular emphasis on the history of symptoms, concomitant diseases, and current medication. The following were measured on physical examination: heart rate (HR), office systolic blood pressure (SBP), office diastolic blood pressure (DBP), and basic body parameters.

Laboratory tests were conducted on fasting peripheral venous blood samples, collected in the morning (7:30–8:30 a.m.). The following hematological and biochemical parameters were measured: hematocrit, as well as hemoglobin, urea, creatinine, N-terminal pro-brain natriuretic peptide (NTproBNP), high-sensitivity troponin T (hsTnT) levels. The estimated glomerular filtration rate (eGFR) was estimated based on the Modification of Diet in Renal Disease (MDRD) study equation [20].

Echocardiographic examinations were conducted with Vivid S6 (GE-Healthcare, USA) and Vivid 7 (GE-Healthcare, USA) ultrasound systems and evaluated cardiac chamber dimensions, left ventricular wall thickness and contractility, ejection fraction with the biplane Simpson's method, as well as valvular structure and function. Echocardiography reports included any moderate-to-severe mitral, tricuspid, and/or aortic regurgitation; severe aortic stenosis; as well as the numerical values of the following parameters: left ventricular end-diastolic diameter (LVEDD), right ventricular end-diastolic diameter (RVEDD), interventricular septum (IVS), left atrial (LA) diameter, measured in the parasternal long-axis view.

Impedance cardiography (ICG). All ICG measurements were performed with the Niccomo™ device (Medis, Germany) within 24 hours of admission, after 10 minutes of rest in a sitting position. Data was recorded during a 10-minute assessment and exported to the dedicated software (Niccomo Software). The final analysis included mean values of hemodynamic parameters, such as: TFC [1/kOhm], calculated from basic impedance (Z0) as its reciprocal: TFC = 1000/Z0; SI, calculated using the Sramek and Bernstein formula for stroke volume (SV) = $VEPT \times dZ_{max} \times LVET/Z0$ and indexed to body surface area to yield SI [mL/m^2]; CI [$(mL/min)/m^2$], calculated as SI×HR; acceleration index (ACI [$1/100*Ohm/s^2$]), expressing the maximum acceleration of blood in the aorta from the moment the aortic valve opens; velocity index (VI [1/1000*Ohm/s] expressing the maximum velocity of blood in the aorta from the moment the aortic valve opens; Heather index (HI [$Ohm*s^2$]), characterizing the maximum contraction force of the left ventricle, corresponding to cardiac inotropism; SVRI [$(dyn \times s)/cm^5/m^2$)], calculated as 80×(MBP–CVP)/CI, where CVP is central venous pressure (with an assumed value of 6 mm Hg).

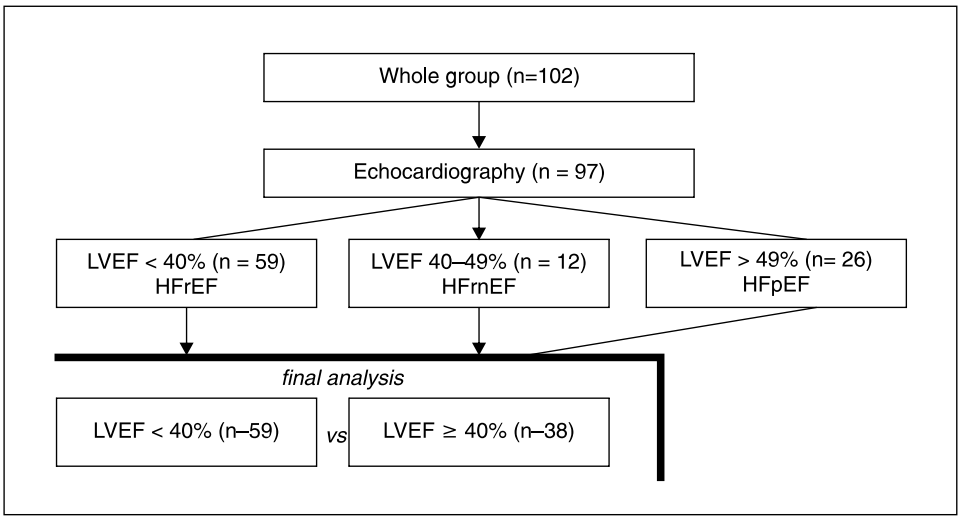

Figure 1. Analysis assumptions — compared subgroups (HFrEF — heart failure with reduced left ventricular ejection fraction; HRmrEF — heart failure with mid-range left ventricular ejection fraction; HFpEF — heart failure with preserved left ventricular ejection fraction, LVEF — left ventricular ejection fraction)

Statistical analysis. The statistical analysis of data was conducted with the use of MS Office Excel 2013 and Statistica 12.0 (StatSoft Inc.). Data distribution was presented on histograms and evaluated visually. The results for qualitative variables were expressed as numbers and percentages; while continuous (quantitative) variables were expressed as means ± standard deviation (SD). For a comparative analysis, the study group was divided into two subgroups: patients with LVEF < 40% (n = 59) and LVEF ≥40% (n = 38) (Figure 1).

Results

Clinical characteristics

The subgroup with LVEF < 40% comprised predominantly men with ischemic HF etiology. These patients were younger than those in the LVEF ≥ 40% subgroup (Table 1). Nonetheless, the two groups showed no significant differences in terms of the New York Heart Association (NYHA) functional class, rates of dyspnea, or a history of edema, or pathological weight gain. Physical examination of patients with higher LVEF showed higher blood pressure values, higher rates of peripheral edema, and lower rates of peripheral hypoperfusion. The subgroups differed only slightly in terms of medication, with higher rates of angiotensin-converting enzyme (ACE) inhibitors in the LVEF ≥ 40% subgroup.

The echocardiographic examination showed the mean LVEF value in the study population of 37.3 ± 14.1%, LVEDD of 59.2 ± 10.2 mm, RVEDD of 35.3 ± 5.7 mm, and LA dimeter of 47.3 ± 0.60 mm. In comparison, patients with LVEF < 40% had larger cardiac chamber dimensions, higher rates of moderate/severe mitral regurgitation, with lower rates of moderate/severe aortic stenosis (Table 2).

The mean NT-proBNP level in the LVEF < 40% subgroup was significantly higher than that in the subgroup with better LVEF (Table 3). There was a significant correlation between LVEF values and NT-proBNP levels (R = -0.38; P < 0.0001). At the same time, patients with LVEF ≥ 40% had significantly lower hemoglobin levels and hematocrit values, with comparable markers of renal function.

The two compared subgroups differed significantly in terms of hemodynamic profiles. Patients with LVEF < 40% exhibited lower SBP values, lower values of cardiac function as a pump (SI, CI, HI, ACI, VI), higher TFC, and a less favourable ratio of pre-ejection period (PEP) to left ventricular ejection time (LVET) (Table 4, Fig. 2). These differences were confirmed when we assessed the correlation of these parameters with LVEF.

Discussion

our findings demonstrated that the clinical presentation of decompensated HFrEF differs from that of HFmrEF/HFpEF. Our observations regarding age differences, sex distribution, HF etiology, echocardiographic findings, and comorbidities are essentially consistent with those presented in earlier reports. Impedance cardiography proved to significantly differentiate patients from the two evaluated subgroups. Patients with higher

Table 1. The comparison between patients with LVEF < 40% and LVEF ≥ 40% — patients characteristics

	LVEF < 40% N = 59	LVEF ≥ 40% N = 38	P	Whole group N = 97
	n (%)/ mean ± SD			
Age, mean ± SD	68.1 ± 13.2	76.7 ± 9.5	0.0005	71.5 ± 12.6
Male, mean ± SD	50 (84.8)	24 (63.2)	0.015	74 (76.3)
NYHA class				
Mean class NYHA [-], mean ± SD	3.32 ± 0.57	3.32 ± 0.52	0.897	3.32 ± 0.55
class III, n (%)	37 (62.7)	25 (65.8)	0.773	62 (63.9)
class IV, n (%)	22 (67.3)	13 (34.2)	0.773	35 (36.1)
HF de novo, n (%)	16 (27.1)	10 (26.3)	0.931	26 (26.8)
Ischemic etiology, n (%)	41 (69.5)	21 (55.3)	0.003	62 (63.9)
CLINICAL EXAMINATION				
Dyspnea at rest, n (%)	26 (44.1)	15 (39.5)	0.655	41 (42.3)
Dyspnea on effort, n (%)	58 (98.3)	38 (100.0)	0.420	96 (99.0)
Orthopnoe, n (%)	45 (77.6)	30 (79.0)	0.875	75 (77.3)
Edema, n (%)	44 (74.6)	31 (81.6)	0.421	75 (77,.3)
Pathological weight gain, n (%)	23 (39.0)	14 (36.8)	0.832	37 (38.1)
PHYSICAL EXAMINATION				
HR [bpm], mean ± SD	89.4 ± 25.3	82.3 ± 20.1	0.220	86.6 ± 23.5
SBP [mmHg], mean ± SD	127.3 ± 25.6	147.2 ± 27.0	0.0001	135.1 ± 27.2
DBP [mmHg], mean ± SD	80.2 ± 12.8	83.3 ± 12.8	0.282	81.4 ± 13.5
BMI [m^2/kg], mean (SD)	28.9 ± 5.8	31.5 ± 6.9	0.094	29.9 ± 6.3
Hypertension (SBP > 140mmHg, DBP >90mmHg), n (%)	6 (10.2)	19 (50.0)	0.00006	25 (25.8)
Hypotension (SBP < 90mmHg), n (%)	3 (5.1)	2 (5.3)	ns	5 (5.2)
Tachypnoe, n (%)	14 (23.7)	6 (15.8)	0.345	20 (20.6)
Rales, n (%)	58 (98.3)	38 (100.0)	0.783	96 (99.0)
Edema, n (%)	40 (67.8)	34 (89.5)	0.014	74 (76.3)
Peripheral hipoperfusion, n (%)	9 (15.3)	1 (2.6)	0.046	10 (10.3)
CONCOMITANT DISEASE				
Prior MI, n (%)	32 (54.2)	10 (26.3)	0.007	42 (43.3)
Hypertension, n (%)	34 (57.6)	30 (79.0)	0.031	64 (66.0)
Atrial fibrillation, n (%)	29 (49.2)	22 (57.9)	0.400	51 (52.6)
Moderate-to-severe valvular disease, n (%)	18 (30.5)	15 (39.5)	0.477	33 (34.0)
Procedure: ICD, n (%)	10 (17.0)	0 (0.0)	0.040	10 (10.3)
Procedure: CRT, n (%)	5 (8.5)	1 (2.6)	0.040	6 (6.2)
Diabetes mellitus, n (%)	29 (49.2)	19 (50.0)	0.935	48 (49.5)
COPD, n (%)	10 (17.0)	5 (13.2)	0.614	15 (15.5)
CKD (stadium ≥ 3), n (%)	16 (27.6)	12 (31.6)	0.674	28 (28.9)
MEDICATION USE BEFORE HOSPITALIZATION (available for 95)				
ACE-I, n (%)	30 (52.6)	28 (73.7)	0.039	58 (61.1)
ARB, n (%)	5 (8.8)	5 (13.2)	0.495	10 (10.5)
B blocker, n (%)	41 (71.9)	33 (86.8)	0,086	74 (77.9)
Aldosterone antagonists, n (%)	22 (38.6)	9 (23.7)	0.129	31 (32.6)
Diuretics, n (%)	40 (70.2)	29 (76.3)	0.511	69 (72.6)
Iwabradine, n (%)	0 (0.0)	2 (5.3)	0.080	2 (2.1)
Digoxin, n (%)	3 (5.3)	3 (7.9)	0.605	6 (6.3)
Amiodarone, n (%)	10 (17.5)	3 (7.9)	0.180	13 (13.7)

ACE-I — angiotensin-converting-enzyme inhibitors; ARB — angiotensin II receptor blockers; BMI — body mass índex; CKD — chronic kidney disease; COPD — chronic obstructive pulmonary disease; CRT — cardiac resynchronization therapy; DBP — diastolic blood pressure; HR — heart rate; ICD — implantable cardioverter defibrillator; MRA — mineralocorticoid receptor antagonista; NYHA — New York Heart Association; SBP — systolic blood pressure

Table 2. The comparison between patients with LVEF < 40% and LVEF ≥ 40% — echocardiography

	LVEF < 40% N = 59	LVEF ≥ 40% n=38	p
	n (%)/ mean ± SD		
LVEDD [mm], mean ± SD	65.0 ± 8.4	51.4 ± 6.6	0.000001
RVEDD [mm], mean ± SD	36.4 ± 6.2	33.7 ± 4.6	0.081
LA [mm], mean ± SD	48.7 ± 5.2	45.6 ± 6.7	0.015
LVEF [%], mean ± SD	27.7 ± 6.5	52.2 ± 8.3	0.000001
MR[85], n (%)	32 (65.3)	14 (38.9)	0.016
AS[85], n (%)	2 (4.1)	7 (19.4)	0.023
AR[85], n (%)	0 (0.0)	2 (5.6)	0.095
TR[85], n (%)	19 (38.8)	15 (41.7)	0.707

Upper index — number of subjects with sufficient valve assessment; AR — aortic regurgitation; AS — aortic stenosis; LA — left atrium; LVEDD — left ventricle end-diastolic dimension; RVEDD — right ventricle end-diastolic dimension; LVEF — left ventricle ejection fraction; MR — mitral regurgitation; TR — tricuspid regurgitation

Table 3. The comparison between patients with LVEF < 40% and LVEF ≥ 40% — laboratory data on admission

	LVEF < 40% N = 59	LVEF ≥ 40% N = 38	p	Whole group N = 97
	mean ± SD			
Creatinine [mg/dl], mean ± SD	1.36 ± 0.49	1.24 ± 0.55	0.148	1.31 ± 0.51
eGFR MDRD [ml/min/1.73 m^2], mean ± SD	61.5 ± 24.2	63.2 ± 23.0	0.644	62.2 ± 23.6
Urea [mg/dl], mean ± SD	55.8 ± 29.4	51.6 ± 21.6	0.615	54.2 ± 26.6
NT-proBNP [pg/ml], mean ± SD	7991 ± 8463	3453 ± 3031	0.0008	6213 ± 7195
hsTnT [ng/l], mean ± SD	124.5 ± 292.2	79.2 ± 212.2	0.219	106.9 ± 263.4
Hb [g/dl], mean ± SD	13.1 ± 2.0	11.8 ± 2.4	0.003	12.6 ± 2.3
Hematocrit [%], mean ± SD	39.8 ± 5.7	36.4 ± 6.6	0,003	38.5 ± 6.2

eGFR — estimated glomerular filtration rate; Hgb — hemoglobina; hsTnT — high-sensitive cardiac troponin T; NTproBNP — N-terminal fragment of the prohormone brain-type natriuretic peptide

LVEF seemed to have less pronounced abnormalities in their hemodynamic profile, with higher values of parameters indicating cardiac function as a pump and lower TFC. However, it is worth noting that the symptoms reported by patients with HFmrEF/HFpEF were not any less pronounced than those reported by patients with LVEF < 40%.

