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Bleeding gastroduodenal ulcers in patients without *Helicobacter pylori* infection and without exposure to non-steroidal anti-inflammatory drugs

Krvareći gastroduodenalni ulkusi kod bolesnika bez *Helicobacter pylori* infekcije i bez upotrebe nesteroidnih antiinflamatornih lekova

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Abstract

Background/Aim. A high risk of bleeding in *Helicobacter pylori (H.pylori)*-negative, non-steroidal anti-inflammatory drugs (NSAID)-negative ulcers highlights the clinical importance of analysis of the changing trends of peptic ulcer disease. The aim of the study was to investigate the risk factors for ulcer bleeding in patients with non-*H. pylori* infection, and with no NSAIDs use. **Methods.** A prospective study included patients with endoscopically diagnosed ulcer disease. The patients were without *H. pylori* infection (verified by pathohistology and serology) and without exposure to NSAIDs and proton pump inhibitors (PPI) within 4 weeks before endoscopy. After endoscopy the patients were divided into 2 groups: the study group of 48 patients with bleeding ulcer and the control group of 47 patients with ulcer, but with no bleeding. Prior to endoscopy they had completed a questionnaire about demographics, risk factors and habits. The platelet function, von Willebrand factor (vWF) and blood groups were determined. Histopathological analysis of biopsy samples were performed with a modified Sydney system. The influence of bile reflux was analyzed by Bile reflux index (BRI). **Results.** Age, gender, tobacco and alcohol use did not affect the bleeding rate. The risk of bleeding did not depend on concomitant diseases (p = 0.509) and exposure to stress (p = 0.944). Aspirin was used by 16/48 (33.3%) patients with bleeding ulcer, as opposed to 7/47 (14.9%) patients who did not bleed (p = 0.036). Abnormal platelet function had 12/48 (25.0%) patients who bled, as opposed to 2/47 (4.3%) patients who did not bleed (p = 0.004). Patients with BRI < 14 bled in 79.2%, and did not bleed in 57.4% of the cases (p = 0.023). There was no statistical difference between groups in regards to blood groups and range of vWF. Antrum atrophy was found in 14/48 (29.2%) patients with bleeding ulcer and in only 5/47 (10.6%) patients who had ulcer without bleeding (p = 0.024). **Conclusion.** Abnormal platelet function, aspirin use and antrum atrophy were the risk factors for ulcer bleeding in non-*H. pylori*, non- NSAIDs ulcer disease.

Key words:
peptic ulcer hemorrhage; helicobacter pylori; anti-inflammatory agents, non-steroidal; risk factors.

Apstrakt

Uvod/Cilj. Visoki rizik od krvarenja *Helicobacter pylori* (*H. pylori*)-negativnih i sa nesteroidnim inflamatornim lekovima (NSAIL)-negativnih ulkusa potencirao je klinički značaj analiziranja novih tendencija peptičke ulkusne bolesti. Cilj ove studije bio je ispitati faktore rizika od nastanka krvarećeg ulkusa kod bolesnika bez *H. pylori* infekcije i bez upotrebe NSAIL. **Metode.** Prospektivna studija obuhvatala je bolesnike sa endoskopski dijagnostikovanom ulkusnom bolešću. Bolesnici su bili bez *H. pylori* infekcije (potvrđeno patohistološki i serološki) i bez upotrebe NSAIL i inhibitora protonske pumpe (IPP) tokom četiri nedelje pre endoskopije. Posle endoskopije bolesnici su bili podeljeni u dve grupe: studijsku grupu od 48 bolesnika sa krvarećim ulkusom i kontrolnu grupu od 47 bolesnika sa ulkusom bez krvarenja. Pre endoskopije bolesnici su popunjavali upitnik o demografskim podacima, faktorima rizika i navikama. Funkcija trombocita, von Willebrand faktor (vWF) i krvne grupe ispitivane su, takođe. Patohistološki su analizirani biopsijski

