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Serum IgG antibodies against *Helicobacter pylori* low molecular weight antigens 50kDa, 30kDa and Urease A 26 kDa, along with vacuolating cytotoxin A are associated with the outcome of the infection

Serumska IgG antitela protiv *Helicobacter pylori* antigena male molekulske mase 50kDa, 30kDa i ureaza A 26kDa, uporedo sa vakuolizirajućim citotoksinom A povezana su sa ishodom infekcije

Nebojša Manojlović*†, Ivana Tufegdžić†‡, Elizabeta Ristanović†§, Dubravko Bokonjić†‖

Military Medical Academy, *Clinic for Gastroenterology and Hepatology, ‡Institute for Pathology, §Institute for Microbiology, ‖National Poison Control Center, Belgrade, Serbia; University of Defence, †Faculty of Medicine of Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. We designed and conducted this study due to the fact that results of the previous studies about seroreactivity to low-molecular-weight *Helicobacter pylori* antigens, cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA) in patients with gastric cancer and peptic ulcer were conflicting. **Methods.** The Western blot test was performed in 123 patients, 31 with gastric cancer, 31 with duodenal ulcer, 31 with gastric ulcer, 30 with gastritis and functional dyspepsia in order to determine IgG antibodies to *H. pylori* antigens (CagA, VacA, Heat shock protein 60kDa, Urease B 66 kDa, Flagellin 55kDa, 50kDa, 30 kDa, Urease A 26 kDa, 24 kDa). In this study we analyzed: seroreactivity to *H. pylori* antigens between group with functional dyspepsia and others; between grades of different histopathological parameters of inflammation of antral and corporal mucosa and between antrum-predominant gastritis and corpus-predominant gastritis + pangastritis groups. **Results.** It was shown that seropositivity to 50 kDa antigen could be used as a biomarker for functional dyspepsia, seropositivity to 30 kDa antigen for antrum-predominant gastritis and *H. pylori* colonization in the antrum, to UreaseA26 kDa antigen for pangastritis and corpus-predominant gastritis and degree of inflammation in the corpus. Seropositivity to VacA was the biomarker for gastric cancer and peptic ulcer taken together and inflammation of antral mucosa. Seropositivity to CagA was associated with more intensive inflammation of antral and corporal mucosa, Urease B66 kDa with inflammation of corpus mucosa, but neither of them with specific outcome of *H. pylori* infection and topographic distribution of gastric inflammation. **Conclusion.** Serum IgG antibodies to *H. pylori* antigens 50kDa, and VacA may represent useful biomarkers for the specific outcome of *H. pylori* infection, while serum antibodies to 30 kDa and UreaseA26 kDa antigens might be used as specific biomarkers for different topographic distribution of inflammation in gastric mucosa.

Key words:
helicobacter pylori; antigens; biomarkers; stomach ulcer; stomach neoplasms; duodenal ulcer; duodenal neoplasms.

Apstrakt

Uvod/Cilj. Do sada objavljene studije o seroreaktivnosti protiv *Helicobacter pylori* antigena male molekulske mase kao i citotoksina povezanog sa genom A (CagA), vakuolizirajućeg citoksina (VacA) kod bolesnika sa karcinomom želuca i peptičkim ulkusom pokazale su protivurečne rezultate, te smo u cilju istraživanja ove pojave dizajnirali i sproveli ovu studiju. **Metode.** Western blot test primenjen je kod 123 ispitanika, 31 sa karcinomom želuca, 31 sa ulkusom duodenuma, 31 sa ulkusom želuca, 30 sa gastritisom i funkcionalnom dispepsijom u cilju određivanja IgG antitela protiv *H. pylori* antigena (CagA, VacA, Heat shock protein 60kDa, Urease B66kDa, Flagelin55 kDa, 50kDa, 30 kDa, Urease

Correspondence to: Nebojša Manojlović, Military Medical Academy, Clinic for Gastroenterology and Hepatology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: nebojsa.manojlovic1@gmail.com

