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The Importance of P-glycoprotein Multidrug Transporter Activity Measurement in Patients with *Helicobacter pylori* Infection

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A B S T R A C T

P-glycoprotein is important in local antibiotic resistance. Aim was to evaluate the role of P-glycoprotein in local antibiotic resistance in patients with antral gastritis during antibiotic therapy to Helicobacter pylori *infection. In the group of 53 patients with pathohistologically verified gastritis and microbiologically confirmed* H. pylori *infection (no signs of antimicrobial resistance) we have determined P-glycoprotein activity in gastric mucosa biopsy specimens, and compared them with the P-glycoprotein activity in 12 control subjects with normal endoscopic findings. The* H. pylori *positive patients were treated according to Maastricht protocol with short-term 7-day therapy consisting of two antibiotics (amoxicillin and azithromycin/metronidazole and clarithromycin) and a proton pump inhibitor. P-glycoprotein activity was determined in rhodamine dye efflux test and quantified by ratio of the mean fluorescence (RMF) in flow cytometry analysis.* H. pylori *was successfully eradicated in the first cycle in 20 patients, whereas therapy was continued in 33 patients. The mean pre-treatment RMF values were higher in patients with* H. pylori *infection then in control subjects ($p<0.0046$). RMF was also higher in patients with multiple therapeutic failure than in those with successful* H. pylori *eradication ($p<0.0001$). RMF increased significantly during the antibiotic therapy ($p<0.05$). P-glycoprotein might be one of the causes of therapy failure in patients with* H. pylori*. Our study confirms the importance of quantitative evaluation of P-glycoprotein expression during antibiotic treatment response.*

Key words: *P-glycoprotein, multidrug resistance,* Helicobacter pylori*, antibiotic therapy*

Introduction

According to the Maastricht Consensus, treatment of *H. pylori* infection implies one antisecretory drug and a combination of two antibiotics[1,2]. About 10% of *H. pylori* treated infections remain positive. Successful *H. pylori* eradication depends on numerous factors. The efficacy of *H. pylori* eradication has been shown to be influenced by patient compliance during treatment[3], gastric pH[4], and geographic location. Local factors have the role on better success in eradication, such as coexistence of antral gastritis, gastritis of the gastric body[5], and in patients with peptic ulcer (cagA and vacAs1 positive strains) then in patients with functional dyspepsia[6–8]. It should be noted that the development of primary and secondary bacterial resistance depends on the length of treatment, combination of antibiotics, and drug concentration[9–10]. *H. pylori* has developed resistance to many drugs, e.g., metronidazole, clarithromycin, etc.[3,7,11]. Treatment failure has been ascribed to metabolic changes in *H. pylori* itself[7,12] and increasing resistance to chemotherapy. Repeat drug administration at a higher concentration has been demonstrated to produce better result[4,10,12], suggesting that local conditions are responsible for therapeutic success[3,12].

The oral bioavailabity or absorption of antibiotics depends on the dosage forms administered, mucosal permeability and absorptive clearence. *H. pylori* poorly responsive to therapy, may relate MDR expression because some antibiotics are known transmembrane transporters substrates. A high concentration of multidrug trans-

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porter (P-glycoprotein (Pgp), that actively pumps out all potentially cytotoxic substances, is found on the gastrointestinal tract epithelium. Their mechanism of action is being based on binding a broad spectrum of drugs to the membrane polypeptide chain and extruding them to the surroundings by use of ATP energy. All ATP dependent efflux proteins belong to the large ABC superfamily[13–15] and are homologous in many animal species[16]. Efflux as a mechanism of antibiotic resistance was demonstrated for gram-negative bacteria in the early 1980's[17]. The antimicrobial efflux transporter for *H. pylori*, LmrA, homologous to human multidrug transporter has only recently been described[18–20].

Materials and Methods

Patients

Gastric biopsy specimens were collected from 65 subjects upon their informed consent in writing according to the Helsinki Declaration and approved by the Hospital Ethics Committee. Fifty-three patients (24 male and 29 female), mean age 47.4 (range 18–75) years, with endoscopically and pathohistologically verified antral gastritis had *H. pylori* infection and were treated as outpatients.