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uzorci po modifikovanom Sydnejskom sistemu. Uticaj bilijarnog refluksa analiziran je pomoću bilijarnog refluksnog indeksa (BRI). **Rezultati.** Godine, pol, pušenje i upotreba alkohola nisu imali uticaj na stopu krvarenja. Rizik od krvarenja nije zavisio od udruženih bolesti (p = 0,509), niti od izloženosti stresu (p = 0,944). Aspirin je uzimalo 16/48 (33,3%) bolesnika sa krvarećim ulkusom, u poredeđenju sa 7/47 (14.9%) bolesnika koji nisu krvarili (p = 0,036). Abnormalnu funkciju trombocita imalo je 12/48 (25,0%) bolesnika sa krvarenjem, u poređenju sa 2/47 (4,3%) bolesnika koji nisu krvarili (p = 0,004). Bolesnici sa BRI < 14 krvarili su u 79,2%, a nisu krvarili u 57,4% slučajeva (p = 0,023). Nije bilo statistički značajne razlike između dve grupe u odnosu na krvne grupe i nivo vWF. Antralna atrofija nađena je kod 14/48 (29,2%) bolesnika sa krvarećim ulkusom i kod 5/47 (10,6%) bolesnika koji su imali ulkus bez krvarenja (p = 0,024). **Zaključak.** Abnormalna funkcija trombocita, upotreba aspirina i antralna atrofija jesu faktori rizika od krvarenja kod *H. pylori*-negativne i NSAIL-negativne ulkusne bolesti.

Ključne reči:
peptički ulkus, krvarenje; helicobacter pylori; antiinflamatorici, nesteroidni; faktori rizika.

Introduction

Peptic ulcer disease has a multifactorial pathogenesis. The discovery of *Helicobacter pylori (H. pylori)* infection has dramatically changed the understanding and management of this clinical entity. In the 1990s a number of reports from around the world defined that *H. pylori* infection was present in more than 90% of patients with duodenal, and in about 85% of those with gastric ulcers [1]. On the other hand, the use of non-steroidal anti-inflammatory drugs (NSAIDs) is the other major cause of peptic ulcers. Several authors have found that NSAIDs were used by 25–75% of patients with *H. pylori*-negative duodenal ulcers (DU) or that NSAIDs are the most frequent identifiable causes of non-infected DU [2].

However, the epidemiology of peptic ulcer has significantly changed in the past decades because of the huge effort made to eradicate *H. pylori* infection [3]. The proportion of peptic ulcers which is unrelated to NSAIDs and *H. pylori* appears to be increasing. In North America, there is good evidence that 20–40% of peptic ulcers are not associated with *H. pylori* or NSAIDs [3–5], whereas in other parts of the world, the proportion of *H. pylori*-negative ulcers remains much lower (less than 4%) [5]. A study from Northern Italy reported the prevalence of only 8% [6, 7]. In Europe, three studies from Scotland, Denmark and Italy showed a prevalence of *H. pylori*-negative duodenal ulcer of 10–15% [1]. Reports from different parts of Asia show a wide variation of its prevalence. The prevalence of *H. pylori*-negative ulcer ranges from 3% in Japan to 29% in Singapore and Pakistan. Recent reports show that the prevalence of *H. pylori*-negative ulcers will also depend upon the background prevalence of *H. pylori* in general population as *H. pylori*-negative ulcers will not exist if everyone has the infection [2, 5]. A meta-analysis of seven randomized double blind trials in North America found that 20% of patients with *H. pylori*-associated ulcers had ulcer recurrence within 6 months, despite successful *H. pylori* eradication and no reported NSAID use [3, 8].

Ulcers attributed to the use of aspirin have risen in number, because aspirin is widely used for the prevention of thrombotic events 9. Also, the proportion of patients taking low-dose aspirin in combination with other antiaggregants, such as clopidogrel is increasingly high [10].

About 25% of patients with idiopathic ulcers also have reactive gastritis, which might be related to bile reflux or prior NSAID use or *H. pylori* infection, so it might be that mucosa does not recover fully and remains vulnerable to other injuries.

Other etiological factors in *H. pylori*-negative, NSAID-negative ulcers are poorly defined, but may include a genetic predisposition, altered acid secretion, rapid gastric emptying, defective mucosal defense mechanisms, psychological stress, smoking, drinking alcohol or gender [11]. A number of studies have demonstrated a relationship between ABO blood group and hemostasis. Indeed, a higher rate of bleeding complications has been described in patients belonging to the group O [12, 13] and blood group O individuals are consistently over-represented in patients with inherited bleeding disorders [12, 14]. Some authors mentioned that individuals with blood group O have a higher risk of bleeding disorders due to low levels of the circulating plasma protein, von Willebrand factor (vWf) [15, 16]. In a large twin study, Orstavik et al. [17] found that 66% of the total variation in plasma vWf levels was genetically determined and 30% of this genetic component was explained by ABO blood group. Plasma levels of vWf and other coagulation factors influence risk of hemorrhage. Also, vWf is involved in platelet adhesion and subsequent platelet aggregation during primary haemostasis [18, 19]. Another reason for ulcer bleeding is probably the platelet dysfunction. It may be acquired, inherited or induced by platelet inhibiting agents, such as acetyl salicylic acid.