A26 kDa, 24 kDa). U ovoj studiji analizirali smo: razlike u seroreaktivnosti na *H. pylori* antigene između grupe sa funkcionalnom dispepsijom i ostalih grupa; između gradusa različitih patohistoloških parametara inflamacije antralne i korpusne mukoze i između antrum predominantnog gastritisa i korpus predominantnog pangastritisa. **Rezultati.** Seropozitivnost protiv 50 kDa antigena pokazala se kao biomarker za funkcionalnu dispepsiju, seropozitivnost protiv 30 kDa antigena bila je biomarker za antrum predominantni gastritis i gradus kolonizacije *H. pylori* u antrumu, protiv Urease A26 kDa antigena za pangastritis i korpus predominantni gastritis i stepen inflamacije u korpusu. Seropozitivnost protiv VacA bila je biomarker za karcinom želuca i peptički ulkus, kada se razmatraju kao jedinstvena grupa, i za inflamaciju antralne mukoze. Seropozitivnost protiv CagA bila je povezana sa intenzivnijom inflamacijom antralne i korpusne mukoze, Urease B66kDa antigena sa inflamacijom korpusne mukoze, ali ne i sa specifičnim ishodom *H. pylori* infekcije i topografskom distribucijom inflamacije želuca. **Zaključak.** Serumska IgG antitela protiv *H. pylori* antigena 50 kDa i VacA mogu predstavljati korisne biomarkere za specifični ishod *H. pylori* infekcije, dok bi antitela protiv 30 kDa i Urease A26 kDa antigena mogla biti specifični biomarkeri za različitu topografsku distrubuciju inflamacije želudačne mukoze.

Ključne reči:
helicobacter pylori; antigeni; biološki pokazatelji; želudac, ulkus; želudac, neoplazme; duodenum, ulkus; duodenum, neoplazme.

Introduction

Helicobacter pylori affects about 50% of the world population [1] and most of them do not develop symptoms and do not have the serious outcome of *H. pylori* infection.

Gastric cancer develops in 1–1.5% of infected people, and about 65–80% of patients with gastric cancer are infected with *H. pylori* [2, 3]. Peptic ulcer, both gastric and duodenal, develops in 10–20% of infected people. Patients with duodenal ulcer are infected with *H. pylori* in 90–100% of cases, and patients with gastric ulcer in 60–100% of cases [4, 5]. About one-quarter of population suffer from dyspepsia, and the majority of them have functional dyspepsia. Patients with functional dyspepsia are infected with *H. pylori* in about 50% of cases. Approximately 25% of the Western population suffer from dyspeptic symptoms each year. Seventy percent of them do not have organic cause and symptoms are related to so-called functional dyspepsia [6, 7].

H. pylori infection outcome is very different and depends on 3 groups of factors: virulence factors of *H. pylori*, host factors, and environmental factors [8].

H. pylori virulence factors could influence the ability of these bacteria to colonize, persist and/or induce severe disorders. Therefore, the status of certain virulence factors might be a potential biomarker to predict consequences of their carriers [9].

The extensive investigations of Cytotoxin associated with gene A (CagA) and Vacuolizing cytotoxin A (VacA) in development of different *H. pylori* associated diseases have been done. CagA has been extensively investigated and designated as an important oncoprotein that induce malignant neoplasm in mammals [10]. CagA producing strains are reported to be associated with severe clinical outcomes, especially in Western countries [11]. On the other hand, meta-analyses performed to estimate the value of serum CagA antibodies as a serum marker for gastric cancer in East Asian countries showed opposite results [12]. Meta-analysis regarding serum VacA antibodies and risk for gastric cancer and peptic ulcer presented significant association [9].