Cell cultures

For determination of Pgp activity, exponentially growing human erythroleukemic K562 cell line with an optimal density of $5x10^5$/mL were prepared as control cells. The cell lines were grown in an RPMI 1640 nutrient mixture supplemented with 2 μM L-glutamine, 1 μM sodium pyruvate, 1000 units/mL penicillin, 100 mg/mL streptomycin, and 10% fetal calf serum (FCS) at 37°C in a humidified atmosphere of 5% CO_2 in air[26]. Negative control: drug-sensitive K562 line was purchased from the ATCC (American Type Culture Collection, Rockville, Maryland, USA). Positive control: the resistant HHT/K562 cell line obtained by continuous exposure to HHT was a kind gift from Professor J.P. Marie (Hotel Dieu, France) and was cultured in the presence of 150 ng/mL of HHT.

Chemicals

Rhodamine-123 (Rh123, 2-6-amino 3 imino 3H xanthene 9 y benzoic acid methyl ester, Sigma, St. Louis, MO, USA) is a cationic lipophilic fluorescent dye frequently used to study Pgp activity. The cells were labeled with 1:100 of stock solution in PBS, a final concentration 20 μg/mL. Non-toxic substrates for Pgp, a fungal metabolite Cyclosporine A (CsA, from Tolypocladium inflantum, Sandoz Pharmaceuticals, Switzerland) and homoharringtonine (HHT, an alkaloid from Cephalotaxus hainanensis, Sigma) were used in a final concentration of 2.5 ng/mL and 150 ng/mL, respectively.

Methods

HP Diagnostic studies

The presence of *H. pylori* was verified by two parallel tests (microbiology and pathohistology). Histologic diagnosis included material staining according to Giemsa[21]. Microbiologic diagnosis included isolation and typing. B-biodisk and Chaves method determined antibiotic sensitivity by E test[22–24]. Control group had 12 patients with dyspepsia as an indication for upper gastrointestinal endoscopy, in whom *H. pylori* infection was neither microbiologically nor histologically demonstrated[21–24]. As the study was not aimed at comparison of different therapeutic protocols, it included a higher proportion of patients with multiple therapeutic failure than would otherwise be expected according to the efficacy of *H. pylori* eradication.

Treatment of H. pylori *infection*

All patients underwent standard short-term triple therapy consisting of pantoprazole (P), a proton pump inhibitor (PPI), at a dose of 40 mg b.i.d., and two antibiotics, according to Maastricht recommendations[1,2,9]. The following antibiotics were used: metronidazole (M), 500 mg; amoxicillin (A), 1000 mg; and clarithromycin (C), 500 mg b.i.d. for seven days, in randomized combinations. The patients were randomly allocated to the PAM (n=39) or PAC (n=14) protocol. Following initial therapeutic failure in eradication of *H. pylori* infection, the patients were administered a new combination of the listed antibiotics for 10 days, or azithromycin (AZ) at a dose of 500 mg s.i.d. for 5 days in addition to one of the listed antibiotics for 10 days[1,2,25]. So, in the second course after initial failure with the PAM protocol, there were 15 patients in the PAC protocol and PAAZ protocol each. Also, in the second course after initial failure with the PAC protocol, there were three patients on the PAAZ protocol[1,2,9,25]. Following second failure of *H. pylori* eradication, a third combination was introduced, consisting of P 40 mg b.i.d., in combination with bismuth citrate (B) t.i.d., M 500 mg s.i.d., and tetracycline (T) 500 mg q.i.d. for 14 days[1,2,25]. This final protocol included 22 patients. The treatment with pantoprazole, 40 g/day, was continued for another 6 weeks after each of these protocols[1,2,9,25]. *H. pylori* eradication was simultaneously assessed by microbiology and pathohistology 7 weeks of the completion of each protocol[1,2,9]. Patients who had been receiving anti-inflammatory or MDR dependent drugs (that may have influenced the efflux) within one year preceding the study were not included[13–20].

Collection of biopsy specimens

Gastric biopsy specimens for microbiology and histology as well as for the assessment of Pgp activity were obtained from the oxyntic area during gastrointestinal endoscopy using an Olympus and Pentax endoscopy video system, in line with recommendations of the Sydney classification[24]. Two specimens from the antrum and body of the stomach each, and one from the angulus were obtained for microbiologic and histologic analysis, whereas two specimens from the anterior and posterior wall of the antrum each were obtained for the study of Pgp activity.