H. pylori-negative ulcers have been shown to have a higher incidence of mortality and recurrent bleeding [20]. Many recent reports suggest that the pathogenesis of bleeding duodenal ulcer is different from that of non-complicated ulcer disease and that other risk factors different from *H. pylori* are perhaps responsible for a relatively high proportion of bleeding ulcers [2]. Study of Hung et al. [21] showed that the incidence of *H. pylori*-negative idiopathic bleeding ulcers has increased in recent years. According to that study, *H. pylori* negative ulcers account for around 16% of bleeding peptic ulcers.

The aim of this study, was to investigate the risk factors for bleeding in *H. pylori*-negative, NSAID-negative peptic ulcers, and to examine the factors that contribute to complicated ulcers, and also to make new efforts to improve the prevention of these conditions.

Methods

The study was conducted in the Clinical Center of Montenegro from January 2010 to January 2012. A prospective study included 95 consecutive patients with endoscopically diagnosed peptic ulcer disease who were without *H. pylori* infection and without exposure to NSAIDs. We also excluded patients who were taking proton pump inhibitors (PPI), anticoagulant drugs and antiplatelet drugs except aspirin. *H. pylori* status was verified by histology (two biopsy specimens taken from the stomach antrum and two from the corpus) and serology (antibodies to *H. pylori*). In cases of bleeding ulcer we performed second look endoscopy within 72 hours from the initial endoscopy and then took biopsies for histopathology analyses. Blood samples were taken from all the patients within 24 hours in order to perform serological analyses in regard to the presence of antibodies to *H. pylori* [22]. Enzyme-linked immunoassay test (ELISA) for the quantitative detection in serum of anti-*H. pylori* IgG antibodies was performed using a commercial test kit, according to instructions of the manufacturer. The results were expressed as: reactive-positive (IgG level > 20 IU/mL); grey zone-equivocal (IgG level: 15–20 IU/mL) and non reactive-negative (IgG level < 15 IU/mL). We included in the study only patients with IgG level < 15 IU/mL (non-reactive). The patients who positively responded to the questions about NSAIDs, PPI, anticoagulant and antiplatelet drugs (except aspirin) use less than 4 weeks before endoscopy were excluded from the study. The patients were divided into two groups. The study group consisted of 48 patients with bleeding ulcer, while the control group of 47 patients also with ulcer, but without sings of bleeding.

Before endoscopy all the patients filled out the questionnaire on demographic data, habits (alcohol consumption and smoking), concomitant diseases, exposure to stress during last year, as well as on taking aspirin during the past 4 weeks. We also analyzed information related to the treatment of *H. pylori* infection in the past. They also responded to questions related to previous treatment of the diseases of upper gastrointestinal tract (gastritis, gastric ulcer and duodenal ulcer) diagnosed by endoscopy over a period of 6 months prior to inclusion in this study.