Investigation of antibodies to low molecular weight antigens as *H. pylori* virulence factors showed interesting, but opposite results, too. Serum antibodies against low molecular-weight-antigens as 19.5kDa [13–22], 26.5kDa [13–16, 20–23], 30kDa [13–21, 23], 35kDa [13–19, 21, 24] and 60kDa [13–15, 20, 25, 26] were associated with serious outcome of *H. pylori* infection in some studies, but the results were conflicting, too. Less extensive investigations of serum antibodies with conflicting results were performed including 37 kDa [17–19] and 45 kDa [14], 54kDa [24], Hsp60 [25, 26] antigens. One study was done for serum antibodies against 46kDa [24], 48kDa [24], 50kDa [27], 53kDa [22], 57kDa [20], 67kDa [27] antigens. Two studies investigating serum antibodies against 54kDa antigen [24, 28] failed to show associated with the serious outcome of infection.

We conducted cross-sectional study in order to investigate the value of seropositivity to low molecular weight antigens, along with CagA and VacA as biomarkers for detection of gastric cancer, and duodenal and gastric ulcer.

Methods

The study was conducted and performed during 2009 in the Clinic for Gastroenterology and Hepatology, the Institute of Pathology and the Institute of Microbiology of the Military Medical Academy (MMA) in Belgrade, Serbia. We selected and enrolled patients with dyspeptic symptoms, different underlying disease [gastric cancer (GCA), duodenal ulcer (DU), gastric ulcer (GU) and gastritis], and actual *H. pylori* infection confirmed by histopathological examination and the anti-*Helicobacter pylori* IgG positive Vira Blot.

We took a medical history from all patients and performed a physical examination, abdominal ultrasound (US) or computed tomography (CT), esophagogastroduodenoscopy (EGDS), complete blood count (CBC), liver and renal chemistry. Inclusion criteria were: presence of dyspepsia symptoms; previously untreated patients due to *H. pylori* infection; patients without proton pump inhibitors and H2 blockers in the last two weeks; absence of malignancy except for gastric cancer; absence of any immunological disorder; informed consent of the patient for EGDS and biopsy; blood sample for analyses; participation in the study; endoscopic and histopathological diagnosis of one of the following diseases: gastric cancer, duodenal ulcer, gastric

ulcer, gastritis; confirmed histopathological diagnosis of *H. pylori* infection; Western blot (ViraBlot) IgG positive for *H. pylori* infection.

EGDS was performed in all our patients in the Endoscopy Section using Olympus (GIFQ165, SN: 2207997, Olympus corporation, Tokyo) forward viewing EGD under local application of xylocaine spray. A minimum four gastric mucosal tissue biopsies (2 each from the antrum and corpus) and additional biopsies from any endoscopically visible lesion were taken. All patients were examined for findings that indicated endoscopic gastritis, such as erythema, hyperemia, atrophy, and mucosal nodularity according to the criteria of the Houston-updated Sydney grading system, and for gastric tumor, duodenal and gastric ulcer [29].

All the obtained biopsies were collected, placed on filter paper, fixed in 10% neutral formalin, and sent for preparation of formalin-fixed, paraffin-embedded tissue blocks. Three-micrometer-thick sections were prepared. One set of tissue sections was stained with hematoxylin and eosin (H&E) and the other with Giemsa stain for histopathological examination including detection of *H. pylori* in the gastric mucosa. The biopsies were evaluated for the intensity of mononuclear inflammatory cellular infiltrates, inflammatory activity (neutrophilic infiltrations), glandular atrophy, metaplasia and *H. pylori* colonization [30]. Additionally, the cases were graded according to the Houston-updated Sydney system [29], which was graded according to the intensity of mononuclear inflammatory cellular infiltrates within the lamina propria: absent inflammation (Grade 0), mild inflammation (Grade 1), moderate inflammation (Grade 2), and severe inflammation (Grade 3) (Table 1). Grading was done for activity, atrophy, intestinal metaplasia and degree of *H. pylori* colonization, also. Additional immunohistochemistry staining was performed in case of the tumor.