Microbiologic and histologic analysis

Density of intraepithelial neutrophils (DIEn) and *H. pylori* density (DHP) were determined in biopsy speci-

mens as sensitive parameters of mucosal lesion and *H. pylori* presence[24] *H. pylori* and neutrophil density was scored 1–4 according to Sydney classification.

Determination of Pap activity

Preparation of gastric biopsy specimens

Upon endoscopy, the gastric biopsy specimens (GBS) were immediately placed in transport media and then into transport containers with ice at a constant temperature of 4°C. GBS was dissociated mechanically by mincing the sample with little scissors, then washed with cold buffer and centrifuged at 3000 rpm for 5 min at 4°C. The viability and yield were assessed by trypan blue exclusion.

Rhodamine efflux measurement

A direct functional assay for Pgp activity was performed by the Rh123 uptake/retention method (27) adapted for stomach tissue analysis. Briefly, aliquoted GBS (5–8x10^5 cells/tube) were stained for 60 min with 5 μL of Rh123 in the presence or absence of MDR-reversing agent CsA (5 μL). After Rh123 uptake, cells were washed and fed with Rh123-free culture medium, cultured for 60 min at 37 °C, again in the presence or absence of CsA to evaluate its effect on Rh123 efflux (retention). Afterwards, GBS was centrifuged in culture medium, resuspended and kept in ice and darkness for 10 min until flow cytometry analysis. As a reference, K562 and HHT 150/K562 cells were processed in parallel with the patient samples. The analysis was performed on an FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with an ultraviolet argon laser (excitation at 488 nm, emission at 530/30 and 570/30 nm band-pass filters). Analysis of 10^4 cells *per* sample was carried out in log histogram. When Rh123 was being diffused into the cell, Pgp actively pumped out the fluorochrome, and cellular fluorescence was determined by the rates of influx and efflux. If another compound, a substrate and/or inhibitor, was presented in the same manner together with Rh123, the efflux was blocked and the fluorescent dye accumulated in the cell, producing higher mean fluorescence intensity (MFI). The result can be quantitatively analyzed and expressed as the ratio of the two mean fluorescences (RMF). RMF represents the ratio of MF of Rh123 with CsA modulator divided by MF of Rh123 without modulator after subtraction of the fluorescence of the blanks (autofluorescence of cells <1%). As the amount of intracellular Rh123 dye content upon the addition of modulator correlated with Pgp activity, RMF ≥1 was considered positive.

Variation due to test conditions

Upon endoscopy, GBS was transported at a constant temperature of 4 °C and analyzed within one hour. Gastric cells were mechanically dissociated by use of small scissors or by enzymatic digestion (trypsin), whereby the viability and gain were controlled by trypan blue exclusion. The cells obtained by mechanical dissociation (mincing with scissors) were more numerous and showed higher viability than those obtained by trypsin enzymatic dispersion. In order to assess the impact of storage on test reproducibility, 12 randomly chosen GBS were divided into two groups. One group was analyzed on the day of sampling, and the other were stored overnight at room temperature in 10 mL of cold buffer. The minimal concentration of GBS was 0.5×10^5 cells/mL.

Statistics

We used STATISTICA software for the analysis of variance, multiple regression analysis and ROC analysis in the interpretation of results. The level of significance was set at $p<0.001$. The mean value of the scores obtained on antrum biopsy (anterior and posterior wall/2) was used for statistical analysis of DIEn and DHP.

Results

The activity of Pgp was measured in gastric biopsy specimens (GBS) of 53 patients with *H. pylori* infection and 12 dyspeptic patients without *H. pylori* infection (control group). We have found no diffeterce in age (47.39 *vs*. 48.33 years) and sex (M/F=5/7 *vs*. 24/29) between this two groups.

Measurement of Pap activity in patients with H. pylori *infection*

Pgp activity was determined in 53 patients with *H. pylori* infection and 12 dyspeptic patients without *H. pylori* infection (control group). The intensity of rhodamine dye extrusion by GBS with *H. pylori* infection was higher as compared with *H. pylori* negative control GBS (0.8±0.30 *vs*. 1.38±0.66; γ=1; F=8.6, $p<0.0046$). *H. pylori* infection stimulated Pgp activity. Based on ROC analysis, RMF≥1 was considered positive.