All the patients were analyzed according to gender and age. The patients who were current smokers for at least 6 months were considered as smokers. Consuming more than 10 g of alcohol/day by women, and more than 20 g/day by men was considered as medically relevant. The impact of concomitant diseases in ulcer disease was determined by the use of individual index of co-existent diseases (ICED). The values of ICED are 0–3, and reflect the impact of concomitant disease to the severity of ulcer disease. The ICED score of 0 indicates that associated diseases have no impact, score 1 indicates a mild impact, score 2 moderate and score 3 serious impact on the occurrence of ulcers [23]. Exposure to a stressful situation was graded by "Holmes and Rahe stress scale". The patients answered whether in the past year they had been exposed to some of the possibly stressful situations. By adding points for all the exposure situations, the total number (score) indicates the impact of stress on health, or occurrence of the disease. Therefore, the score value of 300 and more indicates a risk of disease, score value of 150–299 means that the risk of disease is intermediate (reduced by 30%) and the score value of 150 or less indicates a very low risk of disease [24]. Blood groups were determined by the principle of hemagglutination. The possible impact of duodenogastric biliary reflux was assessed by calculating bile reflux index (BRI) according to Sobala et al. [25]. This index is calculated by the following formula: BRI = (7 × E) + (3 × IM) + (4 × CI) – (6 × Hp), where E is edema in the lamina propria, IM – intratestinal metaplasia, CI – chronic inflammation and Hp represents colonization of the stomach with *H. pylori*. The pathologists were grading each parameter from antral biopsy specimens from 0 to 3. A value of BRI above 14 indicates significant duodenogastric reflux (bile acid level grater than 1 mmol/L, which is the upper limit of normal biliary reflux) with 70% sensitivity and 85%, specificity. Von Willebrand factor plays an important role in both primary hemostasis by the formation of the hemostatic plug (due to its function in platelet adhesion) and in aggregation. We used a Dade Behring vWF Ag test kit intended for *in vitro* diagnostic use with Dade Behring coagulation analyzers for the quantitative determination of vWF Ag in human plasma by immunoturbidimetry. The normal plasma vWF level in adult population is usually in the range of 50–160%. Detection of platelet dysfunction in citrated human whole blood was done by the PFA - 100 system. This system consists of an instrument and two different test cartridges in which the process of platelet adhesion and aggregation following a vascular injury is simulated *in vitro*. The collagen/epinephrine (Col/EPI) test cartridge is the primary cartridge used to detect platelet dysfunction induced by intrinsic platelet defects, vWF, or exposure to platelet inhibiting agents. A reference range for Col/EPI is 84–160. The collagen/ADP (Col/ADP) test cartridge is used to indicate if an abnormal result obtained with the Col/EPI test cartridge may have been caused by the effect of aminosalicylic acid (ASA) or medication containing ASA. A reference range for Col/ADP is 68–121 s. The platelet function test is marked as normal if both Col / EPI and Col / ADP are normal, and when any of them or both abnormal, test is marked as abnormal.

Endoscopy procedure and histopathology examination of mucosal biopsy samples

All endoscopies were done by an Olympus endoscope (GIF TYPE Q 165) and biopsies were taken with standard forceps. Ulcer was defined as any visual loss of mucosal integrity, of a diameter greater than 5 mm, and bleeding stigmata were classified according to the modified Forrest classification [26]. According to that classification all ulcers are divided into those who have not bled, those that have bled and those with bleeding during endoscopy. In cases when ulcer bleeding required endoscopic hemostasis, it was done using either injection technique with diluted epinephrine or by mechanical device (hemoclipping). From any ulcer and surrounding mucosas biopsies were taken to exclude the existence of the diseases which required different therapeutic approaches, such as malignant ulcers, Crohn's disease, lymphoma, etc.At the same time, two biopsies from the antrum and two from the corpus were taken in order to histopathologically confirm the absence

of *H. pylori* infection, and to additionally analyze the type of gastritis. The modified Sydney system for classification of gastritis was used for interpretation of biopsy samples (Hematoxylin and Eosin staining) [27]. Modified staining by Giemsa was used for *H. pylori* determination.

This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved ethically by Ethical Committee, Clinical Center of Montenegro (No 03/01-688/2, from January 28, 2010). All the patients provided a written informed consent.

All data were analyzed using SPSS, version 16.0 (SPSS Inc, Chicago, IL, USA). Values are expressed as means or percentages as appropriate. The χ^2-test was used to analyze the difference between the two groups. Univariate and multivariate logistic regression was used to analyze the risk [odds ratio (OR) and 95% confidence interval (CI)] of developing bleeding ulcer in patients with peptic ulcer disease. The differences were considered significant at the level of $p < 0.05$.

Results

There were 48 patients with bleeding ulcer in the study group and 47 patients with ulcer with no signs of bleeding in the control gruop.

The demographic characteristics, habits and medical histories with the results for the patients in the study and the control group are shown in Table 1. Gender did not affect bleeding. A higher incidence of bleeding peptic ulcer was found in men but with no statistical significance ($p = 0.358$). There was no difference in bleeding ulcer in relation to age groups. There was higher incidence of bleeding ulcers among older patients, but without statistical significance ($p = 0.350$). Bleeding was not affected by smoking. Alcohol consumption was more frequent among patients who bled, but the difference was not significant ($p = 0.115$). Risk of bleeding from ulcer did not depend on concomitant diseases. We had similar findings related to exposure to stress with no significant difference between the investigated groups ($p = 0.944$). The previous treatment of *H. pylori* infection had no effect on new bleeding ulcers. Of the patients covered by the study, 44.8% of those who were previously treated for *H. pylori* infection bled, as opposed to 55.2% of the patients who were also previously treated, but now had ulcer without signs of bleeding ($p = 0.461$). The previous treatment of gastric or duodenal ulcer, did not affect future ulcer bleeding. The history of gastritis was significantly different among the investigated groups ($p = 0.038$). The logistic regression showed that the medical history of gastritis had a protective effect on future ulcer bleeding with relative risk for ulcer bleeding 5 times less than in patients with no previous history of gastritis ($p = 0.017$). Logistic regression model (univariate and multivariate) is shown in Table 2.