Although, the whole study group was predominantly male, the proportion of men was noticeably lower in the HFmrEF/HFpEF subgroup. Data from AHF registries show the proportion of women in this subgroup to range from 53% to 72.4% [21–24].

The patients from the HFmrEF/HFpEF subgroup were older and had higher rates of concomitant chronic conditions and lower rates of post-infarct HF etiology [21]. Patients with HFrEF are known to have higher rates of coronary artery disease, while those with HFpEF have higher rates of atrial fibrillation, hypertension, and anaemia [6, 17, 18, 25]. Particularly interesting were our findings on anaemia, which are consistent with earlier reports on higher rates of this condition in HFpEF [26, 27]. Our findings regarding the rates of chronic kidney disease (CKD) being comparable in both subgroups were also consistent to those reported in many registries [23, 28–31]. However, Bishu et al. [27], who assessed renal function based on cystatin C levels, demonstrated higher rates of CKD in patients with HFmrEF/HFpEF, which was most likely due to the selected diagnostic marker, as cystatin C is highly sensitive [32, 33–36]. Quiroz et al. made similar observations, finding higher admission creatinine levels in patients with LVEF > 50% [21].

In our study, the two subgroups differed the most in terms of the rates of hypertension, with as many as 50% of HFmrEF/HFpEF patients presenting with a blood pressure of over 140/90 mmHg. This is consistent with earlier reports [6, 27, 37] and may be responsible for the higher rates of renin-angiotensin-aldosterone system (RAAS) inhibitors in the subgroup with LVEF ≥ 40%, although some reports have indicated higher rates of calcium-channel blockers and alpha-blockers, rather

Table 4. The comparison between patients with LVEF < 40% and LVEF ≥ 40% — impedance cardiography

	LVEF < 40% N = 59	LVEF ≥ 40% N = 38	p	LVEF vs.	
	n (%)/ mean ± SD			R	P
IMPEDANCE CARDIOGRAPHY					
HR [bpm], mean ± SD	83.8 ± 22.8	77.7 ± 20.1	0.188	-0.03	0.784
SBP [mmHg], mean ± SD	114.6 ± 16.9	136.1 ± 29.5	0.0002	0.38	0.0001
DBP [mmHg], mean ± SD	73.7 ± 11.5	72.2 ± 12.0	0.418	-0.04	0.718
SI [ml*m^{-2}], mean ± SD	36.0 ± 10.4	44.4 ± 13.7	0.004	0.30	0.005
CI [ml*m^{-2}*min^{-1}], mean ± SD	2.86 ± 078	3.12 ± 0,80	0.208	0.24	0.026
HI [Ω•s^{-2}], mean ± SD	7.82 ± 4.77	12.0 ± 6.75	0,002	0,38	0.0003
ACI [1*100^{-1}*s^{-2}], mean ± SD	59.6 ± 23.3	77.1 ± 39.1	0.051	0.25	0.022
VI [1*1000^{-1}*s^{-1}], mean ± SD	38.2 ± 15.7	48.1 ± 25.5	0.120	0.22	0.047
SVRI [dyn*s*cm^{-5}*m^2], mean ± SD	2424 ± 733	2292 ± 802	0.457	-0.17	0.131
TFC [1*kOhm^{-1}], mean ± SD	37.4 ± 8.2	33.7 ± 6.5	0.009	-0.28	0.005
STR [%], mean ± SD	0.54 ± 0.33	0.36 ± 0,13	0.001	-0.38	0.0002
PEP [ms], mean ± SD	137.5 ± 62.1	103.2 ± 34.0	0.003	-0.38	0.0002
LVET [ms], mean ± SD	272.5 ± 47.7	303.1 ± 62.8	0.018	0.18	0.100
SI < 35 ml/m2, n (%)	23 (38.9)	8 (21.1)	0.041	-	-
TFC > 35 kOhm, n (%)	34 (57.6)	12 (31.6)	0.012	-	-

ACI — acceleration time índex; CI — cardiac índex; DBP — diastolic blood pressure; HI — Heather índex; HR — heart rate; LVET — left ventricular ejection time; PEP — pre-ejection period; SBP — systolic blood pressure; STR — systolic time ratio; SVRI — systemic vascular resistance índex; TFC — thoracic fluid contente; SI — stroke índex; VI — velocity index

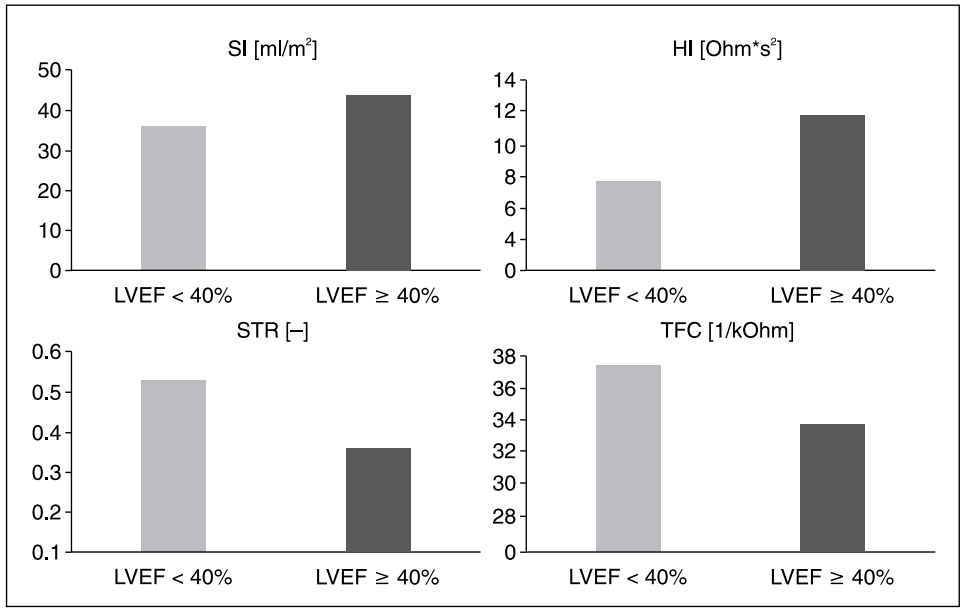

Figure 2. The comparison of hemodynamic parameters between patients with LVEF < 40% and LVEF ≥ 40%

than RAAS inhibitors, in patients with HFpEF [24, 27]. The lower rates of RAAS inhibitor use in patients with HFrEF may have been due to the higher rates of objective clinical contraindications (e.g. hypotension, renal dysfunction, hyperkalemia) in this group.

Our findings demonstrated that, in comparison with patients with HFrEF, patients with HFmrEF/HFpEF had lower natriuretic peptide levels [38–40], which could indicate a lower myocardial load in the latter subgroup. However, the fact that the HFmrEF/HFpEF subgroup

had higher rates of obesity may have also played a role, as low levels of natriuretic peptides may be due to increased natriuretic peptide absorption by adipocytes [41] as well as their reduced production as part of disrupted hormonal metabolism in the obese [42].

Impedance cardiography assessments revealed significant differences in hemodynamic profiles between the study subgroups stratified by LVEF. To our knowledge, this is the first report of this kind. Our findings demonstrated low LVEF to be reflected by lower ICG parameters of cardiac function as a pump (SI, CI, HI, ACI, VI). The values of these parameters in the HFrEF subgroup were comparable to those presented in earlier reports. Kaszuba et al. demonstrated a relationship between the ejection fraction and PEP, LVET, and STR, although that particular study included patients with no manifestations of HF exacerbation [43]. On the other hand, the hemodynamic profile of left ventricular function in the HFmrEF/HFpEF subgroup more closely resembled that in patients with uncomplicated hypertension. Studies evaluating hemodynamic parameters in hypertensive patients showed that even diastolic dysfunction alone was reflected in lower values of SI, VI, ACI, and HI as well as a higher SVRI [44, 45].

It seemed advisable to compare the subgroups also in terms of TFC, a parameter useful in differentiating the causes of dyspnea and assessing pulmonary congestion [46, 47]. We found that, although the rates of patients with elevated TFC were considerable in both subgroups, they were noticeably higher in patients with HFrEF (57.6 vs. 31.6%). Only one in three patients with HFmrEF exhibited marked pulmonary congestion. This indicates that diuretic treatment in this subgroup may not always be as effective as expected.

Our findings clearly showed differences between the hemodynamic profiles of patients with HFmrEF/HFpEF and of those with HFrEF. This suggests that the reported symptoms could be due mainly to other, concomitant conditions (poorly controlled hypertension, arrhythmia, or acute exacerbation of CKD, etc.). An ostensibly "better" hemodynamic profile does not exclude a poor clinical condition and severe symptoms. Moreover, the complexity of potential pathomechanisms makes it more difficult to select optimal treatment. Therefore, the diagnostic assessments in these patients should help select a treatment most suitable for the predominant cause of HF exacerbation.

Our findings may explain why it is so difficult to obtain robust scientific evidence for the effectiveness of selected medications in treating patients with HFmrEF/HFpEF. In such a non-homogeneous group [1, 48] the use of varied regimens, based on individual hemodynamic profiles may be a better management strategy. Therefore, ICG may be a practical tool in this group of patients, as its usefulness in selecting optimal treatments based on the individual hemodynamic disturbances has been already demonstrated in patients with hypertension [49, 50].

Study limitations

One indisputable limitation of our study is the small sample size. The observed differences in hemodynamic profiles may have been partly due to the uneven distribution of the sexes between the two subgroups. However, this fact should not be a significant confounding factor, as both subgroups were predominantly male. Another significant limitation was the 24-hour window allowed for hemodynamic assessment, as the hemodynamic profile may change even within less than an hour of initiating effective treatment. On the other hand, the varied time of echocardiographic examination was less problematic, as the LVEF value during clinical stabilization is considered to be the most reliable prognostic factor.

Conclusion

This study confirms earlier observations on the differences between patients with significantly impaired left ventricular systolic function versus those with mildly impaired and preserved left the ventricular systolic function. Despite the fact that left ventricular function does not determine the severity of clinical presentation in patients with decompensated HF, the observed differences in hemodynamic parameters demonstrated a non-homogeneity of the pathomechanisms and causes of decompensated HF. These findings prompt further studies on the use of ICG in patients hospitalized due to HF exacerbation.

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List of abbreviations

ACEI — angiotensin-converting-enzyme inhibitors
ACI — acceleration index
AHF — acute heart failure
AR — aortic regurgitation
ARB — angiotensin II receptor blocker
AS — aortic stenosis
BMI — body mass index
CI — cardiac index
CKD — chronic kidney disease
COPD — chronic obstructive pulmonary disease

CRT — cardiac resynchronization therapy
CVP — central venous pressure
DBP — diastolic blood pressure
EF — ejection fraction
eGFR — estimated glomerular filtration rate
ESC – European Society of Cardiology
Hgb — hemoglobin
HF — heart failure
HFmrEF — heart failure with mid-range ejection fraction
HFpEF — heart failure with preserved ejection fraction
HFrEF — heart failure with reduced ejection fraction
HI — Heather index
HR — heart rate
hsTnT — high-sensitivity troponin T
ICD — implantable cardioverter defibrillator
ICG — impedance cardiography
IVS — interventricular septum
LA — left atrium
LVEDD — left ventricular end-diastolic diameter
LVEF — left ventricular ejection fraction
MDRD — modification of diet in renal disease
MRA — mineralocorticoid receptor antagonist
NTproBNP — N-terminal pro-brain natriuretic peptide
NYHA — New York Heart Association
PEP – pre-ejection period
RVEDD — right ventricular end-diastolic diameter
SBP — systolic blood pressure
SD — standard deviation
SI — stroke index
STR — systolic time ratio
SVRI — systemic vascular resistance index
TFC — thoracic fluid content
TRC — the time interval between the R-wave peak (in ECG) and the C-point (in ICG)
TR — tricuspid regurgitation
VI — velocity index

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VIA MEDICA

ORIGINAL ARTICLE

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The possible use of the blood serum concentration measurements of sHLA-G in women with endometrial and cervical cancers during radiotherapy as an indicator of the status of the tumour microenvironment

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ABSTRACT

Background: The selective suppression of cytotoxic immune cells constitutes a crucial event in the development of malignancy. This phenomenon increases in accordance with the growth of a tumor and is just one result of the increased expression in the cancer milieu of those proteins, such as human leukocyte antigen G (HLA-G) and its soluble form (sHLA-G). Given that radiotherapy may influence immune system activity, we aimed to measure (sHLA-G) serum levels both before and after the radiotherapy due to endometrial or cervical cancer.

Methods: We assessed the sHLA-G blood serum concentration levels in a group of 43 patients (28 and 15 diagnosed with cervical cancer and endometrial cacer respectively), who received primary or adjuvant radiotherapy. We assessed the blood serum concentrations of the sHLA-G through a series of measurements taken before and four days after the latest radiation dosage using an ELISA kit.

Results: Median serum sHLA-G levels significantly decreased after radiotherapy (5.63 U/ml; range 0.00 – 344.55; vs 5.57 U/ml; 0.00 –94.02; P = 0.045). The changes of sHLA-G levels didn't influence patients' survival. Pretreatment and post-treatment sHLA-G levels were negatively correlated with patients' age (R Spearman = -0.45, P = 0.041; R Spearman = -0.46. P = 0.038).

Conclusions: The detected levels of sHLA-G blood serum concentrations may supply clinically applicable information regarding the status of the tumor microenvironment — that is, the size and the degree of suppression of the tumor environment — where the tumor-immune cell interaction is realized. Finally, this information may also prove helpful in the treatment of cancer.