Antibiotic therapy stimulaed Pgp activity. Irrespective of therapeutic success in ten randomly chosen *H. pylori* positive patients pre- and post-therapeutic Pgp activity was assessed. After treatment (7-weeks), stronger GBS extrusion of rhodamine dye was recorded in 6/10 patients. Wilcoxon pair test yielded a significant difference between the pre- and post-therapeutic values (t=9.0; z=1.885; $p<0.05$).

Neither is repeat therapy efficacious in enhanced Pgp activity

The course of therapy was not affected by either age or sex. The patients requiring multiple courses at *H. pylori* eradication (group B, n=33) initially had a more severe infection (higher *H. pylori* concentration and intraepithelial neutrophil infiltration), however, neither these variables were found crucial for course of treatment. Therapeutic success was influenced by Pgp activity: the patients with failure of *H. pylori* eradication had a statistically higher ($p<0.00001$) pretherapeutic Pgp activity (1.647±0.65) than either patients with efficient *H. pylori* eradication (0.889±0.28) or control group (0.787±0.29, Table 1).

Other parameters vs. *Pgp in* H. pylori *infection*

Multiple regression analysis yielded a significantly highest beta coefficient for RMF and DIEn ($p<0.001$).

TABLE 1
CORRELATION OF ELEVATED PRETHERAPEUTIC PGP ACTIVITY VRS OTHER PARAMETERS ACCORDING TO NEEDS FOR MULTIPLE THERAPIES

Variables	Tharapy cycle			*H.pylori* positive	
	H. pylori negative	*H. pylori* positive			
	NO	One=I	Two or more=II	P^a	beta P^b
widctlpar	(n=12)	(n=20)	(n=33)		
Age (yrs)	48.3±16.5	47.5±8.8	47.3±13.7	0.973	0.152
Sex (M/ F)	5/7	9/11	15/18	0.975	0.153
DHP	1.0±0.0	2.75±0.91	2.90±0.97	0.00001	0.00065
DIEn	1.0±0.0	2.65±0.81	3.06±0.86	0.00001	0.450
RMF ($\overline{X}$±SD)	0.787±0.29	0.889±0.28	1.647±0.65	0.00001	0.000005

NO = no therapy *H. pylori* positive patients: A = one therapy cycle, B = two or more therapy cycles; M = male; F = female; DHP = *H. pylori* density; DIEn = density of intraepithelial neutrophil infiltration; RMF = ratio of mean fluorescences, P^a for analysis of variance, P^b for multiple regression analysis

ROC analysis demonstrated higher sensitivity, specificity and accuracy for RMF (90.90%, 71.87% and 81.54%, respectively, for borderline RMF value of 1.0) than for DHP (48%, 71% and 60%, respectively, for borderline DHP of score 2), and DIEn (66%, 71% and 69.23% respectively, for borderline DIEn of score 2).

Combined therapy has no effect in case of enhanced Pgp activity

The number of patients included in the study was too small for statistical analysis of each antibiotic group. The antibiotics used appeared to differ in stimulating Pgp activity, i.e. the first successful course was for PAM or PAC (RMF±SD for PAM 0.83±0.3 and for PAC 0.95±0.25) was less dependent on Pgp activity than the repeat combination with azithromycin (RMF±SD 1.706±0.62; n=18). The patients with multiple attempts at *H. pylori* eradication had higher pretherapeutic RMF values, however, there was no significant difference in the choice of antibiotics (between-group analysis of variance: f=5.031; p<0.000898).

Factors influencing therapeutic efficacy

There was no significant difference in pretherapeutic Pgp activity between the patients with ultimately successful and unsuccessful *H. pylori* eradication (1.26±0.715 *vs.* 1.54±0.66; p<0.13418, Table 2). As there was no such difference in other clinical parameters either (age, sex, DIEn and DHP, p<0.189), it seems that, although influencing the duration and course of treatment, Pgp cannot be used to predict the ultimate treatment outcome.

Test conditions and Pgp meaurement

The activity of Pgp was measured in gastric biopsy specimens (GBS) of 53 patients with *H. pylori* infection and 12 dyspeptic patients without *H. pylori* infection (control group). The two patient groups were matched according to mean age (47.39 *vs.* 48.33 years) and sex (M/F=5/7 *vs.* 24/29).