The blood samples were obtained from all of them and frozen at -20°C. Using the Western blot detection system (ViraBlot), IgG anti VacA 87 kDa, CagA 136kDa, Urease B 66 kDa (UreB 66), Heat shock protein 60 kDa (Hsp60), Flagellin 55kDa (Fla 55), 50 kDa, 30 kDa, Urease A 26 kDa (UreA 26) and 24 kDa *H. pylori* antigens were identified. *H. pylori* antigens of ViraBlot represent a combination of German patient isolates of highly antigenic *Helicobacter* strains. Bands for diagnosis of *H. pylori* infection were divided into highly specific as CagA 136kDa, VacA 87kDa, 30kDa, UreA 26kDa, 24kDa and less specific as Hsp 60kDa and 50kDa.

Diagnosis of GCA was established in 31 patients, DU in 31 patients, and GU in 31 patients, whilst in 30 patients gastritis with functional dyspepsia (FD) was diagnosed.

According to manufacturer guideline for use, the test was considered negative if there were no bands or there were nonspecific bands such as UreB 66 kDa, Hsp 66 kDa, Fla 55 kDa, 50 kDa. The test was possibly positive if there was one clear specific band of 30kDa, UreaA 26 kDa, 24 kDa. Test was positive if there was at least one band of following two specific CagA 136 kDa or VacA 87 kDa or at least one clear band of 30kDa, Urea A 26, 24 kDa or one clear band of 30 kDa, UreA 26 kDa, 24 kDa and one clear band of Hsp 60 kDa, 50 kDa.

All patients included in our cross sectional study were classified and analyzed in several ways.

The first, according to baseline diagnosis, patients were divided in four groups: GCA, DU, GU, and FD.

The second, all parameters of gastric and corpus inflammation according to Houston-updated Sydney classification: inflammation, activity, atrophy, and intestinal metaplasia and *H. pylori* colonization on the basis of seroreactivity to *H. pylori* antigens in ViraBlot [29].

The third, classification was made on the basis of predominantly located inflammation irrespective of baseline diagnosis: antrum-predominant gastritis and pangastritis along with corpus-predominant gastritis. Because of a small number of patients with corpus-predominant gastritis (only 4 participants) we made one group with pangastritis (45 participants) and corpus-predominant gastritis.

The fourth, two groups were divided on the basis of the presence of GCA and peptic ulcers as one group and FD as the other group.

Statistical analysis

Complete statistical data analysis was done with the statistical software package, SPSS Statistics 18.

Most of the variables were presented as frequency of certain categories, so *t*-test of proportion or cross-tabulation analysis [odds ratio (OR), 95% confidence intervals (CI)] were done for calculation of statistical significance of differences between groups.

In case of continuous data, variables were presented as median, minimal and maximal values (range).

All the analyses were estimated at minimal $p < 0.05$ level of statistical significance.

Table 1

Demographic and clinical characteristics of the patients

Groups	Gender (n)		Age (years)	
	male	female	median	range
GCA (n = 31)	10	21	65.0	40–85
DU (n = 31)	13	18	54.0	21–87
GU (n = 31)	12	19	67.5	34–81
FD (n = 30)	13	17	63.5	21–80
Total (n = 123)	48	75	63.0	21–87

GCA – gastric cancer; DU – duodenal ulcer; GU – gastric ulcer; FD – functional dyspepsia.

Results

Four groups of patients with GCA, DU, GU and upper FD were comparable regarding sex and age (Table 1).

The initial analysis was performed in four groups comparing antibody to all separate antigens of ViraBlod test. The frequency of serum antibody positivity to CagA was not different among the four groups. The immunoreactivity to VacA was found to be less frequent in the group of patients with FD (32%) as compared with other groups, what was statistically insignificant. The immunoreactivity to UreB66, Hsp60 and Fla 55 antigens was high in all groups of patients without any differences among them. Immunoreactivity to 24 kDa antigen was generally less frequent than other antigens, but there were no differences amonggroups. The immunoreactivity to 50 kDa antigen occurred significantly more frequently in the group of patients with FD than in the other groups ($p = 0.02$ for GCA and DU; $p = 0.01$ for GU), (Table 2).