Key words: Ovarian cancer, endometrial cancer, sHLA-G

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Introduction

The suppression of the immune system constitutes a crucial event in the development of malignancy. It is known that this phenomenon increases in accordance with the growth of a tumour as a result of the increased expression in the cancer milieu of the proteins responsible for the evasion of cancer cells from immune system surveillance. The suppressive environment is related not only to the membrane form of expression, but also to the secretion of the soluble form of these proteins, such as Fas-L [1], RCAS1 [2–3], and HLA-G [4], to the

extracellular matrix of the cancer microenvironment. This profile of the microenvironment is also determined by the infiltration of immune regulatory cells (e.g., Treg) into the cancer microenvironment itself [5] and to the polarization of the tumour microenvironment by an increase in Th2 cytokine (e.g., IL-10) concentration [6]. Tylor *et al.* have demonstrated that the recruitment of Tregs to the cancer microenvironment inhibits an effective antitumor immune response [7]. It has been shown that radiotherapy affects the function of the immune system. Muroyama *et al.* have shown that RT-induced proliferation of Treg cells and the post-RT intratumoral Treg cells have a suppressive function [8]. Generally, the clinical studies suggest that radiotherapy increase the production of Tregs and their recruitment to local tumour microenvironment [9].

The presence of these factors in the tumour microenvironment may be discerned in the cancer milieu. Furthermore, the levels of its expression or the concentrations of its soluble forms can be determined not only in the tumour microenvironment but also in the peripheral blood. Since these factors are crucial for the development of the phenomenon, the possibility of assessing the levels in women treated for gynaecological malignancies would seem to constitute a clinically applicable indicator of the status of the tumour microenvironment — that is, the size and the degree of suppression of the tumour environment. Most likely, the use of the information deriving from the interactions between cancer and its stroma and the immune cells in the tumour microenvironment will spur improvement in the therapy for gynaecological malignancies. As of now, treatment for endometrial cancers remains a clinical problem [10].

We focused on our studies on the cancer microenvironment and on the proteins present there and deriving from the cancer milieu. Most likely such proteins like HLA-G can be used as biomarkers not specifically linked with the particular type of gynaecological malignancy, as they have also been found under normal physiological conditions in women's reproductive tracts (e.g., in the feto-maternal interface) [11]. The blood serum profiles of these biomarkers may supply interesting information for clinicians, such as the fact that developing cancer modifies its own microenvironment.

HLA-G is an antigen whose participation in the regulation of the immune system has been well documented [11]. HLA-G is one of the proteins involved in the regulation of the interaction of the tumour and immune cells that takes place in the microenvironment of a growing tumour [12, 13]. HLA-G is believed to protect the target cells that are deficient in HLA class I antigens from NK-dependent lysis by interacting on their surfaces with killer-inhibitory receptors. Tumour cells may, therefore, be excluded from the host immune response. Expression of the suppressive molecule HLA-G differs from that found in other types of malignancies. In general, an increased expression of HLA-G is known to be associated with disease progression not only in ovarian [14], endometrial [15], and breast [16] cancers, but also in non-gynaecological types of malignancies, such as bladder cancers [17], and retinoblastoma [18]. Park *et al.* have demonstrated that cells can generate the soluble HLA-G (HLA-G1 and HLA-G5) by the dual mechanism of alternative splicing and proteolytic shedding and that the soluble form of HLA-G is able to inhibit the lytic activity of NK cells [11]. The concentration of the soluble form of sHLA-G in the peripheral blood could demonstrate the level of suppression by the tumour environment. Ben Yahia *et al*. have demonstrated the growth of sHLA-G in early stages (Stages I and II) as well as the correlation with grading of endometrial cancer. The alteration of the level of sHLA-G was associated with the rapid spread of the disease [19].

The presence in the peripheral blood proteins such as sHLA-G might be related to the suppressive influence of cancer cells on the immune system. This information detected in peripheral blood is valuable and applicable especially from a clinical point of view. For this reason, we decided in our study to evaluate the sHLA-G blood serum concentration levels both before and after the radiotherapy in patients treated for cervical and endometrial carcinomas.

Material and methods

Human subject

In the case of early-stage cervical cancer patients (up to and including IB1 according to FIGO classification) a radical hysterectomy and adnexectomy with pelvic and paraaortic lymphadenectomy were performed followed by brachytherapy and external beam radiation. More advanced stages were treated with primary radiotherapy with or without concurrent chemotherapy. Patients with endometrial cancer were treated surgically in all cases and simple, extrafascial hysterectomy with bilateral ovariectomy with pelvic lymphadenectomy was performed. All of the analyzed patients with endometrial cancer were treated with adjuvant external beam radiation and brachytherapy with or without chemotherapy. The mean age of the patients included was 58 (range 35–84 years). The patients had undergone treatment in the Gynecologic Oncology Department of the M. Sklodowska-Curie Memorial Institute or in the Gynecology and Oncology Department of the Lukaszczyk Oncological Center, respectively in Krakow and Bydgoszcz between January 2007 and September 2010. The patient's consent was obtained in each case. Prior to the study, the approval of the Jagiellonian Uni-

versity Ethical Committee (KBET/135/B/2007) was also obtained. Information on all the patients who died was retrieved from the database of the Kujawsko-Pomorski and Malopolski regional office of the National Health System of Poland. We have analyzed long-term outcomes after radiotherapy regarding overall survival (OS).

ELISA

The blood was collected to a serum collection tube both directly prior to radiotherapy and on the fourth day following the last radiation dosage. A clot was allowed to form at room temperature for 30–60 minutes. The tube was placed on ice for 30 minutes in order to contract a clot. The serum samples were then centrifuged at 3000xg for 10 minutes at room temperature. The supernatants 1.0–2.0 ml were collected and stored at -80°C. The analysis of sHLA-G concentration in the serum samples was performed in the Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics, and Biotechnology, Jagiellonian University. The soluble human leukocyte antigen-G (sHLA-G) was detected using the sHLA-G sandwich ELISA kit (BioVendor-Exibo, Czech Republic). Briefly stated, the blood plasma samples were diluted twice and incubated for 1 hour in the 96-well microplate precoated with the monoclonal anti-sHLA-G antibodies. Following incubation, the wells were washed and then filled with the monoclonal anti-human beta-2-microglobulin antibodies labeled with horseradish peroxidase. After an additional 1 hour of incubation, the wells were again washed, and the colour reaction was developed using tetramethyl benzidine (TMB) substrate. The absorbance values were measured at 450 nm on a microplate reader followed by the calculation of the sHLA-G concentrations. The assay was calibrated using a set of sHLA-G standards provided by the producer of the kit.

Statistical analysis

The distribution of variables in the study groups of women checked with the use of the Shapiro-Wilk test showed that each of the women was different from normal. Pre- and postoperative sHLA-G concentrations levels were analyzed using the Wilcoxon test. The statistical significance in the levels of sHLA-G, both pre- and post treatment, between cervical cancer and endometrial cancer patients groups was determined by the Mann-Whitney Test. The Mann-Whitney test was also used for the calculation of differences related to FIGO stage and cancer grade. For survival evaluation, Kaplan-Meier curves analysis was performed.

Results

The statistically significant differences in sHLA-G blood serum concentration level were identified before radiotherapy and on the fourth day following the collection of the last dosage of radiotherapy (Median 5.63 U/ml; range 0.00 – 344.55; vs 5.57 U/ml; 0.00–94.02; p = 0.04, respectively). The results are summarized in Table 2.

No statistically significant differences were identified in the sHLA-G blood serum concentration levels with respect to the clinicopathological parameters, such as FIGO stage and tumour grade. Similarly, there were no differences in pre- and post-treatment sHLA-G levels between cervical and endometrial cancer patients. Pre-treatment sHLA-G levels in the sera of patients with I and II stage disease was not significantly different from patients with stage III and IV disease (5.63 U/ml, range 0–71.82, vs. 5.45 U/ml, range 0–344.55 U/ml, P = 0.735). Similarly, the difference in post-treatment levels of sHLA-G was not statistically significant (5.75 U/ml, 0–41.28 vs 5.09 U/ml, 0–94.02, P = 0.474). The difference in sHLA median levels between well and moderately differentiated tumors was not different from poorly differentiated tumors, both in pre- and post-treatment evaluation (5.63 U/ml, 0–71.82 vs. 5.45 U/ml, 0–344.55; P = 0.71 and 5.75 U/ml, 0–41.28 vs. 5.09 U/ml, 0–94.02 U/ml, P = 0.622).

We have found significant, negative correlation between patients' age both pre- and postreatment and sHLA-G levels (R Spearman = -0.45, P = 0.041; R Spearman = -0.46. P = 0.038 respectively).

No statistically significant differences were identified in the sHLA-G blood serum concentration levels before and after radiotherapy with respect to a long-term outcome. When patients were divided into two groups: patients with decreased or stable post-treatment sHLA-G levels (Group 1, n = 31) and patients with increased post-treatment sHLA-G levels (Group 2, n = 12), there were no statistical significant difference in patients' survival (1592 vs. 657 days, P = 0.60; Figure 1).

Discussion

We have found statistically significant differences between the levels of the blood serum concentrations of sHLA-G as measured before and after radiotherapy in patients treated for cervical and endometrial carcinomas. Radiotherapy induces single- and double-stranded DNA breaks leading to apoptosis [20], but it has been shown that RT affects the immune system activity, including the induction of a systemic antitumor response, with a pro-inflammatory activity and an abscopal effect [21, 22]. RT has also been demonstrated

Table 1. Clinicopathological characteristics and treatment modalities of the patients

In this study, we analyzed the blood serum samples obtained from 43 patients, including No	Age	Cancer	FIGO	Histopathology	Grade	Surgery	Chemo-therapy
1	50	Cervical cancer	IB1	squamous cell carcinoma	3	Yes	No
2	46	Cervical cancer	IB1	squamous cell carcinoma	3	Yes	Yes
3	35	Cervical cancer	IB2	squamous cell carcinoma	2	No	No
4	50	Cervical cancer	IIA	squamous cell carcinoma	2	No	Yes
5	51	Cervical cancer	IIA	squamous cell carcinoma	3	No	Yes
6	48	Cervical cancer	IIB	squamous cell carcinoma	2	No	No
7	65	Cervical cancer	IIB	adenocarcinoma	2	No	Yes
8	65	Cervical cancer	IIB	squamous cell carcinoma	2	No	Yes
9	52	Cervical cancer	IIB	squamous cell carcinoma	2	No	Yes
10	52	Cervical cancer	IIB	adenocarcinoma	1	No	Yes
11	62	Cervical cancer	IIB	squamous cell carcinoma	3	No	Yes
12	42	Cervical cancer	IIB	squamous cell carcinoma	3	No	Yes
13	48	Cervical cancer	IIIB	squamous cell carcinoma	2	No	No
14	63	Cervical cancer	IIIB	squamous cell carcinoma	1	No	No
15	58	Cervical cancer	IIIB	squamous cell carcinoma	3	No	Yes
16	72	Cervical cancer	IIIB	squamous cell carcinoma	3	No	Yes
17	48	Cervical cancer	IIIB	squamous cell carcinoma	2	No	No
18	52	Cervical cancer	IIIB	squamous cell carcinoma	2	No	Yes
19	49	Cervical cancer	IIIB	adenocarcinoma	2	No	Yes
20	47	Cervical cancer	IVB	squamous cell carcinoma	2	No	Yes
21	58	Cervical cancer	IIIB	squamous cell carcinoma	3	No	Yes
22	43	Cervical cancer	IIIB	squamous cell carcinoma	2	No	Yes
23	52	Cervical cancer	IIB	squamous cell carcinoma	2	No	Yes
24	68	Cervical cancer	IIA	squamous cell carcinoma	2	No	Yes
25	62	Cervical cancer	IIB	squamous cell carcinoma	3	No	Yes
26	46	Cervical cancer	IB1	squamous cell carcinoma	3	Yes	Yes
27	68	Cervical cancer	IIA	squamous cell carcinoma	2	No	Yes
28	48	Cervical cancer	IIIB	squamous cell carcinoma	2	No	No
29	66	Endometrial cancer	IB	endometrioid adenocarcinoma	3	Yes	Yes
30	79	Endometrial cancer	IB	endometrioid adenocarcinoma	2	Yes	No
31	74	Endometrial cancer	IB	endometrioid adenocarcinoma	3	Yes	Yes
32	52	Endometrial cancer	IB	endometrioid adenocarcinoma	2	Yes	No
33	61	Endometrial cancer	IIIA	endometrioid adenocarcinoma	2	Yes	Yes
34	66	Endometrial cancer	IIIB	endometrioid adenocarcinoma	1	Yes	No

→

Table 1 cd. Clinicopathological characteristics and treatment modalities of the patients

In this study, we analyzed the blood serum samples obtained from 43 patients, including No	Age	Cancer	FIGO	Histopathology	Grade	Surgery	Chemo-therapy
35	74	Endometrial cancer	IIIC1	endometrioid adenocarcinoma	3	Yes	Yes
36	61	Endometrial cancer	IIIB	endometrioid adenocarcinoma	2	Yes	No
37	52	Endometrial cancer	II	endometrioid adenocarcinoma	1	Yes	No
38	46	Endometrial cancer	IIIA	serous adenocarcinoma	3	Yes	Yes
39	78	Endometrial cancer	IIIA	serous adenocarcinoma	3	Yes	Yes
40	84	Endometrial cancer	II	undifferentiated carcinoma	3	Yes	No
41	70	Endometrial cancer	II	endometrioid adenocarcinoma	1	Yes	No
42	74	Endometrial cancer	IIIC1	endometrioid adenocarcinoma	3	Yes	Yes
43	70	Endometrial cancer	II	endometrioid adenocarcinoma	1	Yes	No

Table 2. The difference in pre- and post-treatment serum sHLA-G levels

	Median	Range	P-value
Pretreatment	5.63 U/ml	0.00–344.55	P = 0.04
Post-treatment	5.57 U/ml	0.00–94.02	

to exert an immunosuppressive effect, increased levels of functionally active Treg lymphocytes were detected following radiotherapy [23]. Ionizing radiation has also been demonstrated to modulate the HLA-G expression. Michelin *et al*. have demonstrated that irradiation downregulated cell surface and total HLA-G levels and increased sHLA-G1 in the medium of the melanoma cell line. Authors concluded that radiotherapy might induce a proteolytic cleavage of this molecule [24]. Most probably decreasing serum level of sHLA-G observed after radiotherapy is linked with reduction of the tumour mass that the patients underwent during surgery before adjuvant radiotherapy.

The suppression of the immune system constitutes a crucial event in the development of malignancy, particularly in cases of cancer relapse. Surgery, chemo- and radiotherapy all have different effects on tumour and immune cell interaction [25, 26]. Since, on the one hand, the immune system can demonstrate anti-tumour activity, but, on the other hand, can promote tumour growth, the degree of the suppressive influence of cancer cells on the immune system may be able to determine the success of the treatment for a cancer relapse [2]. Nevertheless, it is not common practice to evaluate the suppressive influence of cancer cells on the immune system. This detection could be helpful in monitoring treatment processes and might reflect the influence of this treatment on the restoration of proper immune system activity. sHLA-G, however, has been observed in blood sera of patients with gynaecological malignancies [15]. Furthermore, sHLA-G has not yet been studied in relation to the applied surgery in cases of gynaecological malignancies.