Table 1

Demographic sharacteristics, habits and medical history of patients

Characteristics of the patients	Study group n (%)	Control group n (%)	χ^2-test (p)
Total number	48 (50.5)	47 (49.5)	
Gender			
men	30 (62.5)	25 (53.2)	0.358
Age, years ≥ 50	33 (68.8)	28 (59.6)	0.351
Medical history			
gastritis	10 (20.8)	19 (40.4)	0.038*
gastric ulcer	5 (10.4)	6 (12.8)	0.72
duodenal ulcer	13 (27.1)	7 (14.9)	0.145
Smoking			
yes	22 (45.8)	22 (46.8)	0.924
Alcohol user			
yes	24 (50.0)	16 (34.0)	0.115
Exposure to stress [24]			
< 150	15 (31.3)	15 (31.9)	0.944
150–299	22 (45.8)	20 (42.6)	
> 300	11 (22.9)	12 (25.5)	
Concomitant diseases			
yes	33 (68.7)	36 (76.6)	0.391
Previous treament of *H. pylori*			
yes	13 (44.8)	16 (55.2)	0.461

***statistically significant difference**

Table 2

Logistic regression model for bleeding ulcers

Characteristics of the patients	Univarate Logistic Regression		Multivariate Logistic Regression	
	Exp B (95% CI)	p	Exp B (95% CI)	p
Aspirin use	2.857 (1.048–7.786)	0.040*	1.758 (0.423–7.295)	0.437
Previous gastritis	0.388 (0.156–0.962)	0.041*	0.197 (0.052–0.746)	0.017*
Platelet dysfunction	7.500 (1.576–35.683)	0.011*	20.703 (1.724–48.643)	0.017*
Bile reflux index ≥ 14	0.355 (0.144–0.878)	0.025*	0.144 (0.040–0.514)	0.003*
Antrum atrophy	3.459 (1.132–10.566)	0.029*	9.075 (1.768–46.576)	0.008*

***statistically significant difference**

The influence of aspirin and platelet function on ulcer bleeding can be seen in Table 3. Aspirin consumption was a significant predictive factor for bleeding in univariate logistic regression, but with no significance in multivariate regression model (Table 2). The platelet function was abnormal in 12 (25.0%) patients with bleeding peptic ulcer, as opposed to 2 (4.3%) who had abnormal function of platelet and did not bleed (χ^2 test; $p = 0.004$). At the same time, relative risk for ulcer bleeding in the patients with platelet dysfunction was very high: 20.7 ($p = 0.017$) (Table 2).

Table 3

Effects of aspirin and platelet function on bleeding ulcer

Use of aspirin and platelet function	Study group n (%)	Control group n (%)	χ^2-test (p)
Drug aspirin use	16 (33.3)	7 (14.9)	0.036*
Platelet function abnormal	12 (25.0)	2 (4.3)	0.004*

*statistically significant difference

The effects of blood groups and serum levels of vWF on ulcer bleeding are presented in Table 4. Most of the patients with bleeding ulcer had blood group O (45.8%), while blood group A was the most frequent among the patients without ulcer bleeding (40.4%). There was no significant difference between the study and control patients regarding blood groups ($p = 0.268$). The serum level of vWF did not affect the bleeding rate, since the most of the patients in both groups had normal level of vWF and similar proportion in both groups had lower level of vWF ($p = 0.935$).

Histopathological analysis of gastric mucosa and its impact on ulcer bleeding are shown in Table 5. Of all the parameters of histopathological analysis of gastritis, only atrophy of the antrum was significant predictive factor for ulcer bleeding (χ^2; $p = 0.024$). Relative risk for ulcer bleeding among patients with gastric antrum atrophy was 9.075 ($p = 0.008$). The presence of significant duodenogastric bile reflux seemed to be protective against bleeding since almost half of patients in the control group (42.6%) and only 20.8% from the study group had BRI >14 (χ^2-test; $p = 0.023$). Relative risk for ulcer bleeding was almost 7 times less in patients with high value of BRI ($p = 0.003$) (Table 2).