TABLE 2
IMPACT OF PRETHERAPEUTIC VALUES ON *H. PYLORI* ERADICATION EFFICACY

Variable	Final eradication of *H. pylori*		p
	Successful (n=31)	Unsuccessful (n=22)	
Age (yrs)	46.54±12.51	48.54±11.44	0.556
Sex (M/F)	15/16	9/13	0.598
DHP	2.90±0.97	2.77±0.92	0.626
DIEn	2.77±0.846	3.09±0.86	0.189
RMF ($\overline{X}$±SD)	1.26±0715	1.54±0.66	0.13418

M = male; F = female; DHP = *H. pylori* density according to Sydney criteria; DIEn = density of intraepithelial neutrophil infiltration according to Sydney criteria; RMF = ratio of means

In order to assess the impact of storage on test reproducibility, as shown in Table 3, the mean RMF was 1.427±1.034 and 2.521±2.067 on day 1 and 2, respectively. RMF was elevated in most of the samples (10/12; t test for dependent samples: t=1.932; γ=11; p<0.0795; SIGN test: z=1.443; p<0.148). In the samples with the addition of modulator, fluorescence varied on day 2 and it seemed that there was no overexpression of Pgp function, although the cells were viable. As storage results in changed drug transport functions, for reasons of cellular metabolic and membrane integrity it is better to use fresh cells, within a few hours of sampling.

Discussion

Many questions about the mechanisms of drug failures to cure *H. pylori* infection the remain to be answered. The patient compliance, as antimicrobial resistance is the most important factor determining the outcome of antibiotic therapy. The increased prevalence of antibiotic-resistant *H. pylori* strains has serious implications[28,29].

TABLE 3
EFFECT OF SAMPLE STORAGE

H.Pylori Positive Patinet (N=12)	Day 1			Day2		
	Rh 123	Rh123+CyA	RMF	Rh 123	Rh123+CyA	RMF
	MF	MF		MF	MF	
$\overline{X}$±SD	102.39±69.96	117.73±77.80	1.42 ±1.034	79.40±90.92	138.65±101.06	2.522±2.067
Confid. -95.00%	57.93	68.30	0.66	21.63	74.43	1.21
Confid. +95.00%	146.84	167.1730	2.02	137.17	202.86	3.8352
Minimum	5.47	17.86000	0.24	17.25	4.28	0.15
Maximum	262.30	225.3500	3.40	308.40	326.90	6.97

Day 1 = fresh cells, several hours of sampling; day 2 = cells stored overnight at room temperature; Rh123 = rhodamine; CyA = cyclosporine A; MF = mean fluorescence; RMF = ratio of mean fluorescence
Day 1 *vs*. Day 2 results:for Rh123 v < V = 41.44% Z = 0.28 p<0.77; for Rh123+Cy v < V =58.33 Z = 0.28 p<0.77; for RMF v > V = 83.33 Z = 2.02 p<0.043

The present article speculates about the potential mechanism limiting the gastrointestinal availability of antibiotics by P-glycoprotein (Pgp) mediated decrease in drug accumulation. In humans, Pgp is expressed on a wide variety of normal cells. Pgp is especially present in secretory tissues including the liver, intestinal tract epithelia from jejunum and colon, adrenal cortex, kidney, certain capillary endothelia, peripheral blood lymphocytes and hemopoietic precursor cells[30]. In gastrointestinal tract, Pgp acts as an ATP-consuming efflux pump extruding the natural toxins across the epithelial surface to the intestine, hepatocytes in biliary canaliculi and small ductules of the pancreas[31]. In the gastrointestinal tract there is regional variation in Pgp expression: being maximally expressed in the epithelial cells of illeum with a gradual decrease proximally into the jejunum duodenum and stomach[32]. Expression of Pgp on the luminal surface of epithelial cells in gastrointestinal tract suggest a role in decreasing[33], absorption[34] and secretion of endogenous and exogenous amphipathic toxins[35].