HLA-G expression has been observed in endometrial cancer and can be compared with the expression reported in various other malignances. In immunohistochemical staining, HLA-G expression varied between 40% and 55% according to different studies [15, 27]. This discrepancy could be the result of the different characteristics of the patients included in the study, such as being in an advanced stage of the disease or having a tumor with non-endometrioid histology. Barrier *et al.* found a correlation between HLA-G expression and increasing FIGO stage which could serve as a pre-operative indicator of dissemination [15]. Contrary to our study we did not observe a correlation between the FIGO stage and the blood serum concentration levels of sHLA-G. Most likely this has to do with the character-

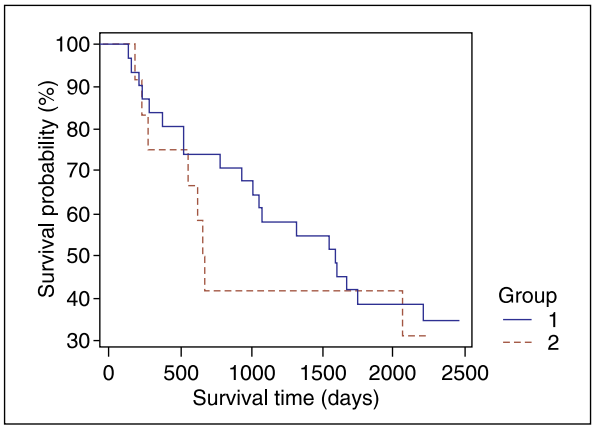

Figure 1. Kaplan-Meier survival curves for analyzed patients. Group 1: patients with decreased or stable post-treatment sHLA-G levels; Group 2: patients with increased post-treatment sHLA-G levels (1592 vs 657 days, P = 0.60)

istics of the patients included in the study, where most patients have advanced diseases [11].

HLA-G expression was associated with disease progression in patients with cervical cancer. In the study of Li *et al.*, the expression of HLA-G molecule gradually increased from preinvasive stages to advanced cancers, indicating the role of HLA-G in tumour progression [28]. Additionally, Dong et al., indicates, that the HLA-G expression is related to HPV16/18 infection [29]. However, tissue expression of HLA-G may not directly reflect serum levels of soluble HLA-G. Similarly to our study, in the paper by Samulels *et al.*, patients survival was not influenced by serum sHLA-G levels. Additionally, the authors also did not find any association between sHLA-G levels and clinicopathological characteristic of cervical cancer [30].

This serial method of measurement may help to reveal the relationship between the applied therapy and the size and degree of the suppression of the tumour environment. Furthermore, such results could indicate that a change in therapy is needed; it could also provide a strong, clinical indication and foundation for the earlier application of molecular therapies, such as immunotherapy.

Conclusions

The detected sHLA-G blood serum concentrations may supply clinically applicable information regarding the status of the tumour microenvironment — that is, the size and the degree of suppression of the tumour environment — where the tumour-immune cell interaction is realized. Finally, this information may also prove helpful in the treatment of cancer.

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Statement of competing interests

The authors declare no competing financial interest and no conflicts of interests.

List of abbreviations

HLA-G — uman leukocyte antigen G
sHLA-G — Soluble Human Leukocyte Antigen-G
RCAS1 — receptor-binding cancer antigen expressed on SiSo cells
Treg — Regulatory T cells
RT — radiotherapy

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VIA MEDICA

ORIGINAL ARTICLE

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A preliminary study on MTDH expression as a potential prognostic cancer marker

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ABSTRACT

Background: Clinical studies have revealed that MTDH is overexpressed in various malignancies and is associated with disease progression and poor clinical outcomes. In order to study MTDH prognostic potential, we decided to evaluate MTDH expression changes using cancerous and non-cancerous cells lines. Secondly, for the first time, we evaluated MTDH expression in prostate cancer cell lines representing different metastatic potential in vivo.

Methods: MTDH and PBGD (control) genes expression were measured by reverse transcription-quantitative polymerase chain reaction assay using Universal Probe Library in cancerous and non-cancerous cell lines.

Results: MTDH gene expression analysis showed a decrease in colorectal cancer cell lines (Caco2, HT29) compared to non-cancerous cells lines (VH10, VH25, Hek293). The mean level of the MTDH mRNA expression, normalized in relation to the reference gene PBGD, increased in the prostate cancer cell lines as follows: PC3 (0.62 ± 0.07), PC3M (1.02 ± 0.17), PC3MPro4 (1.20 ± 0.22), and reached the highest value in PC3M4 (1.86 ± 0.48). In VH10, the expression of this gene was at 1.0 ± 0.07.

Conclusions: Our MTDH gene expression data are consistent with Mtdh protein expression analyzed in The Human Protein Atlas (HPA). Increasing MTDH expression is a promising prognostic factor.

Key words: MTDH (Metadherin), prostate cancer cell lines, gene expression

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Introduction

Bioinformatics analysis of pharmacogenetic (60 cell lines, including 58 cancer lines, with a specific cytogenetic profile and the gene expression profile, tested for the resistance or sensitivity to the 24,000 new chemicals) identified the 8q22 region as potentially associated with response to chemotherapy [1]. Studies using high-throughput technologies allowed the characterization of the high correlation between the amplification of genes on chromosome 8q22 and early recurrence of cancer [2–4].

There are 13 known genes in that region of chromosome, including MTDH which plays a key role in the metastasis [4]. Interestingly, MTDH has 30–40% higher expression in breast cancer what accelerates the formation of metastasis and increases chemoresistance [4]. The MTDH gene was first identified in PHFA cells (Primary Human Fetal Astrocytes) after inducing with HIV-1 (Human Immunodeficiency Virus-1) or exposure to HIV-1 envelope glycoprotein [5, 6].

MTDH (Metadherin) firstly called AEG-1 (Astrocyte Elevated Gene-1), then LYRIC (3D3 and LYsine-RIch CEACAM1 co-isolated) is a gene that encodes a protein involved in physiological processes, such as regulating differentiation and proliferation of progenitor cells at early stages of embryonic development as part of the RNA-induced silencing complex (RISC) that participates in gene silencing. However, the protein is also involved in some pathological conditions, including cancer metastasis, increased proliferation and multidrug resistance [7–9].

Recently, clinical studies have revealed that MTDH is overexpressed in various cancers and associated with disease progression and poor clinical outcomes [10]. Moreover, MTDH has been found to promote cancer metastasis, chemoresistance, invasion, angiogenesis [7–9] and a key player in radioresistance regulation [11].

In this study, we analyzed the expression of the MTDH gene in the cancerous and noncancerous cells. Moreover, we decided to check how the expression of MTDH changes with the advancement of

prostate cancer. For this purpose, we used a research model of cell lines derived from the PC3 line: PC3M, PC3MLN4 and PC3MPro4 ref: Pettaway CA, Pathak S, 1996 https://www.ncbi.nlm.nih.gov/pubmed/9816342), which present various degrees of advancement of the same cancer.

Methods

Cell line cultures

The PC3, PC3M, PC3MLN4 and PC3MPro4 cell lines [12] were cultured in the RPMI medium, while the VH10, CACO2, HT29 and Hek293 cell lines were cultured in the DMEM medium (both from BioWest SAS (France) with 10% fetal bovine serum (FBS) (Pan-Biotech GmbH, Germany) and a 5% antibiotic and antimycotic solution (BioWest SAS) at 37°C in a 5% CO2 atmosphere at a constant humidity.

mRNA isolation

mRNA isolation was performed using the High Pure RNA Isolation Kit from Roche Diagnostics GmbH (Germany) according to the manufacturer's protocol. The method includes lysis of cells and subsequent separation of RNA on silica columns. RNA from each cell line was isolated in triplicate.

Reverse transcription reaction

cDNA synthesis was carried out using the Transcriptor High Fidelity kit cDNA Synthesis Kit from Roche Diagnostics GmbH according to the manufacturer's protocol, using two types of primers: Anchored oligo (dT) 18 Primer and Random Hexamer Primer.

Real-Time PCR

Real-time PCR was carried out using primers with the following sequences: reverse primer 5'-gaaactggctcagcagtagacc -3'; forward primer 5'- actggagatgctaatacaaatgga -3 ', and the TaqMan UPL 60 probe. The primers were selected using the free Universal Probe Library Assay Design Center application available online at www.universalprobelibrary.com. The porphobilinogen deaminase (PBGD) gene (Universal Probe Library Human PBGD Gene Assay, Roche, Life Science) was used as a reference gene for the normalization of the obtained data. The real-time PCR reaction was performed using Light Cycler 2.0 PCR System Roche Diagnostics GmbH (Germany) in the following conditions: one cycle at 95°C/10 min; 45 cycles of denaturation (95°C/10 sec), annealing (60°C/30 sec) and extension (72°C/1 sec) with data collection in two channels: 530 nm for FAM and 610 nm for Light Cycler® Yellow 555. Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

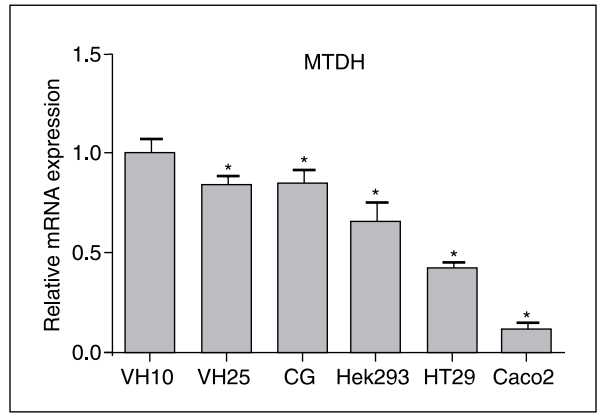

Figure 1. Results of the MTDH gene expression. Analyses were performed three times in three biological replicates (9 repetitions in total) and results were normalized with PBGD as the reference gene. The graph presents cell lines showing a decrease in the MTDH gene expression in colorectal cancer cell lines compared to non-cancerous cells lines (VH10, VH25, Hek293)

Results

MTDH gene expression was measured in cancerous (Caco2, HT29, PC3, PC3M, PC3MLN4, PC3MPro) and non-cancerous cell lines (HEK293 and fibroblasts: VH10 and VH25) using quantitative qPCR. All results were performed three times in three biological replicates and normalized with PBGD expression. We observed a statistically significant decrease of MTDH expression in Caco2, HT29 and PC3 cells in comparison to fibroblasts. Furthermore, our analysis conducted in the PC3, Caco2 and non-cancerous cell lines HEK293 cell demonstrated levels of the MTDH gene expression similar to those reported by the HPA (Human Protein Atlas) project available online.

The mean level of the *MTDH* mRNA expression, normalized in relation to the reference gene *PBGD*, increased in the prostate cancer cell lines as follows: PC3 (0.62 ± 0.07), PC3M (1.02 ± 0.17), PC3M-Pro4 (1.20 ± 0.22), and reached the highest value in PC3M4 (1.86 ± 0.48). In VH10, the expression of this gene was at 1.0 ± 0.07 (Fig. 2).

Discussion

Overexpression of *MTDH* gene was noticed in multiple cancers including colorectal cancer [13] breast cancer [14], prostate cancer [15] and glioma [16].

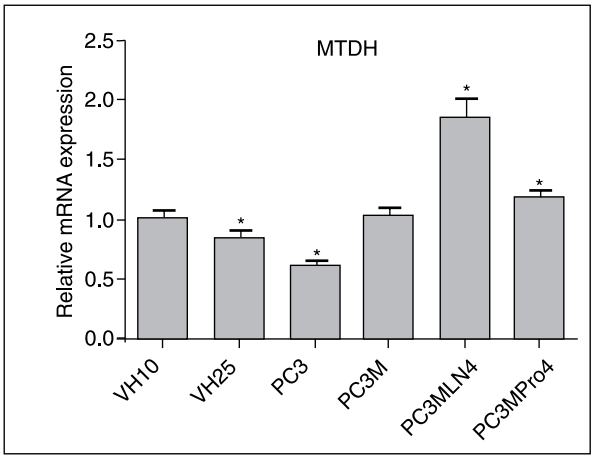

Figure 2. Relative levels of the *MTDH* gene expression in prostate cancer cell lines and fibroblasts. The level of expression was normalized in relation to the reference gene *PBGD*, with values standardized in relation to VH10. The graph shows mean values from 9 analysis; ± SD. Asterisks indicate statistically significant values ($p \leq 0.05$) compared to VH10. The graph presents cell lines showing an increase in the MTDH gene expression in PC3MLN4, PC3MPro compared to VH10 fibroblasts.

The expression of this gene was also analyzed as part of the large-scale project — The Human Protein Atlas (HPA) [17] at the protein as well as mRNA level in various tissues and cell lines. In our study, we compared *MTDH* mRNA expression between cancerous (CG, HT29, Caco2, PC3, PC3M, PC3MLN4, PC3Mpro4) and noncancerous cell lines (VH10, VH25, Hek293). Some of these cell lines were included in HPA, which gave us the opportunity to assess the compliance of our results. HPA included six different fibroblast cell lines, of which five were foreskin. We also chose two human foreskin fibroblasts: VH10 and VH25 as wild-type reference cell lines. Another noncancerous cell line, Hek293, was underexpressed compared to fibroblasts in HPA as well as in our analysis.

Our results of *MTDH* expression pattern in cancerous cell lines were also consistent with the previous results. Caco2 was one of the cell lines analyzed in HPA with the lowest expression of *MTDH* gene. We also noticed the lowest *MTDH* expression in Caco2. On the other hand, PC3 cell line showed a slightly decrease in *MTDH* expression in this study as well as in HPA.

The consistency of the results obtained in this work with the previous research encouraged us to study other cell lines: CG and HT29. HT29 is another cell line derived from colorectal adenocarcinoma and, like Caco2, showed statistically significant lower expression level of *MTDH* compared to VH10. Moreover, we analyzed *MTDH* expression in CG cell line. It was between VH10 and Hek293, with was consistent with results of *MTDH* expression in cell lines derived from brain obtained in HPA.

Finally, we assessed whether the expression of *MTDH* increases with the progression of the disease. For this purpose, we used prostate cancer malignant transformation model, cell lines derived from PC3 (PC3M, PC3MLN4 and PC3MPro4). The highest level of metastatic capabilities in a mouse model of human prostate cancer reflects PC3MLN4. We found that this cell line showed the highest level of *MTDH* expression. We confirmed that metastatic capabilities of prostate cancer cell lines correlated with an increase of *MTDH* expression.