Table 4

The influence of blood groups and serum levels of von Willebrand factor (vWF) on ulcer bleading

Characteristics of patients	Study group n (%)	Control group n (%)	χ^2-test (p)
Total number	48 (50.5)	47 (49.5)	
Blood groups			
A	12 (25.0)	19 (40.4)	
B	8 (16.7)	9 (19.1)	0.268
AB	6 (12.5)	6 (12.8)	
0	22 (45.8)	13 (27.7)	
(vWF)			
normal	29 (60.4)	28 (59.6)	
lower	5 (10.4)	6 (12.8)	0.935
higher	14 (29.2)	13 (27.7)	

Table 5

The influence of histopathological characteristics on ulcer bleeding

Characteristics of the patients	Study group n (%)	Control group n (%)	χ^2-test (p)
Total number of patients	48 (50.5)	47 (49.5)	0.05
Antrum histology (present)			
intestinal metaplasia	13 (27.1)	18 (38.3)	0.244
inflammation	46 (95.8)	46 (97.9)	0.570
atrophy	14 (29.2)	5 (10.6)	0.024*
activity	34 (70.8)	25 (53.2)	0.076
Corpus histology (present)			
intestinal metaplasia	10 (20.8)	12 (25.5)	0.587
inflammation	46 (95.8)	44 (93.6)	0.629
atrophy	13 (27.1)	13 (27.7)	0.950
activity	24 (50.0)	30 (63.8)	0.174
Biliary reflux index > 14	10 (20.8)	20 (42.6)	0.023*

*statistically significant difference

Discussion

The prevalence of *H. pylori* infection is changing and proportion of ulcers that are *H. pylori*-negative and NSAID-negative seems to be increasing. Some authors observe that these "idiopathic" peptic ulcers seem to be more resistant to standard therapy, may be associated with more frequent complications and these that relapse may require long-term maintenance therapy [1, 2, 11, 28]. However, as antisecretory medications are often less effective in controlling gastric pH in *H. pylori*-negative patients, it is recommended to prescribe full doses of these drugs as maintenance therapy in this scenario [2].

The incidence of bleeding ulcers is between 32 and 51 *per* 100 000 *per* year [29]. Bleeding represents about 70% of all ulcer disease complications with the highest morbidity and mortality [30].

It seems that the prevalence of *H. pylori* infection is lower among patients with bleeding ulcer than in patients with ulcer disease without bleeding. Hung et al. [20] show that among 638 patients with bleeding ulcers there was 18.8% *H. pylori* negative patients.

One explanation for the increase in bleeding rate among *H. pylori*–negative, NSAIL-negative ulcers is stress-related ulcerogenesis due to other medical conditions [3, 31]. Between 5% and 20% of patients with gastric or duodenal ulcer lack an identifiable organic etiology and data available in studies published worldwide suggest that psychosocial factors play a significant role [31–33]. Wong et al. [34] have shown that exposure to a stressful situation plays an important role in the pathogenesis of *H. pylori* negative ulcers, with a high risk of rebleeding and mortality. Even though we could not find that exposure to stress is related to bleeding peptic ulcers, we observed that almost two thirds of patients with bleeding ulcer (68.7%) had the score higher than 150, graded by "Holmes and Rahe stress scale".

Patients with non-NSAID and non-*H. pylori* ulcers are often older, sicker and more frequently experience bleeding episodes while in hospital [28]. Xia et al. [35] have shown that 17% of duodenal ulcers were *H. pylori*- and NSAID-negative and revealed that the presence of concomitant diseases was an independent predictor for those ulcers. Some other studies gave the same results [36–38]. Na et al. [39] compared geriatric (older than 65) and non-geriatric patients with peptic ulcer bleeding and found that there was no difference in the incidence of *H. pylori* negative ulcers between compared groups. In the same time, geriatric patients with bleeding ulcers had much higher rate of cardiovascular and pulmonary concomitant diseases compared to the group of non-geriatric patients.

Our results are not in accordance with these findings especially in regard to concomitant diseases where we found that even greater percentage of patients with other diseases did not have ulcer bleeding. At the same time, patients older than 50 were more frequent in our study group than in the control group, again with no significant difference, probably because of a small number of patients included in the present study.