The seroreactivity to 30 kDa antigen was observed to be significantly more frequent in patients with upper FD than in those with GCA ($p = 0.01$), and more frequent than in GCA group without reaching statistically significant difference (Table 2).

CagA seroreactivity was associated with more intensive lymphocyte infiltration of the antral and corporal gastric mucosa ($p = 0.034$; $p = 0.016$) (Table 3). UreB66 antigen seroreactivity was associated with more intensive lymphocyte infiltration of the corpus mucosa ($p = 0.04$) (Table 4).

VacA seroreactivity was associated with more intensive lymphocytic infiltration of the antral mucosa ($p = 0.014$), and there was a trend towards more intensive lymphocyte infiltration of corporal mucosa ($p = 0.061$) (Table 3). Seroreactivity to 30 kDa antigen was associated with more intensive colonization of *H. pylori* in the antral mucosa ($p = 0.01$), and seroreactivity to UreA 26kDa antigen was associated with more intensive lymphocyte infiltration of the corpus mucosa ($p = 0.005$) (Table 4).

Serum antibodies to all *H. pylori* antigens in ViraBlot test were analyzed in patients with antrum-predominant gastritis v.s. pangastritis and corpus-predominant gastritis. A significant difference was found only in antibodies to 30kDa antigen which was more frequent in the group with antrum-predominant gastritis ($p = 0.025$), (Table 4), and to UreA 26 kDa antigen which was more frequent in pangastritis and corpus-predominant gastritis ($p = 0.01$) (Table 3).

Table 2

Seroreactivity against *H. pylori* antigens in four groups of patients

WB IgG	Groups, n (%)					SP	FD v.s. others		
	GCA 31 (100)	DU 31 (100)	GU 31 (100)	FD 30 (100)	Total 123 (100)		GCA	DU	GU
CagA	30 (97)	28 (90)	29 (93)	26 (84)	113 (92)	*p* OR CI	ns	ns	ns
VacA	18 (58)	18 (58)	17 (55)	10 (32)	63 (51)	*p* OR CI	ns	ns	ns
UreB66	28 (90)	29 (93)	23 (74)	26 (84)	106 (86)	*p* OR CI	ns	ns	ns
Hsp60	30 (97)	30 (97)	25 (81)	28 (93)	113 (92)	*p* OR CI	ns	ns	ns
Fla55	30 (97)	29 (93)	29 (93)	27 (90)	116 (94)	*p* OR CI	ns	ns	ns
50 kDA	15 (48)	15 (48)	14 (45)	23 (77)	67 (54)	*p* OR CI	0.02 0.29 0.1–0.9	0.02 0.29 0.1–0.9	0.01 0.23 0.09–0.8
30 kDA	17 (55)	24 (77)	14 (45)	23 (77)	78 (63)	p OR CI	ns	ns	0.01 0.25 0.08–0.7
26 kDA	26 (84)	27 (87)	23 (74)	27 (90)	103 (85)	*p* OR CI	ns	ns	ns
24 kDA	14 (45)	13 (42)	15 (48)	12 (40)	54 (44)	*p* OR CI	ns	ns	ns

WBIgG – Western blot immunoglobulin G; GCA – gastric cancer; CagA – Cytotoxin-associated with gene A; VacA – Vaculoating cytotoxin A; UreB – urease B 66 kDA; Hsp60 – Heat shock protein 60 kDa; Fla55 – Flagellin 55 kDa; GCA – gastric cancer; DU – duodenal ulcer; GU – gastric ulcer; FD – functional dyspepsia.
SP – statistical parameters (*p* – probability; OR – odds ratio; CI – 95% confidence intervals; ns – not significant).