Recently we have found that several sources reported about the presence of direct evidence that Pgp inhibits the gastrointestinal absorption of orally administered drugs[35–39]. To the best of our knowledge, this is the first report analyzing Pgp activity in *H. pylori* infection. We evaluated Pgp function in gastric cells from patients with *H. pylori* infection by means of intracellular accumulation and efflux of a fluorescent dye. In the GBS loaded with Rh123 dye an increased efflux was observed in 33 *H. pylori* positive patients who failed to respond to treatment. The patients with enhanced Pgp activity (RMF >1.5) failed to respond to either first or second therapy with the same or another antibiotic. As Pgp activity is low (RMF<1) in patients who respond favorably to the first course of treatment, it is of most importance to assess Pgp activity before therapy introduction and to monitor it before repeat antibiotic cycles. Pgp activity is generally low at the time of diagnosis, i.e. before the patient receives any treatment. These findings indicate that an increased Pgp activity may contribute to the drug-induced effect on gastric cells long after therapy completion. Although *H. pylori* infection *per se* stimulates Pgp pump activity, this activity seems to additionally rise during the course of disease, because RMF index is lower at the disease onset. And the last but not the least, the lower RMF values recorded in patients with remission support the hypothesis that inappropriate therapeutic combinations lead to eradication failure. Therefore, Pgp activity testing might be used as a sign of therapeutic failure and need of multiple therapeutic protocols in a particular patient. Although Rh123 efflux could not predict final outcome of the attempts at *H. pylori* eradication, it did show that elevated RMF values had greater impact on *H. pylori* eradication failure with the use of AZ than with other antibiotics. Other parameters of *H. pylori* infection (DHP, DIEn) did not prove useful in predicting either the need of an increased number of therapeutic protocols or the final treatment outcome. We can sugest pretherapeutic testing of Pgp in *H. pylori* infected patients, and repeated measurement of Pgp in patients with unsucesfull eradication. In this way we will be able to detect patients who will need repeated therapies, and in those patients antimocrobial in vitro testing will be needed.

At the end, for those who intend to start measurement of Pgp in gastric cells, we can sugest that storage results in changed drug transport functions, for reasons of cellular metabolic and membrane integrity it is better to use fresh cells, within a few hours of sampling.

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VAŽNOST ODREĐIVANJA AKTIVNOSTI P-GLIKOPROTEINSKOG PRIJENOSNIKA ZA VIŠE LIJEKOVA U BOLESNIKA S *HELICOBACTER PYLORI* INFEKCIJOM

SAŽETAK

P-glikoprotein je važan čimbenik u neuspješnosti liječenja infekcije antibioticima. Cilj studije bio je istražiti važnost P-glikoproteinskog prijenosnika za više medikamenata u bolesnika s antralnim gastritisom tijekom antibiotske eradikacijeke terapije *Helicobacter pylori* infekcije. U skupini od 53 bolesnika s patohistologijski dokazanim gastritisom i mikrobiologijski potvrđenom *Helicobacter pylori* infekcijom, a bez znakova za antimikrobnu rezistenciju, mjerili smo aktivnost P-glikoproteina u želučanoj sluznici dobivenoj nakon biopsije, te smo je uspoređivali s aktivnosću P-glikoproteina u 12 ispitanika s urednim endoskopskim i patohistologijskim nalazom. Bolesnici s *Helicobacter pylori* infekcijom liječeni su 7-dnevnom terapijom, a prema protokolu iz Maastrichta, koja se sastojala od dva antibiotika (amoksicilin i azitromicin/metrronidazol i klaritromicin) i inhibitora protonske pumpe. Aktivnost P-glikoproteina određivana je rhodamine dye efflux testom i kvantificirana je srednjom vrijednošću florescencije (RMF), koja je mjerena protočnim citometrom. *Helicobacter pylori* je uspješno eradiciran u prvom pokušaju u 20 bolesnika, dok je terapija trebala biti nastavljena u 33 bolesnika. Prije početka liječenja, bila je viša srednja vrijedonst RMF-a u bolesnika s *Helicobacter pylori* infekcijom nego u kontrolnoj skupini ($p<0,0046$). RMF je također bio viši u bolesnika s višestrukim neuspijesima u eradikadciji *Helicobacter pylori* infekcije nego u onih s uspješnom eradikacijom ($p<0,0001$). RMF je značajno poratao tijekom antibiotskog liječenja ($p<0,05$). Možemo zaključiti kako P-glikoprotein može biti jedan od uzroka neuspijeha u eradikaciji *H. pylori* infekcije. Naša studija potvrđuje važnost mjerenja i kvantitativne evaluacije ekspresije P-glikoproteina tijekom antibiotske terapije.