The relationship between blood group antigens and peptic ulcer disease, especially with upper gastrointestinal bleeding was widely evaluated in the past and one of the studies found that the blood group O had higher frequency in the group with bleeding ulcer than in the control group compared to other blood groups [40]. The reason for bleeding is due to the level of the circulating plasma protein von Willebrand factor [15]. It seems that vWf levels are 25% higher in non-O compared to group O individuals [16]. An interesting finding is that the rebleeding rate between patients with different blood groups was similar [40]. At the same time, some other studies find determination of blood groups not being a useful tool to determine the individual risk for gastroduodenal ulcer and ulcer bleeding [41, 42]. In our study we found that the most patients in the bleeding group had blood group O and blood group A in the control group, but there was no significant difference between the two groups in regard to blood groups.

Although a number of studies have demonstrated the influence of ABO blood group on the levels of von Willebrand factor, the nature of this association and its clinical importance is still largely unknown. A deficiency of vWF is responsible for a hemorrhagic diathesis [12]. In our study low level of vWf did not affect ulcer bleeding.

Platelet aggregation can be inhibited by aspirin. Thrombocytopathy or impaired platelet function leads to the commonest gastrointestinal complication – bleeding [43, 44]. Kang et al. [44] found that the most important risk factor for bleeding peptic ulcers in *H. pylori* negative patients was a history of aspirin and/or antiplatelet agent use. We have also shown that abnormal platelet function was a significant risk factor for ulcer bleeding. In the present study, one third of patients who were taking aspirin had bleeding ulcer, and only 14.9% in the group of patients who were taking aspirin and had ulcer disease without signs of bleeding. Regular or periodical use of aspirin, other antiplatelet agents and/or anticoagulant drugs is an important reason for ulcer bleeding.

After *H. pylori* cure, gastric acid hypersecretion is not a risk factor for bleeding from duodenal ulcer, neither among patients with previous bleeding episodes. However, duodenal ulcer recurrence with bleeding may occasionally occur in patients cured of *H. pylori*, even if acid output is normal [45]. Kang et al. [44] found that patients with bleeding peptic ulcer had a higher proportion history of peptic ulcer disease. In our study, neither previous treatment of *H. pylori* infection, nor previous ulcers existence of any localization had any significant effect on new bleeding ulcer. Mc Coll [5] reported that ulcers recurring after *H. pylori* eradication are most probably equivalent to ulcers in patients without evidence of current or previous infection and should be managed in the same way. In our study we had 44.8% of patients with bleeding ulcer who were previously treated for *H. pylori* infection, as well as 55.2% of those with ulcers without bleeding and with previous *H. pylori* infection treatment. Eradication of *H. pylori* infection did not seem to protect our patients from developing bleeding ulcer disease. It is interesting that patients with previously treated gastritis had a less risk of ulcer bleeding in our study.

More studies founded that the long-standing mucosal inflammation caused by *H. pylori* infection can weaken the gastric barrier against acid or bile reflux [3]. Bile and duodenal contents have chronic noxious effects on both stomach and esophagus. Long-term exposure, proximal reflux of duodenal juice can damage unprotected mucosa and can cause dysplasia, intestinal metaplasia and ulcers. The bile reflux index is derived from observed changes in tissue histology and is, thus, an important tool that can reflect mucosal changes caused by the bile [46]. In the present study we showed that the possibility for ulcer bleeding was less if BRI was higher. It seems that the alkaline content of bile may have a protective effect on bleeding.

Kemppainen et al. [47] showed that antrum atrophy was associated with a higher risk of bleeding ulcer. While *H. pylori* infection is associated with chronic active antral gastritis, a lower prevalence of *H. pylori* is found in patients with atrophic gastritis and intestinal metaplasia. This study also showed that atrophy of the antrum was a significant risk factor for ulcer bleeding.

Conclusion

The etiopathogenesis of non-*H. pylori* and non-NSAID ulcers and ulcer bleeding in that setting has not been established yet. Our results suggest that different factors were involved in the etiopathogenesis of bleeding in *H. pylori*-negative, NSAID-negative peptic ulcers. Relative risk for bleeding from ulcer was significantly higher among aspirin users, patients with abnormal platelet function and patients with antrum atrophy. A high bile reflux index and previous treatment of gastritis had a protective effect on ulcer bleeding among patients with non-*H. pylori* and non-NSAID ulcers.

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