High expression of MTDH was also detected in the tissues and other cancer prostate cell lines. Kikuno [18] showed MTDH overexpression in clinical PC tissue samples and cultured PC cells compared to benign prostatic hyperplasia tissue samples and normal prostate epithelial cells. They proposed MTDH as a novel genetic biomarker to serve as an attractive molecular target for new anticancer agents to prevent PC cell progression and metastasis. High MTDH expression also was observed in another prostate cancer cell lines: LNCaP, DU145 [18, 19].

In addition to gene expression evaluation — Mtdh protein level was measured using human prostate tissue microarray [15]. It showed that Mtdh distribution is in the cytoplasm and cell membrane and was concluded that subcellular distribution can predict Gleason grade and survival. Furthermore, Mtdh was also found in the bone metastases of prostate cancer (81.8%) [15]. Erdem et al. [20] examined 97 prostatectomy samples and showed that MTDH coupled with the main fibroblast growth factor is an independent prognostic parameter. These results suggest that MTDH expression, both on RNA and protein level seems to be a potential prognostic factor in prostate cancer. Moreover, the possible signalling pathways in prostate cancer cell lines, in which MTDH might be involved and that can be potential therapeutic targets for antineoplastic agents, have been studied. Inhibition of the Mtdh expression proved to be one of the molecular events demonstrating an antineoplastic effect [19]. In order to implement MTDH expression as a prognostic factor, further studies with consistent results are needed - gene expression analysis using digital droplet PCR and measurement of the potential biomarker in plasma or urine collected from prostate cancer's patients - should also be taken into account.

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VIA MEDICA

ORIGINAL ARTICLE

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Rationale and design of PREvalence of DyspneA in patients treated with TicagrelOR (PREDATOR) program

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ABSTRACT

Background: Ticagrelor, a reversible P2Y12 inhibitor, is a mainstay of antiplatelet strategy in patients with acute coronary syndrome (ACS). However, a large number of ticagrelor-induced dyspnea decrease patients' adherence and reduce an overall efficacy of the therapy.

Design: The PREDATOR program consists of phase III and IV, multicenter, randomized, double-blind, placebo-controlled clinical trials and preceding pilot studies that assesses the prevalence and treatment of ticagrelor-induced dyspnea in coronary artery disease (CAD) and ACS patients. The PREDATOR LD is designed to evaluate the occurrence of dyspnea after 180 mg ticagrelor loading dose, and relief of dyspnea by theophylline administration in low-to-high risk acute coronary syndromes without ST-segment elevation (NSTE-ACS) and stable CAD designated to undergo invasive treatment. The PREDATOR MD is a cross-over trial in stable CAD patients 1 year after percutaneous coronary intervention for ACS. Enrolled patients will be randomized to one of four antiplatelet treatment regimens (ticagrelor 2x90 mg, ticagrelor 2x60 mg, ticagrelor 2x45 mg or clopidogrel 75 mg [morning]+placebo [evening]) or placebo and will be assessed for dyspnea at the day 7, then undergo a switch of treatment and reassessment at day 14. The sample size will be estimated based on preceding pilot studies.

Discussion: The PREDATOR LD is expected to prospectively assess dyspnea rate with a loading dose of ticagrelor, and analyze a potential of theophylline to elevate symptoms of ticagrelor-induced dyspnea, while the PREDATOR MD will prospectively assess dyspnea and adverse events rate with a maintenance dose of P2Y12 inhibitors prospectively assess. All evaluations will be conducted using standardized metrics for dyspnea quantification.

Key words: antiplatelet, ticagrelor, dyspnea, loading dose, maintenance dose, rationale, trial

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Introduction

Antiplatelet therapy with P2Y12 inhibitors is a mainstay strategy for the management of acute coronary syndrome (ACS) patients. Ticagrelor, novel and reversible P2Y12 inhibitor, is recommended as a class I therapy for 12 months after an ACS event by the European Society of Cardiology and the American College of Cardiology/American Heart Association guideline committees [1–3]. The optimal antiplatelet effect prevents from thrombotic complications, including devastating in consequences stent thrombosis that drives cardiovascular mortality in the early stage post percutaneous coronary intervention (PCI) [4–7]. The potency of antithrombotic strategy with novel P2Y12 inhibitor, ticargelor, as compared to its irreversible counterpart clopidogrel, is well established [8–10]. In large randomized controlled trial (RCT) ticagrelor (90 mg or 60 mg, twice daily) in combination with aspirin significantly reduced the risk of cardiovascular death, myocardial infarction (MI), or stroke as compared with clopidogrel in patients who had prior MI 1–3 years earlier [11]. Bleeding and respiratory adverse events, however, were more frequently observed with ticagrelor use.

One of the reasons of ineffectiveness of antiplatelet therapy originates from low adherence and discontinuation of the therapy due to adverse events [12–17]. Dyspnea is a relatively frequent adverse event ob-

served during ticagrelor therapy, and while it is usually reported as "mild" or "moderate" in intensity among patients experiencing it, it remains an overt reason for discontinuation of the assigned treatment [18]. In the secondary analysis of the PEGASUS-TIMI 54 Trial the rate of discontinuation due to an adverse event was the highest in the treatment arm receiving 90 mg of ticagrelor (19%), followed by the treatment arm receiving 60 mg of ticagrelor (16%), both of which were significantly higher than that in the placebo arm (9%), with the differences most marked within the first year from randomization [19]. A dose-dependent relative increase of both dyspnea and dyspnea requiring discontinuation was present, however, the discontinuation rate resulting from dyspnea among those subjects, who reported dyspnea, was 5-flod higher than observed in the PLATO trial. Interestingly, discontinuation due to dyspnea (6.2% ticagrelor 90 mg bid *vs* 4.3% ticagrelor 60 mg bid *vs* 0.7% placebo) had the same prevalence as the disocntinuation due to bleeding (6.5% ticagrelor 90 mg bid *vs* 5.1% ticagrelor 60 mg bid *vs* 1.2% placebo). The increased risk of bleeding is on the other side of spectrum of thrombotic milieu and is inevitable in successful antithrombotic management; dyspnea, however, remains an adverse event with a high potential of prevention [14]. Ticagrelor-induced dyspnea exerts generally no effort on the patient's activities of daily life and can be resolved quickly, as it does not influence the patient's cardiac nor pulmonary function. Taking together not sufficient awareness of the recognition and management of ticagrelor-related dyspnea, especially among elderly ACS patients treated with several drugs, there exists a global underestimation and mismanagement of this adverse event [20]. For a proper management of patients complaining of dyspnea when taking ticagrelor, all clinically plausible causes of dyspnea must be considered before attributing it to medication itself [21]. Episodes of dyspnea tend to occur during first days after the initiation of the therapy [18]. Dyspnea may have devastating effects when leading to treatment discontinuation, especially in the case of frail population as ACS patients. In the first multicenter observational prospective study to investigate rate of ticagrelor-related dyspnea and its impact on daily life in patients in their first month post PCI, ticagrelor was withdrawn in 16.7% patients during the 1st month of follow-up, with ticagrelor-related dyspnea at the rate of 55.6% of all withdrawal reasons [22]. To provide further optimization of the antiplatelet strategies it is mandatory to avoid discontinuation due to a preventable adverse event.

A number of potential mechanisms could cause ticagrelor-induced dyspnea. It has been hypothesized that the sensation of dyspnea is related to reversibility of the P2Y12 inhibiting agent origins from increased levels of adenosine, which cellural reuptake is impaired [23]. Pulmonary vagal C fibres are stimulated by increased adenosine levels through the A_1R and $A_{2A}R$ receptors on vagal sensory C fibers, which mediate the sensation of dyspnea. The increase in serum adenosine is higher with reversible (i.e. ticagrelor) rather than irreversible P2Y12 inhibitors (clopidogrel) [24]. Therefore, potential treatment options for ticagrelor-associated dyspnea may include adenosine antagonism, such as aminophylline or theophylline. The cases of successful reversal of ticagrelor-induced dyspnea have been described in literature [25]. Their role is still not yet fully addressed in a large clinical study. In a promising randomized trial TROCADERO (TRial Of Caffeine to Alleviate DyspnEa Related to ticagrelOr) the primary objective was to assess the efficacy of caffeine in reducing symptoms of ticagrelor-induced dyspnea, measured by the visual analog scale area under the curve (VAS AUC), in patients after an ACS [26]. However, out of 514 subjects who participated in the questionnaire survey, only a small number of patients experienced dyspnea that required an intervention and were randomized to one of the strategies: 13 to caffeine, and 10 to placebo. In this context, dyspnea and treatment to evelate its symptoms were not prospectively assessed in a large study with a use of standardized tool, thus, its prevalence and severity is not well established.

In the PREDATOR program, we aim for the first time to 1) prospectively assess dyspnea rate with a loading dose of reversible P2Y12 inhibitor, ticagrelor, 2) analyze a potential of theophylline to elevate symptoms of ticagrelor-induced dyspnea, 3) prospectively assess dyspnea and adverse events rate with a maintenance dose of P2Y12 inhibitors. All evaluations will be conducted using standardized metrics for dyspnea quantification.

Methods

Program design and objectives

The PREvalence of DyspneA in patients treated with TicagrelOR (PREDATOR) is an international, multicenter program consisting of two trials preceded by pilot studies (Figure 1). The inclusion and exclusion criteria for both PREDATOR Loading Dose (LD) Study and PREDATOR Maintenance Dose (MD) Study are presented in Table 1. The same criteria will apply to the pilot studies. The main objective of the program is to prospectively and quantitatively assess, with the use of a standardized score, prevalence of dyspnea with ticargelor and a potential of theophylline administration on dyspnea cessation among ACS and stable CAD patients. The study will be conducted in accordance with the principles contained in the Dec-

laration of Helsinki, and every study site will receive an approval from the Local Ethics Committee. Each patient will provide a written informed consent to participate in the study.

Statistical design and analysis

Since the rate of dyspnea with ticagrelor use varies in definitions and rates across previously published literature, we decided to perform preceding pilot studies to estimate the sample size.

PREDATOR LD

The PREDATOR LD is a double-blind, placebo-controlled, randomized trial in low-to-high risk acute coronary syndromes without ST-segment elevation (NSTE-ACS) and stable coronary disease designated to undergo invasive treatment. The primary objective of the study is to evaluate the occurrence of dyspnea after 180 mg ticagrelor loading dose, with following secondary objective of assessing relief of dyspnea by theophylline administration. Patients will undergo two stages of randomization — first randomization to 180 mg ticagrelor LD *vs.* placebo followed by a switch of therapy after 2 hours in patients without dyspnea, and second randomization to theophylline p.o. (loading dose 5 mg/kg, maintenance dose 100 mg tid) *vs.* placebo p.o. of only patients with dyspnea in 1st randomization stage (Figure 2). The study follow-up will be stratified to three durations, driven by randomization: 1) 1st follow-up, within 2 hours after first randomization (patients with dyspnea directly undergo second randomization, while patients without dyspnea by the end of follow-up have the allocated therapy switched), 2) 2nd follow-up, within 2 hours after the 1st follow-up, up to 4 hours after first randomization (patients with dyspnea undergo second randomization, while patients without dyspnea undergo coronary angiography/PCI), 3) 3rd follow-up, within 1 hour after second randomization, and up to 5 hours after first randomization (all patients undergo coronary angiography/PCI, while for patients in whom dyspnea have occurred at first follow-up period the study treatment will be unblinded after termination of the protocol and before PCI; patients in placebo arm

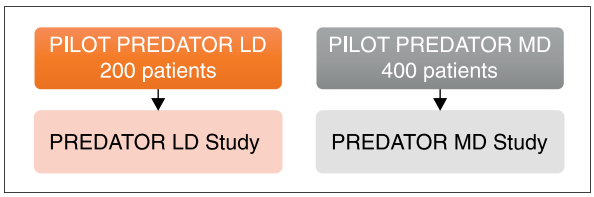

Figure 1. Schematic design of the PREDATOR program

Table 1. Inclusion and exclusion criteria of PREDATOR LD Study and PREDATOR MD Study

	PREDATOR LD Study	PREDATOR MD Study
Inclusion criteria	• diagnosis of low, intermediate or high risk NSTE-ACS designated to invasive treatment or diagnosis of stable coronary disease designated to invasive treatment	• diagnosis of stable coronary disease 1 year after PCI for acute coronary syndrome with known anatomy of coronary arteries designated to invasive treatment
	• male or non-pregnant female • age > 18 years old • provision of informed consent for angiography and PCI • provision of written informed consent to participate in the study	
Exclusion criteria	• diagnosis of STEMI • diagnosis of very high risk NSTE-ACS designated to immediate invasive treatment • dyspnea present at screening • contraindications for ticagrelor or theophylline • second or third degree atrioventricular block during screening for eligibility	• diagnosis of acute coronary syndrome • PCI with coronary stenting during the last 12 months • chronic dyspnea present at screening (e.g. due to severe COPD) • contraindications for ticagrelor, prasugrel, clopidogrel • history of second or third degree atrioventricular block without implanted pacemaker
	• treatment with ticagrelor or theophylline within 14 days before the study enrolment • current treatment with oral anticoagulant or chronic therapy with low-molecular-weight heparin • active bleeding • history of intracranial haemorrhage • recent gastrointestinal bleeding (within 30 days) • history of coagulation disorders • history of moderate or severe hepatic impairment • history of major surgery or severe trauma (within 3 months) • patient required dialysis • manifest infection or inflammatory state • respiratory failure • current therapy with strong CYP3A inhibitors or strong CYP3A inducers	

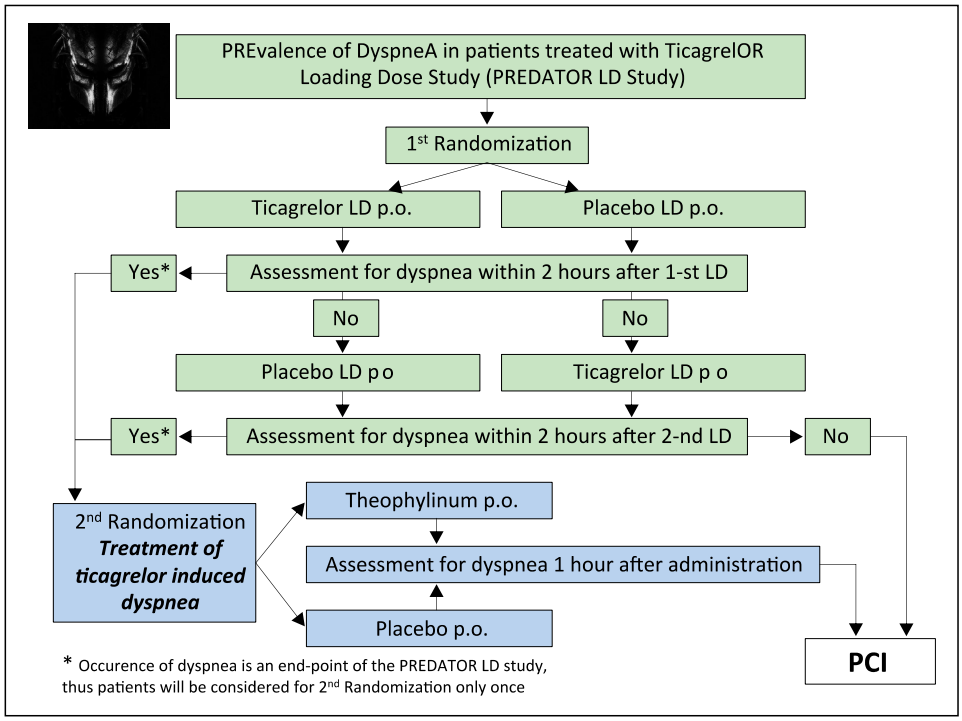

Figure 2. PREDATOR LT Study design

will unblindly receive ticagrelor LD). Dyspnea, as well as the relief from it, will be quantified with the Modified Borg Dyspnoea Score (Table 2). The exact number of study population will be determined based on the PREDATOR LD pilot study including 200 patients.