Table 3

Seroreactivity to *H. pylori* antigens and grade of inflammation (INF), activity (ACT), atrophy (ATR), intestinal metaplasia (IM), *H. pylori* (HP) colonization in the antrum (A) and corpus (C)

WB IgG*	INF-A	ACT-A	ATR-A	IM-A	HP-A	INF-C	ACT-C	ATR-C	IM-C	HP-C
	WB IgG + *vs* WB IgG- (probability)									
CagA	0.034	ns	ns	ns	ns	0.016	ns	ns	ns	ns
VacA	0.014	ns	ns	ns	ns	0.061	ns	ns	ns	ns
UreB66	ns	ns	ns	ns	ns	0.04	ns	ns	ns	ns
Hsp60	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fla55	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
50kDa	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
30kDa	ns	ns	ns	ns	0.01	ns	ns	ns	ns	ns
26kDa	ns	ns	ns	ns	ns	0.005	ns	ns	ns	ns
24kDa	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

For abbreviations see under Table 2.

Table 4

Seroreactivity to different *H. pylori* antigens in patients with different topographic distribution of gastritis

WB IgG	APG, n (%) (n = 74)	CPG and PG, n (%) (n = 49)	Total, n (%) (n = 123)	*p*	OR	95% CI
CagA	67 (90)	46 (94)	113 (92)	ns	na	na
VacA	34 (46)	28 (57)	62 (50)	ns	na	na
Ure B66	62 (84)	44 (90)	102 (86)	ns	na	na
Hsp60	68 (92)	44 (90)	112 (91)	ns	na	na
Fla55	72 (97)	46 (94)	118 (96)	ns	na	na
50kDa	44 (59)	23 (45)	67 (54)	ns	na	na
30kDa	51 (65)	23 (47)	84 (68)	0.025	0.39	0.19–0.84
26kDa	57 (77)	46 (94)	103 (84)	0.01	na	na
24KDa	36 (49)	20 (41)	56 (45)	ns	na	na

APG – antrum-predominant gastritis; CPG – corpus-predominant gastritis; PG – pangastritis.
***p* – probability; OR – odds ratio; CI – confidence intervals; na – not available.**
For other abbreviations see under Table 2.

Table 5

Seroreactivity against different *H. pylori* antigens in groups of patients with gastric cancer (GCA) and peptic ulcers (PU), and gastritis (G) and functional dyspepsia (FD)

WB IgG	GCA & PU, n (%) (n = 93)	G & FD, n (%) (n = 30)	Total, n (%) (n = 123)	*p*	OR	95% CI
CagA	87 (93)	26 (87)	113 (92)	ns	na	na
VacA	53 (57)	10 (32)	63 (51)	0.024	2.33	0.9–5.5
UreB66	80 (86)	26 (87)	106 (86)	ns	na	na
Hsp60	85 (91)	28 (93)	113 (92)	ns	na	na
Fla55	89 (96)	27 (90)	116 (94)	ns	na	na
50kDa	44 (47)	23 (77)	67 (54)	0.009	0.27	0.11–0.7
30kDa	55 (59)	23 (77)	78 (63)	0.08	0.44	0.11–1.13
26kDa	76 (82)	27 (90)	103 (85)	ns	na	na
24kDa	42 (45)	12 (40)	56 (45)	ns	na	na

For abbreviations see under Tables 2 and 3.

Serum antibodies to all *H. pylori* antigens in ViraBlot test were analyzed in the group of patients with GCA and both peptic ulcers v.s. the group with gastritis and FD. A significant difference was found only in antibodies to VacA which appeared more frequent in the GCA & peptic ulcer groups ($p = 0.024$, OR = 2.3), and seroreactivity to 50kDa antigen was more frequent in the gastritis-FD group ($p = 0.009$; OR = 0.27) (Table 5).