PREDATOR MD

The PREDATOR MD is a double-blind, placebo-controlled, cross-over, randomized trial conducted in stable coronary artery disease (CAD) patients 1 year after PCI for ACS. Enrolled patients will be randomized to one of four antiplatelet treatment regimens (ticagrelor 2x90 mg or ticagrelor 2x60 mg or ticagrelor 2x45 mg or clopidogrel 75 mg [morning] + placebo [evening]) or placebo (1:1:1:1:1 ratio), and will be assessed for dyspnea at the day 7 of the allocated therapy. On the same day all patients will undergo a switch of treatment and will be reassessed for dyspnea at day 14. The primary objective of the study is to evaluate the occurrence of dyspnea with different maintenance dose of antiplatelet treatment regimens. Dyspnea will be quantified with the Modified Borg Dyspnoea Score. The secondary endpoints of this study, evaluating the safety of P2Y12 inhibiting therapy, include major bleedings according to the Thrombolysis In Myocardial Infarction (TIMI) criteria, minor bleedings according to TIMI criteria, clinically relevant bradycardia, level of serum uric acid, neutrophil count, overall premature discontinuation of the study drug and discontinuation of the study drug due to dyspnoe. Discontinuation is defined as study drug cessation following medical contact. The exact number of study population will be determined based on the PREDATOR MD pilot study including 400 patients.

Table 2. Modified Borg Dyspnoea Scale

Rate	Symptoms
0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight
3	Moderate
4	Somewhat severe
5	Severe
6	
7	Very severe
8	
9	Very, very severe (almost maximal)
10	Maximal

Study organization

The executive committee is responsible for the overall design, conduct, and supervision of the study,

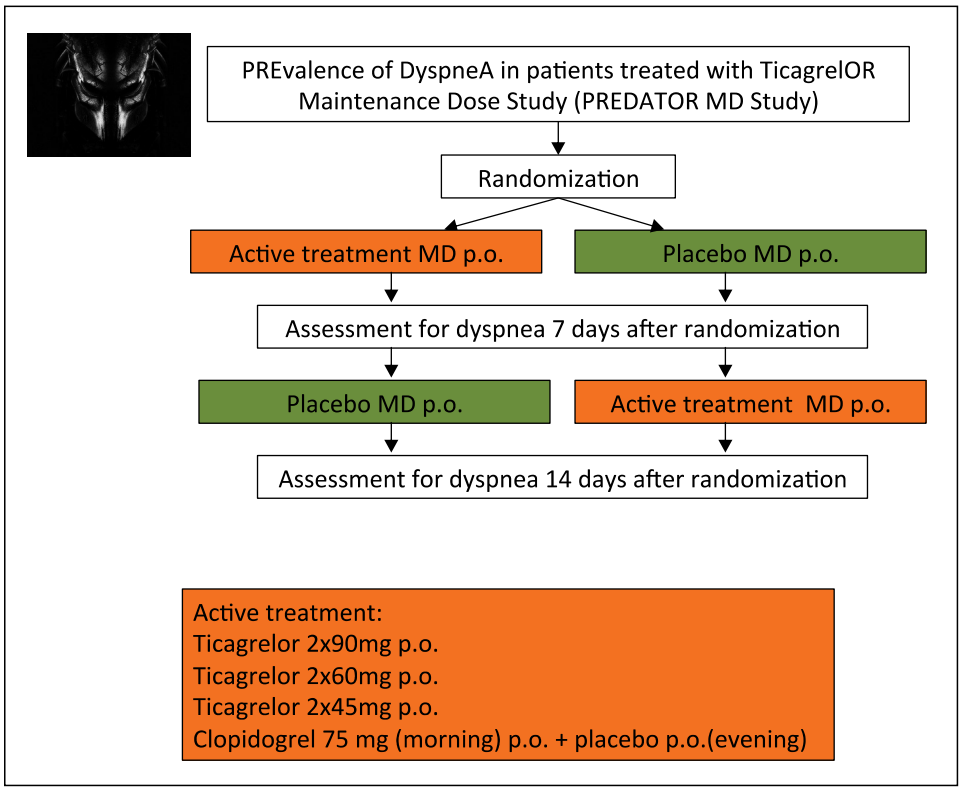

Figure 3. PREDATOR MD Study design

including the development of any protocol amendments. The independent academic statistician will perform or confirm all statistical analyses of the final data. A National Leaders Committee, composed of lead investigators from each participating country, works in tandem with the Executive Committee. The executive committee is composed of representatives from the Collegium Medicum of Nicolaus Copernicus University (Bydgoszcz, Poland), and other collaborating centers. A clinical events committee reviews and adjudicates each suspected clinical end point event in a blinded fashion.

Conclusions

The PREDATOR program includes phase III and IV, multicenter, randomized, double-blind, placebo-controlled clinical trials that assesses the prevalence and treatment of ticagrelor-induced dyspnea in CAD/ACS patients. Those trials will provide important information regarding the impact of ticagrelor loading and maintenance dose on the occurrence and severity of dyspnea, prospectively assessed by a standardized score. A disclosure of such information could provide medical practitioners with more effective tools preventing from consequences of therapy discontinuation due to mild adverse events.

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Statement of competing of interests

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List of abbreviations

ACS — acute coronary syndrome, CAD — coronary artery disease, LD — loading dose, MD — maintenance dose, MI — myocardial infarction, NSTE-ACS — acute coronary syndromes without ST-segment elevation, PCI — percutaneous coronary intervention, RCT — randomized controlled trial, STEMI — ST-segment elevation

myocardial infarction, TIMI — Thrombolysis In Myocardial Infarction, VAS AUC — visual analog scale area under the curve

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VIA MEDICA

CASE REPORTS

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The evolving clinical picture of seronegative spondyloarthropathy based on the example of ankylosing spondylitis in a patient with a primary diagnosis of the SAPHO syndrome — case presentation.

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ABSTRACT

Seronegative spondyloarthropathies are a group of diseases characterized by several clinical features such as inflammatory back pain, sacroiliac joint inflammation, and the presence of the HLA B27 antigen, which occurs more frequently than the general population. Non-specific bowel inflammation, skin lesions, particularly psoriasis or uveitis, are also typical in patients or their family members [1]. In many situations, clinical progression of spondyloarthropathies can be assessed by imaging of the sacroiliac joints [2, 3]. SAPHO syndrome (Synovitis, Acne, Pustulosis, Hyperostosis, Osteitis), is a rare disease, classified as seronegative spondyloarthropathy due to many typical clinical features for this group of diseases. A large variety of symptoms and atypical clinical picture of SAPHO syndrome causes significant diagnostic difficulties [3, 4]. Presented here is a case report of a 41-year-old male patient with a twenty-year history of the disease. Initially, his disease took the form of chronic, recurrent multifocal osteomyelitis (CRMO), which finally turned into a spondyloarthropathy that met the classification criteria for ankylosing spondylitis.

Key words: seronegative spondyloarthropathy, ankylosing spondylitis, SAPHO syndrome, osteomyelitis, CRMO, spondylodiscitis

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INTRODUCTION

SAPHO syndrome is a rare clinical syndrome with co-occurrence of synovitis, skin lesions such as acne, palmoplantar pustulosis (PPP), excessive bone formation and osteomyelitis. According to current knowledge, it is not possible to clearly determine the pathogenetic mechanisms of the disease. Environmental factors, e.g. infections (most often Propionibacterium acnes), or a genetic predisposition, have been considered [2, 4]. The inclusion of SAPHO syndrome into spondyloarthropathies is supported by the presence of sacroiliitis, vertebral osteomyelitis, discitis, psoriasis and inflammatory bowel diseases that occur more frequently than in the general population, similarly to the presence of HLA B27 antigen, which occurs in about 30% of cases [2–4]. According to some authors, there are two subtypes of SAPHO syndrome, one of which is a form of spondyloarthropathy coexisting with hyperostosis and skin lesions, mostly with morphology of palmo-plantar pustulosis or pustular psoriasis [2]. This form is dominated by symptoms of arthritis in the anterior chest wall and inflammation of the sternocostal joints [2, 5]. Inflammatory changes also often occur in the sacroiliac joints, spinal joints and/or intervertebral discs as well as in of hands and feet joints, often asymmetrically [1, 2, 5]. The other type presents with chronic, recurrent, multifocal inflammation of the bone and bone marrow (CRMO), also defined as CNO (chronic, non-bacterial osteomyelitis) [2, 6]. This syndrome, until recently, has been mainly observed in children and young adults, and is characterized by recurring inflammation of the

Table 1. Diagnostic criteria of SAPHO syndrome according to Kahn [7, 10]

Chronic and recurring inflammations of bone and bone marrow (CRMO/CNO)
• Usually aseptic (seldom propionibacterium acne in PCR) • with the involvement of the spine and peripheral bones • with or without skin lesions
Aseptic, acute or chronic arthritis coexisting with:
• pustular psoriasis of hands and feet (PPP) • hidradenitis • acne (conglobata, ulcerans)
Each inflammation of bones and marrow coexisting with :
• pustular psoriasis of hands and feet (PPP) • hidradenitis • acne (conglobata, ulcerans)

bones and/or bone marrow, especially in the epiphyses of long bones (femur and tibia), intervertebral discs, collarbones, mandible and the pelvis [7–9].

The diagnostic criteria of SAPHO syndrome were presented in 1994 by Kahn (Tab.1). Meeting one of the proposed criteria is necessary in order to diagnose the syndrome [7, 10].

SAPHO syndrome diagnosis is difficult due to the frequently atypical clinical picture, the occurrence of different symptoms at various stages of the disease, and the lack of a specific biomarker. However, specific changes are often found in imaging studies [4]. The most frequently observed laboratory abnormalities are elevated ESR and CRP or increased activity of alkaline phosphatase [2].

In imaging studies, subchondral bone sclerosis may be observed, as well as erosions in the anterior chest wall joints, sometimes with periosteal inflammation. Pericostal ossification and hyperostosis are frequent phenomena, particularly in the collarbone area, as well as osteosclerosis of bone bodies, osteophytosis and syndesmophytes in the thoracic and lumbosacral spine [2, 5]. Inflammation of the sacroiliac joints occurs in about 35% of the cases. [3, 7] Inflammation of intervertebral discs is found in about 25–35% of patients and is also part of the spine changes related to the disease.

Imaging is very helpful for SAPHO syndrome diagnosis and differentiation between the observed structural changes. Classical radiography, computed tomography, scintigraphy and magnetic resonance (especially in early stages of the disease) are used. Histopathological tests are essential to determine the nature of bone tissue changes [5, 8, 10].

Differential diagnostics is based, mainly, on the exclusion of an underlying infection of the bones and/or bone marrow, proliferative processes, or histiocytosis [2, 5]. In the diagnosis of SAPHO syndrome, it has become crucial to consider diseases such as ankylosing spondylitis, psoriatic arthritis or arthritis accompanying inflammatory bowel diseases. All of these conditions are characterized by the similarities of symptoms, as well as a potential evolution of the clinical picture during the course of the disease [2, 3].

This paper discusses the case of a 41-year-old male patient with a long-term history of osteomyelitis symptoms and inflammation of the intervertebral discs, in whom symptoms of spondyloarthropathy developed over the last twenty years of the disease. The patient on admission to the Rheumatology Ward, fulfilled the American College of Rheumatology (ACR) modified New York criteria of ankylosing spondylitis, established in1988.

Case description

We presented the case of a 41-year-old male patient with a history of recurrent episodes of inflammation of the body and left ramus of the mandible, occurring since the age of 19. Inflammation of intervertebral discs and progressive, multilevel ossification of the thoracic and lumbar spine segments was observed in the subsequent years. He was admitted to the Rheumatology Ward with significantly increased pain in the spine and hip joints, which remained constant despite the time of day, physical activity, and experienced long-lasting morning stiffness. He had no history of comorbidities, and no significant family history for any diseases, important for further diagnosis. His multiannual history required retrospective analysis of medical records provided, concerning the years between 1992 and 2013. The occurrence of inflammation of the mandible, which appeared between 1992 and 1995, and affected the body and the left mandible ramus, was described there. During several hospital admissions to the dental surgery clinic, laboratory tests showed only a slight elevation in inflammatory markers. X-ray of the mandible was described as: “several osteolytic foci and necroses within the body and the left mandibular ramus”. Histopathological assessment in 1995 (during the third year of the disease), corresponded to chronic inflammation of the bone with possible complications related to fibrous dysplasia; intertrabecular bone remodelling, multiplication of fibrous tissue and vast inflammatory infiltrations were also described. Multiple antibiotic therapies (including lincomycin, vancomycin, tobramycin, pefloxacin, sulfamethoxazole with trimethoprim, oxacillin, amoxicillin with clavulanic acid), and non-steroidal anti-inflammatory drugs (NSAIDs) were administered to this patient several times between 1992 and 1995. In the subsequent years, no recurrences of the inflammatory changes of the jaw bone were observed. He had his teeth extracted on multiple occasions due to advanced decay.

Back pain, without typical symptoms of inflammation, as well as progressive limitation of thoracic spine mobility, became dominant in the clinical picture since 2006, which forced the patient to take NSAID that exceeded the maximum doses. This led to recurring erosive gastritis with mild anaemia, in subsequent years. MRI tests showed hyperintense areas of the end plates of TH12-L5 bodies in the T-1 and T2-weighted images that, according to the description, *"correspond to Scheuermann's disease or bone fragility due to metabolic reasons".*

Owing to the suspicion of a proliferative disease, the patient was extensively examined, including undergoing a bone marrow histopathological assessment in the oncology dept., where the neoplastic disease was excluded.

Due to the increasing pain and further reduction in the spine mobility after the subsequent 7 months, the patient was admitted in the rheumatology ward. There, the progression of thoracic spine changes in the MRI with uneven contours of the end-plates and losses at the levels of Th10/Th11/Th12 were found. Compression of Th12 body with marginal osteosclerosis and similar, multilevel changes in the lumbar part were also described. In the radiologist's opinion, this picture supported an inflammatory background, most probably haematogenous. During this time, no inflammatory changes in the sacroiliac joints were found. Laboratory tests showed ESR 72 mm, CRP 157mg/l, Hb 10.4 G/dL, and ALP 409 U/L, characteristic for an active inflammatory process. The high level of alkaline phosphate was assessed as not linked to liver pathology. HLA B27 antigen detection and the TST (tuberculin skin test/Mantoux test) were negative. Following neurosurgical consultation, the patient was transferred to the neurosurgery ward with a diagnosis of infectious spondylodiscitis. There, antibiotic therapy, subcutaneous administration of calcitonin, and a plaster cast corset were applied. In subsequent years' constant spinal pain in the thoracic and lumbar areas continued. As well as kyphoscoliosis, deterioration of the strength of his lower extremities occurred.

In 2010, subsequent MRI of the thoracic spine described: *"…there is most probable an inflammatory mass surrounding the vertebral bodies from the front and lateral side with a 12 mm thick muff along the thoracic spine, penetrating and surrounding the costovertebral joints and transverse processes, there is an inflammatory infiltration in the epidural space, the spinal cord is compressed*" showing the progression of the inflammatory changes and risk of occurrence of neurological symptoms resulting from the compression of the spinal cord. This, alongside consistently elevated ESR, prompted subsequent antibiotic therapy.

Spastic paresis of the muscles occurred in this patient in 2013. A multilevel laminectomy procedure was performed in the neuro-orthopaedics dept. Intraoperative biopsy and inflammatory tissue culture were performed during the procedure and revealed no bacterial growth. The subsequent MRI showed vast ossification of the majority of motion segments in the cervical, thoracic and lumbar spine. This clinical picture was deemed as a consequence of advanced ankylosing spondylitis or vast, chronic, infectious inflammatory changes. During hospitalization, SPECT (single-photon emission computed tomography) examination was performed, and accumulation of a marker in the thoracic spine, heel bones, pelvis and the sacroiliac joints were observed.

There was no clinical improvement in subsequent months, despite daily treatment with NSAID (400 mg of ketoprofen), and 400 mg of tramadol. Increasing pain and decreased mobility of the lumbosacral spine and pelvis resulted in the patient becoming wheelchair bound. He returned to the rheumatology ward in April 2014.

Physical examination showed intense pain and multidimensional limitation of mobility in the thoracic and lumbosacral spine, as well as in the hip joints. Tests assessing the sacroiliac joints were uninterpretable due to hip joints involvement. There were no inflammatory symptoms in other peripheral joints. During the physical examination, no cutaneous or nail lesions, no digestive tract involvement symptoms, as well as any other significant findings, were observed. Numerous abnormalities were found in laboratory tests: ESR 58 mm/h, CRP 81.8 mg/L, ALP 772 U/L, Hb 12.8 g/dL, hypergammaglobulinemia, with no presence of monoclonal protein, low 25OHD concentration (25-hydroxy-vitamin D), decreased urinary calcium excretion, increased type 1 C-terminal telopeptide and osteocalcin, increased testosterone concentration, normal concentration of the parathyroid hormone. Rheumatoid factor and anti-citrullinated peptide antibodies, as well as anti-nuclear antibodies, were absent. The Quantiferon Gold test and Brucella antibodies were negative.

In the X-ray of the spine (Fig. 1), multilevel syndesmophytes (picture of a "bamboo spine") in the thoracic and lumbosacral sections of the spine were demonstrated, in addition to lesions related to the laminectomy. Pelvis X-ray (Pic.2) showed remodelling of the bone structure, uneven surfaces of the femoral heads, protrusion, and significant bilateral hip joint space narrowing. In the sacroiliac joints, narrowed, blurred and uneven joint spaces with subchondral bone sclerosis were found.

MRI of the pelvis revealed narrowing and blurred sacroiliac joint spaces (mainly in the lower part of the joint), with periarticular bone marrow oedema in the sacrum and ilium, which was enhanced with contrast

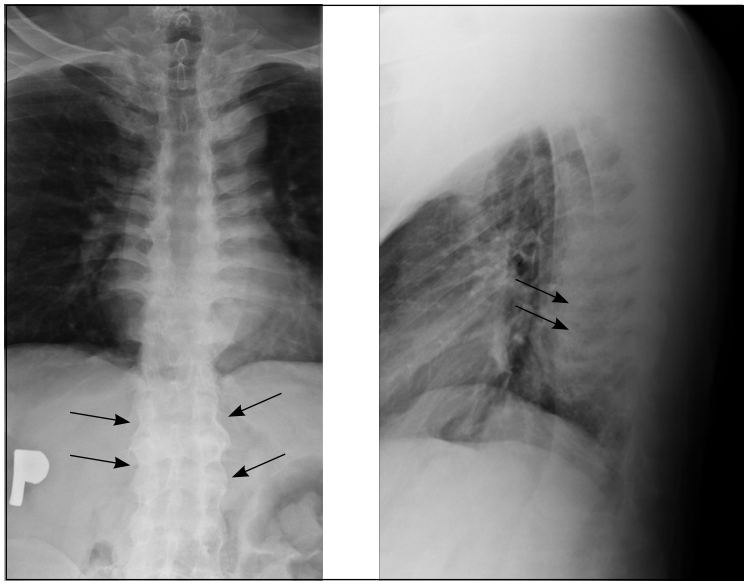

Figure 1. Changes in the spine of the „bamboo spine" type (arrows)

administration. Additionally, bilateral deepening of the acetabulum, with the femoral head bulging into the small pelvis were described. Bilateral deformation of the head and neck of the femur and bone remodelling were also found. Also described were reduced signal from the pelvic cartilage with bone ankylosis, effusion in the periarticular bursae and synovial overgrowth. The clinical picture corresponded to the ankylosing spondylitis accompanied by hip joint inflammation.

The full doses of NSAID, opioid analgesics, including buprenorphine, myorelaxants were implemented. Vitamin D3 supplementation was also applied. BASDAI (Bath Ankylosing Spondylitis Activity Score) index was scored as 5.8. The spine pain VAS (Visual Analogue Scale) was scored as 76 mm.

After supplementing vitamin D deficiency, the patient was administered intravenous infusion of 90 mg Pamidronate. However, the patient refused continuation of this treatment due to lower extremity pain and malaise.

During treatment, clinically significant improvement was not observed. Follow-up BASDAI after monthly therapy according to the above schedule was scored 8.9, and pain of the spine VAS was checked as 90 mm.

According to the observed activity of the disease and risk of complete loss of hip structures, treatment with an anti-TNF-alpha drug was started. The patient was administered with 50 mg of golimumab, and a significant reduction in the symptoms of the inflammatory disease was achieved within 3-months. During the regular assessments of the therapy course, there were a reduction in ESR values, CRP concentration and improvement in BASDAI and VAS values of spinal pain (Tab.2).

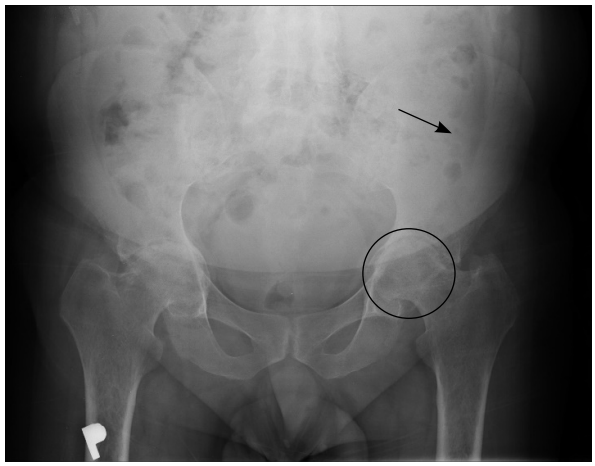

Figure 2. Inflammatory changes in the sacroiliac joints (arrows). Inflammatory changes in the pelvis (ring)

During anti-TNF-alpha therapy, no side effects were observed. Treatment was stopped due to low disease activity in the 18th month. However, after about 8 weeks the patient suffered from a recurrence disease activity symptoms (shown by the highlighted line in Table 2.). An improvement in laboratory parameters was achieved and low disease activity was observed after therapy with golimumab restarting on the 23rd week of treatment.

Discussion

Diagnosis of seronegative spondyloarthropathy, according to the criteria of ASAS (*Assessment of Spondy-*

Table 2. List of assessments of the disease activity in subsequent months of anti-TNF- alpha therapy. Highlighted the line of the table presents the assessment after the treatment with golimumab discontinuation

Month of therapy	ESR	CRP	VAS of the spine pain	BASDAI
3	4 mm/h	9.4 mg/L	60 mm	3.40
6	5 mm/h	4.2 mg/L	30 mm	3.10
18	2 mm/h	6.5 mg/L	15 mm	2.20
20	59 mm/h	121 mg/L	80 mm	6.60
23	8 mm/h	9.5 g/dL	10 mm	1.40

loArthritis International Society) from 2010 is possible on the basis of the typical clinical symptoms, co-occurring with (often discrete) imaging changes, or the presence of HLA B27 antigen [2, 3]. Many symptoms typically related to spondyloarthropathies, such as the spine joints or the sacroiliac joints inflammation, coexisting psoriasis or the inflammatory bowel disease, justify the classification of SAPHO syndrome into this group [2]. Similarly, like many other spondyloarthropathies, SAPHO syndrome can also evolve in its course. Changes in the involved joints, develop over the years. In some cases, the patient initially diagnosed as SAPHO syndrome, during the course of the disease, fulfil the modified New York diagnostic criteria for ankylosing spondylitis, or classification criteria of CASPAR (Classification Criteria for Psoriatic Arthritis) [3, 11].

SAPHO syndrome is a rare disease, and often its atypical course, "stretching over the time" of particular symptoms, as well as a lack of specific biomarkers of the disease, makes the correct diagnosis difficult to establish. According to related publications, there is often a long diagnostic delay for cases of SAPHO syndrome [4, 7]. Diagnostic difficulties may particularly refer to cases which run as chronic, recurring (in many cases recurrences can be easily overlooked), multifocal osteomyelitis (CRMO). Vertebral bodies and the intervertebral discs or, as in this case, the mandible, are frequent locations of the inflammatory changes. An acute course of the disease often presents with inflammation of intervertebral discs, which can lead to a significant deterioration in the patient's mobility, especially, if there are complications, such as spinal cord compression [6]. Laboratory abnormalities accompanying the disease, and the observed imaging changes may suggest a developing proliferative process or infectious etiology. As a result, it often causes complex, time- and cost-consuming diagnostics being performed, or exposes patients to long-term antibiotic therapy. [12–14].

In the present state of knowledge, there are no uniform SAPHO syndrome treatment recommendations developed [15]. NSAIDs are commonly used and in many cases are effective as the disease course is often a relatively mild. In many situations, particularly with coexisting skin lesions, antibiotics, for example, azithromycin or doxycycline are used in therapy. Disease-modifying antirheumatic drugs (DMARDs), such as methotrexate or sulfasalazine are also useful in cases when the disease manifests in peripheral joints, however, information on their effectiveness varies [14, 15]. Many bisphosphonate studies demonstrate good results of such therapy, but they are rather limited to reduce the extent of bone changes. In publications, intravenous administration of pamidronate in doses between 30 to 90 mg is most frequently described with positive results reported [16]. Glucocorticosteroids play a relatively insignificant role, similarly to other spondyloarthropathies. Their application is limited to local or oral administration in acute presentations of the disease [17]. In recent years there have been some publications discussing the positive effects of SAPHO syndrome therapy with anti-TNF-alpha agents [18, 19]. Most frequently, publications describing positive results with this group refer to Infliximab, administered in a typical for spondyloarthropathies dose, 5 mg/kg of body weight at 0, 2 and 6 weeks [19, 20]. Unfortunately, along with increased use of TNF-alpha inhibitors for SAPHO syndrome, there are a number of the publications revealing an increased risk of exacerbation of skin manifestations, which suggests their distinctness from osteoarticular symptoms [21].

Conclusions

Presented case is an example of the SAPHO syndrome, commencing with typical CRMO symptoms which, over time evolving into seronegative spondyloarthropathy, in the course of which the clinical symptoms and advanced imaging changes allow diagnose of disease as ankylosing spondylitis. Symptoms of the mandible and intervertebral disc inflammation required the exclusion of a proliferative process or an infectious disease. Repeated, with no positive result antibiotic therapy is characteristic in the history of this patient, similarly to other cases described. Special emphasis

should be put on the fact of non-occurrence of psoriasis or above-mentioned skin lesions, which often coexist with SAPHO syndrome, the absence of symptoms of other diseases, such as inflammatory bowel disease or uveal diseases, in the patient as well as in his family history. The absence of HLA B27 antigen in this patient, despite various symptoms of ankylosing spondylitis, is also worth mentioning.

In this case, the decision to administer TNF-alpha inhibitor was a result of the disease evolution to the typical clinical picture of ankylosing spondylitis. Involvement of the hips, which could result in a complete destruction of these joints' structures, as well as the total ineffectiveness of the initial treatment, predisposed patient to a risk of permanent disability. Observing the clinical response to golimumab administration, and particularly the rapid recurrence of the symptoms after the therapy interruption, which is commonly observed during therapy of spondyloarthropathies with anti-TNF alfa agents, seem to confirm, that this case describes an evolution of typical SAPHO syndrome to ankylosing spondylitis.