Seroreactivity to 30 kDa antigen was more frequent in the gastritis-FD and there is a trend towards significance ($p = 0.08$; OR = 0.44).

Discussion

Our report is the first one regarding seroreactivity to different *H. pylori* antigens present in the ViraBlot in the Serbian population except for CagA and VacA. Our analysis of seroreactivity to different *H. pylori* antigens in four groups (GCA, DU, GU, gastritis with FD) showed significant difference only with 50kDa and 30 kDa antigens. The frequency of antibodies to all other *H. pylori* antigens in the ViraBlod test was not different among baseline groups.

Serum antibodies to 50 kDa antigen were significantly more frequent in gastritis with FD than in groups with GCA, DU and GU. Seroreactivity to 50 kDa antigen was not associated with the grade of any parameter of gastric inflammation in the antral and corporal gastric mucosa, and was more frequent in antrum-predominant gastritis, but without reaching statistical significance. There are scarce literature data regarding immunoreactivity to 50kDa antigen. Seroreactivity to 50kDa antigen was significantly more frequent in infected persons with *H. pylori* than in noninfected ones [31], in *H. pylori* line Hpu24 in GCA than in GU ulcer, but it was not the case with line NCT11 and Hcp29 [32]. In a study from Turkey, serum antibodies to 50 kDa antigen were not different between GCA patients and patients without cancer [27]. 50kDa antigen was not highly specific for *H. pylori*, but in all our analyses it appeared as a biomarker for *H. pylori* gastritis in FD, but this association could not be explained on the basis of features of the inflammatory process in the gastric mucosa.

Seroreactivity to 30 kDa antigen was significantly more frequent in patients with FD than in GU ($p = 0.01$). 30 kDa seroreactivity was equally frequent in patients with DU as in FD, and less frequent in GCA, but without statistical significance. On the other hand, it was associated with grade of *H. pylori* colonization in the antral mucosa, and antrum-predominant gastritis, which could explain the association with DU and FD, where we could expect the antral predominant type of gastric inflammation and intensive *H. pylori* colonization in the antral mucosa.

Outer membrane protein (OMP)-30kDa antigen is specific for *H. pylori* infection Immunoreactivity to 30 kDa antigen is significantly more frequent in infected subjects with *H. pylori* than in noninfected ones [31]. A presence of serum antibodies to 30kDa OMP was investigated in 10 studies [13–21, 23]. In 4 studies, seroreactivity to 30 kDa antigen showed significant association with specific outcome of *H. pylori* infection [14, 20–23]. In Croatian population, it was associated with higher degree of antrum and corpus inflammation in the stomach [20]. In Australian population, it was associated with healthy blood donors [14], and in Lithuanian population with GCA [23]. In Thai population not actually infected with *H. pylori* it was associated with GCA [21]. In 6 studies serum antibodies to 30 kDa antigen showed no association with specific outcome of *H. pylori* infection [13, 15–19]. Four studies with Thai population did not find association of antibodies to 30 kDa antigen with GCA, DU, GU, mucosa-associated lymphoid tissue (MALT) lymphoma, and FD [13, 16, 18, 19]. In a study from Iran, there was no difference between GCA and FD [17], and in a study from France there were no differences among GU, DU, gastric erosions, MALT lymphoma and FD [15].

Serum antibody to VacA was associated with GCA and peptic ulcer after meta-analysis [9]. Our results are concordant with the previous result from Serbia and Montenegro [33] where VacA was associated with peptic ulcer, and from Croatia where serum antibodies to VacA were associated with DU [20]. Results of our study confirmed association of seroreactivity to VacA with serious infection outcome, but statistical significance was reached only when the groups with GCA and peptic ulcer were joined and tested v.s. gastritis and FD.