Disclosure of interest: Authors report no competing interests

Abbreviations

ACR — American College of Rheumatology
ALP — alkaline phosphatase
ASAS — Assessment of Spondyloarthritis International Society
BASDAI — Bath Ankylosing Spondylitis Activity Score
CASPAR — Classification Criteria for Psoriatic Arthritis
CNO — chronic, non-bacterial osteomyelitis
CRMO — chronic, recurrent multifocal osteomyelitis
CRP — C-reactive protein
ESR — erythrocyte sedimantation rate
Hb — hemoglobin
HLA B27 — Human Leukocyte Antigen B27
MRI — magnetic renosance imaging
NSAIDs — non-steroidal anti-inflammatory drugs
PCR — polymerase chain reaction
PPP — palmo-plantar pustulosis
SAPHO — Synovitis, Acne, Pustulosis, Hyperostosis, Osteitis
SPECT — single-photon emission computed tomography
TNF alpha — tumor necrosis factor alpha
TST — tuberculin skin test
VAS — Visual Analogue Scale

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VIA MEDICA

RESEARCH ARTICLE

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The influence of naloxone on pharmacokinetics and pharmacodynamics of ticagrelor in patients with unstable angina pectoris receiving concomitant treatment with morphine — a protocol of a randomized trial

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ABSTRACT

Rapid platelet inhibition plays pivotal role in contemporary treatment of patients presenting with acute coronary syndromes. Morphine, the most commonly used analgesic has been proven to impair both absorption and onset of action of P2Y12 receptor inhibitors, which can be described as "the morphine effect". Most negative effects of morphine are caused by its undesirable influence on gastrointestinal tract. We hypothesized that naloxone, widely administered intravenous opioid reversing drug, may turn out to be beneficial if given orally in acute coronary syndrome patients previously treated with morphine. Therefore, a phase IV, randomized pilot study was designed so as to evaluate the impact of naloxone administration on pharmacokinetics and pharmacodynamics of P2Y12 inhibitor, ticagrelor in unstable angina patients. A group of 30 consecutive unstable angina patients treated with ticagrelor and morphine will be randomized in a 1:1 ratio into the study arms. To the best of our knowledge, no such approaches to overcome negative influence of morphine in acute coronary syndrome patients have been described in literature so far.

Key words: ticagrelor, morphine, naloxone, pharmacokinetic, pharmacodynamics

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Introduction

Contemporary pharmacological treatment of acute coronary syndromes (ACS) is based on rapid platelet inhibition. According to the latest European Society of Cardiology (ESC) Guidelines for the management of ACS dual antiplatelet therapy with aspirin and a potent P2Y12 receptor inhibitor, preferably ticagrelor, is a recommended approach. Administration of prasugrel is limited to patients whose coronary angiography has been completed and constitutes a strong indication for coronary angioplasty [1]

Throughout the years, patients presenting with symptoms of ACS have been administered morphine as the first line treatment of pain. Pain alleviation is vital not only for humanitarian reasons, but also because of the association of pain with the sympathetic activation leading to vasospasm and increasing the ischemic burden to the heart [2, 3].

Opioid drugs exert their effect through interaction with 3 types of transmembrane G-protein-coupled opioid receptors: μ, δ and κ. The analgetic effect of opioids is mainly attributed to interaction with μ opioid receptors in the central nervous system. Activation of opioid receptors located in smooth muscles and terminal portions of the peripheral sympathetic and sensory nerves of the gastro-intestinal tract depresses gastrointestinal motility and secretion through inhibition of neuronal release of acetylcholine and VASP [5]. Opioid drugs also enhance fluid and electrolyte absorption, mainly through activation of the μ and δ opioid receptors [5]. As a consequence, side-effects such as nausea, vomiting, prolonged gastrointestinal passage, constipation and abdominal discomfort are evoked.

The latest ESC guidelines for the management of patients presenting with ST-segment elevation myocardial infarction (STEMI) lowered the class of recommendation for morphine administration as its negative influence on the pharmacokinetics and pharmacodynamics of ticagrelor has recently been discovered [6, 7]. Morphine has been found to intensify the muscle tone of the gastrointestinal tract, especially the sphincters, which inhibits gastric emptying and weakens intestinal peristalsis, which in turn may lead to the impairment of intestinal absorption and thus to the decrease of peak plasma levels of oral medications [8, 11]. The described phenomenon, which can be called "the morphine effect" is particularly undesirable in patients treated for STEMI, as this population requires immediate platelet inhibition to increase the chance of successful therapy [12–14].

Optimal methods of overcoming "the morphine effect" are yet to be discovered. Several approaches have already been described in literature. These methods include crushing the tablets of P2Y12 receptor inhibitor, which can be useful due to the acceleration of platelets inhibition as a consequence of faster drug absorption or co-administration of prokinetic drug, e.g. metoclopramide so as to speed up the antiplatelet agent absorption [15–18].

The aim of our study is to evaluate the influence of naloxone on the pharmakodynamics and pharmakokinetics of ticagrelor in patients with unstable angina (UA), pretreated with morphine. According to the design of the study, the participants will additionally receive naloxone – a selective opioid receptor antagonist [19], capable of reversing the actions and effects of opioids and commonly used in a variety of clinical scenarios such as:opioid action reversal in opioid intoxication orduring anaesthesia, opioid substitution therapy in opioid addiction and reversal of neonatal respiratory center depression secondary to opioid administration to the mother. In opioid-naive patients, naloxone exerts no significant pharmacological effects. The usual route of administration of naloxone is parenteral. When administered orally or sublingually, naloxone is subjected to very intensive first pass metabolism, resulting in final bioavailability of as little as 2-3%, making its plasmatic concentration barelly detectable [20]. There are literature reports on successful oral administration of naloxone forsevere opioid-related constipation in patients with neoplasmatic diseases, without compromising the analgetic effect of opioids [2, 3, 21, 22], due to local inhibition of intestinal wall opioid receptors [19].

Methods

The study was designed as a phase IV, single-center, randomized, investigator-initiated, parallel-group, open-label, interventional trial. The study protocol was approved by the Ethics Committee of Nicolaus Copernicus University in Toruń, Ludwik Rydygier Collegium Medicum in Bydgoszcz (approval number KB 339/2016). The investigation site will be the Department of Cardiology, Antoni Jurasz University Hospital No. 1 in Bydgoszcz, Poland.

This study was designed to evaluate the impact of naloxone administration on the pharmacodynamics and pharmacokinetics of ticagrelor and its active metabolite AR-C124900XX in patients with UA pectoris who received opioid analgesic in the initial phase of treatment. All enrolled participants will be assigned randomly in a 1:1 ratio to one of the following 2 groups: 1) a group receiving a combination of 180 mg of crushed ticagrelor administered orally followed by intravenous administration of 5 mg of morphine or 2) a group treated with 180 mg of crushed ticagrelor administered orally followed by intravenous administration of 5 mg of morphine and oral administration of 1 mg of naloxone (Figure 1).

Provision of informed consent is mandatory to participate in the study. The main inclusion criterion will be the diagnosis of UA pectoris with a mortality risk of < 140 points according to the GRACE Score. Other inclusion criteria comprise men or non-pregnant women aged 18–80 who have given their informed consent to undergo coronary angiography and percutaneous coronary intervention if required. The main exclusion criteria include recent treatment with any P2Y12 receptor inhibitor, anticoagulants, opioid receptor agonists or potent CYP3A4 inhibitors within 14 days preceding screening, active bleeding, current treatment for malignancy, coagulation disorders, past intracranial hemorrhage, gastrointestinal hemorrhage within 30 days preceding screening, respiratory failure, thrombocytopenia below 100.000/IU or anemia (hemoglobin concentration below 10 g/dL). Also, known hypersensitivity to the administered substances is a disqualifying factor. A complete list of inclusion and exclusion criteria is presented in Table 1.

Sample size calculation

Based on our previous study evaluating the impact of metoclopramide administration on pharmacokinetics (PK) and pharmacodynamics (PD) of ticagrelor and its active metabolite in patients with UA, we assumed that a group of 15 participants in each study arm should provide sufficient data for further analysis [17, 18, 23].

Endpoints

The primary endpoint is the time needed to achieve the maximum plasma concentration of ticagrelor and its active metabolite AR-C124900XX. The secondary

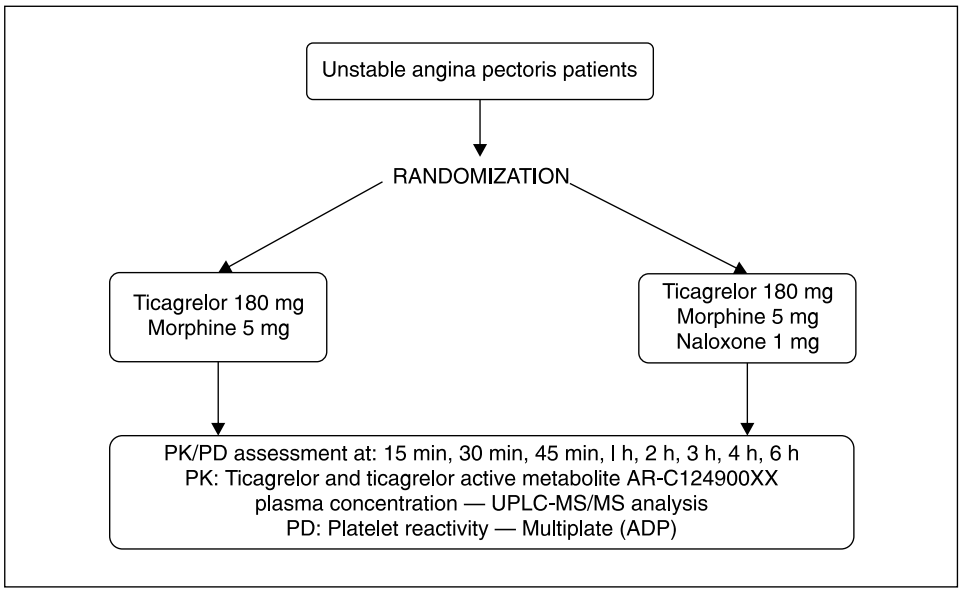

Figure 1. Trial flowchart. PD - pharmacodynamic PK – pharmacokinetic

Table 1. A complete list of inclusion and exclusion criteria for participation in the study

Inclusion criteria:
– Provision of informed consent prior to any study specific procedures
– Diagnosis of unstable angina
– Male or non-pregnant female, aged 18-80 years
– Provision of informed consent for angiography and PCI
– GRACE score < 140 pts
Exclusion criteria:
– Treatment with ticlopidine, clopidogrel, prasugrel or ticagrelor within 14 days before the study enrollment
– Current treatment with morphine or any opioid “mi” receptor agonist
– Hypersensitivity to ticagrelor
– Current treatment with oral anticoagulant or chronic therapy with low-molecular-weight heparin
– Active bleeding
– History of intracranial hemorrhage
– Recent gastrointestinal bleeding (within 30 days)
– History of coagulation disorders
– Platelet count less than < 100 x 10^3/mcl
– Hemoglobin concentration less than 10.0 g/dl
– History of moderate or severe hepatic impairment
– History of major surgery or severe trauma (within 3 months)
– Risk of bradycardic events as judged by the investigator
– Second or third degree atrioventricular block during screening for eligibility
– History of asthma or severe chronic obstructive pulmonary disease
– Kidney disease requiring dialysis
– Manifest infection or inflammatory state
– Killip class III or IV during screening for eligibility
– Respiratory failure
– History of severe chronic heart failure (NYHA class III or IV)
– Concomitant therapy with strong CYP3A inhibitors (ketoconazole, itraconazole, voriconazole, telithromycin, clarithromycin, nefazadone, ritonavir, saquinavir, nelfinavir, indinavir, atazanavir) or strong CYP3A inducers (rifampicin, phenytoin, carbamazepine, dexamethasone, phenobarbital) within 14 days and during study treatment
– Body weight below 50 kg

Table 2. A complete list of study endpoints; AUC – area under the plasma concentraton-time curve of ticagrelor; MEA-multiplate electrode aggregometry

Primary endpoint of the study
— Time to maximum concentration (tmax) for ticagrelor and AR-C124900XX [Time frame: 6 hours]
Secondary endpoints of the study
— Maximum ticagrelor and AR-C124900XX concentration [Time frame: 6 hours]
— Area under the plasma concentration-time curve for ticagrelor (AUC 0–6 h)
[Time frame: pre-dose and 15 min, 30 min, 45 min, 1, 2, 3, 4, 6 hours post dose]
— Area under the plasma concentration-time curve for AR-C124900XX (AUC 0–6h)
[Time frame: pre-dose and 15 min, 30 min, 45 min, 1, 2, 3, 4, 6 hours post dose]
— Platelet reactivity assessed by MEA [Time frame: pre-dose and 30 min, 1, 2, 3, 4, 6 hours post dose

endpoints include ticagrelor and AR-C124900XX maximum plasma concentration, area under the plasma concentration-time curve (AUC) for ticagrelor and AR-C124900XX (AUC 0–6h) and platelet reactivity assessed with Multiple Electrode Aggregometry (MEA) using the Multiplate Analyzer prior to and within the time frame of six hours following tikagrelor loading dose (LD). The summary of all study endpoints is presented in Table 2.

The blood sample collection as well as pharmacokinetic and pharmacodynamics assessment have been previously described [7, 17, 18, 24].

Safety assessment

Only patients with low or intermediate risk of mortality defined as less than 140 points in the GRACE Score will be enrolled in the study. The subjects will be treated with a set of standard medications according to the ESC guidelines for the treatment of ACS, including dual anti-platelet therapy, ACE-inhibitor, beta-blocker and statin. When mandated by clinical deterioration requiring urgent coronary angiography, patients will be excluded from further participation in the study and will be immediately transported directly to cathlab. Morphine will be given in a small, single dose after informing the patient about potential consequences associated with treatment with opioids and after acquisition of informed consent from the patient. In case of occurrence of adverse effects after morphine administration, morphine reversing agent, i.e. intravenous naloxone will be given.

Discussion

According to our knowledge, the study is the first attempt to evaluate the influence of naloxone co-administration on the absorption and antiplatelet action of ticagrelor in patients with UA pectoris who received morphine. We hope that the results of our research will broaden the knowledge regarding optimization of ACS treatment and will lead to the development of more effective and safer therapeutic approaches for patients presenting with myocardial infarction.

The study status

The study is currently recruiting participants. It has been registered in clinicaltrials.gov under the identification number NCT02939248.

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Conflict of interest

All authors declare no conflict of interest.

List of abbreviations:

ACS — acute coronary syndromes
AUC — area under the plasma concentraton-time curve of ticagrelor
ECS — European Society of Cardiology
LD — loading dose
MEA — multiplate electrode aggregometry
PD — pharmacodynamic
PK — pharmacokinetic
STEMI — ST-segment elevation myocardial infarction
UA – unstable angina

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