In our study, seroreactivity to UreA26 kDa antigen was associated with the intensity of lymphocyte infiltration in the corpus mucosa, and it was more frequent in pangastritis and corpus-predominant gastritis. Association UreA26kDa antigen seroreactivity with gastric inflammation could be related to the severe outcome of infection, but there was no association between GCA and peptic ulcer. Seroreactivity to 26 kDa antigen was previously investigated in 6 studies apart from ours [13–16, 20, 27]. In three studies, two from Thai population, it was associated with alone analyzed GCA [13], and simultaneously with CagA [16], and in one from Turkey with noncancer patients [27]. Three studies were not find association of seroreactivity to UreA26 kDa with the specific outcome of the infection [14, 15, 20]. There was no such association in Croatia, among GCA, DU and GU patients [20], in France among GU and DU patients, gastric erosions, MALT lymphoma and FD patients [15], and in mixed Australian-Chinese population among those with GCA, DU, healthy blood donors and FD patients [14, 31–35].

Our study highlighted that serum antibody to CagA was almost ubiquitous, and there were no differences among GCA, DU, GU and upper FD, between the group of patients with antrum-predominant gastritis and that with pangastritis and corpus-predominant gastritis, as well as between the group with peptic ulcer and GCA and the group with gastritis with upper FD. Our results confirmed previously published data about antibodies to CagA, to DU and gastritis, adding the data about GU and GCA in Serbian population [33]. It was associated with more intensive lymphocyte infiltration of the antrum and corpus gastric mucosa. Similar results regarding antibodies to CagA gastric cancer, peptic ulcer and inflammation of the gastric mucosa were found in Croatian population who are from the same geographic area [20].

Seroreactivity to UreB66 antigen was associated with more intensive lymphocyte infiltration of the corpus mucosa, but neither with specific topographic distribution of gastritis nor with specific outcome of infection regarding four baseline groups or GCA and peptic ulcer groups *vs.* FD. There are scarce literature data about seroreactivity to UreB66 kDa. It was investigated only in two studies. In the first Turkish study, results showed association with GCA [27], and the second Croatian study [20] showed no association with GCA and both peptic ulcer and parameters of inflammation of the gastric mucosa. Results from our study are more close to Croatian ones (two neighboring population), showing association with more intensive inflammation of the corporal mucosa, but with no significant difference in corpus-predominant gastritis and also with no significant difference between GCA and FD.

There are no other data about seroreactivity to Fla55 antigen and 24kDa antigen, and in our study we showed that both antigens were equally distributed among four investigated groups.

Seroraectivity to Hsp60 antigen in our study was present in the majority of investigated participants in groups

made on the base of different criteria, without any significant difference. Hsp60 kDa seroreactivity was more frequent in individuals infected with *H. pylori* than in noneinfected ones [31]. Other authors found association with the grade of inflammation particularly in the antral mucosa, and *H. pylori* colonization in the antrum and corpus in Estonian population [25], and with gastric atrophy in British population [26], but not with the specific outcome of infection considering GCA, gastric MALT lymphoma, both peptic ulcers, non ulcer dyspepsia and asymptomatic carriers in Thai [13], France [24], Australian and Chinese population [14].

Limitations of our study represents a relatively small number of patients, and German ViraBlot with *H. pylori* strains of German patients (not Serbian patients).

Conclusion

A presence of serum VacA antibodies was significantly associated with increased risks of peptic ulcer disease, GU and DU compared with gastritis and FD controls. The significant association was also found between serum VacA antibodies and GCA risk. Serum VacA antibodies might be a potential biomarker for the prediction of peptic ulcer disease and GCA risks. Serum antibodies to *H. pylori* antigen 50 kDa might be a potential biomarker for FD while serum antibodies to *H. pylori* antigens 30kDa and 26 kDa might be biomarkers for specific topographic distribution of inflammation in the gastric mucosa. Further investigation of serorectivity to selected *H. pylori* antigens 26kDa, 30kDa, 50kDa and VacA separately and simultaneously as biomarkers for the specific outcome of *H. pylori* infection should be justified.

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