Tohoku J. Exp. Med., 2021, **253**, 85-94

High Body Mass Index Is Correlated with the Success of Vonoprazan-Based Second-Line Therapy for *Helicobacter Pylori* Infection

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Eradication of *Helicobacter pylori* (Hp) is necessary for preventing peptic ulcers and stomach cancer. The potassium-competitive acid blocker vonoprazan is a gastric acid secretion inhibitor that improves the success rate of Hp eradication through its immediate and persistent inhibition of acid excretion. In Japan, first-line treatment involves a regimen in which vonoprazan is combined with amoxicillin and clarithromycin, while second-line treatment involves vonoprazan combined with amoxicillin and metronidazole. However, in contrast to the vonoprazan-based first-line therapy, no studies have investigated the factors influencing the success of vonoprazan-based second-line therapy. In this study, we therefore aimed to investigate factors related to the success of vonoprazan-based second-line therapy. We analyzed the association between the success of Hp eradication and patient factors including metronidazole/amoxicillin minimal inhibitory concentrations (MICs). MICs were measured using the Hp isolated from each patient. A receiver operating characteristic (ROC) analysis was conducted to examine continuous variables and eradication success. We reviewed the records of 33 patients (age: 34-79 years, male/female: 22/11, and body mass index (BMI): 16.1-28.8 kg/m^2) who underwent vonoprazan-based second-line therapy after failure of first-line therapy at seven Japanese facilities between October 2018 and June 2019. The eradication success rate was 81.8% (27/33). ROC analysis revealed an area under the curve and BMI cutoff value of 0.796 and 23.8 kg/m^2, respectively. The eradication success rate was higher in patients with high BMI than in those with low BMI (p = 0.007). Our findings indicate that higher BMI is correlated with the success of vonoprazan-based second-line therapy.

Keywords: amoxicillin resistance; body mass index; eradication therapy; *Helicobacter pylori* infection; metronidazole resistance
Tohoku J. Exp. Med., 2021 February, **253** (2), 85-94.

Introduction

Helicobacter pylori (Hp) are gram-negative flagellated spiral bacteria first detected by Warren and Marshall (1983). Hp infection is a chronic infectious disease that induces gastritis, peptic ulcers, stomach cancer, and mucosa-associated lymphoid tissue lymphoma (Asaka et al. 1996; Uemura et al. 2001; Malfertheiner et al. 2017). To prevent recurrence of peptic ulcers and reduce the risk of stomach cancer, Hp eradication therapy is recommended for patients

Received October 9, 2020; revised and accepted January 14, 2021. Published online February 2, 2021; doi: 10.1620/tjem.253.85.
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with confirmed Hp infection (Leodolter et al. 2001; Lee et al. 2016). Regimens for Hp eradication involve treatment with gastric acid secretion inhibitors and antimicrobials, and regimens with an eradication success rate greater than 90% are recommended (Graham and Fischbach 2010).

Various eradication therapies for Hp have been implemented worldwide (Hu et al. 2017). The conventional regimen involving proton pump inhibitors (PPIs) combined with amoxicillin and clarithromycin therapy (hereafter, PPI-based first-line therapy) is utilized for first-line eradication therapy in Japan. However, decreases in the eradication success rate due to increases in antibiotic-resistant bacteria represent a major issue (Graham and Fischbach 2010; Thung et al. 2016). Recent studies have reported that the eradication success rate of PPI-based first-line therapy has fallen from 90% to 70% in Japan (Nishizawa et al. 2015; Murakami et al. 2016). However, the potassium-competitive acid blocker vonoprazan, a novel gastric acid secretion inhibitor, represents a potential solution to this problem.

Similar to PPIs, vonoprazan inhibits gastric hydrogen/potassium-ATPase, an enzyme that catalyzes the final step in the gastric acid secretion pathway. However, unlike PPIs, vonoprazan inhibits the enzyme in a potassium-competitive and reversible manner (Andersson and Carlsson 2005). Vonoprazan has a potent and a long-lasting antisecretory effect due to its high accumulation and slow clearance from gastric tissue (Hori et al. 2010, 2011; Shin et al. 2011).

Sufficient suppression of gastric acid secretion is known to influence the success of Hp eradication therapy. Previous studies have reported that suppression of gastric acid secretion increases the sensitivity of Hp to antimicrobials and increases the concentration of antimicrobials in the gastric mucus, thereby improving the Hp eradication success rate (Villoria et al. 2008; Marcus et al. 2012). Compared with conventional PPIs, vonoprazan is associated with increases in the eradication success rate through its potent, immediate, and persistent inhibition of acid excretion (Suzuki et al. 2016; Kusano et al. 2018; Lyu et al. 2019). Vonoprazan combined with amoxicillin and clarithromycin (hereafter, vonoprazan-based first-line therapy) has been associated with an eradication success rate of 88%, which is superior to that of PPI-based first-line therapy (Jung et al. 2017). Second-line eradication therapy is recommended for patients in whom first-line therapy has failed. Although a regimen involving PPIs combined with amoxicillin and metronidazole therapy (hereafter, PPI-based second-line therapy) is conventionally utilized for second-line eradication therapy in Japan, PPIs may be swapped out for vonoprazan. A recent study reported a 90% eradication success rate for vonoprazan combined with amoxicillin and metronidazole (hereafter, vonoprazan-based second-line therapy), which is superior to that reported for PPI-based second-line therapy (Shinozaki et al. 2020). Given these findings, vonoprazan-based eradication therapy is recommended by the Japanese guidelines (Kato et al. 2019). However, Hp eradication fails in some patients despite treatment with vonoprazan-based eradication therapy.

Several studies have investigated the clinical factors related to the success of vonoprazan-based first-line therapy. However, to the best of our knowledge, no studies have investigated the patient factors related to the success of vonoprazan-based second-line therapy. The significance of conducting antimicrobial susceptibility tests is also unknown in the context of vonoprazan-based second-line therapy. Thus, the present study aimed to investigate the associations between patient factors, including metronidazole/amoxicillin susceptibility, and the success of vonoprazan-based second-line therapy.

Materials and Methods

Patients

The present study is a subgroup analysis of the primary study, "Seven-day vonoprazan and low-dose amoxicillin dual therapy as first-line *Helicobacter pylori* treatment: a multicenter randomized trial in Japan (Suzuki et al. 2020)." The primary study was registered in the University Hospital Medical Information Network (UMIN) on September 14, 2018 (clinical trial registration number: UMIN000034140). The primary study was comprised of a multicenter randomized controlled trial that utilized two-drug therapy (vonoprazan 20 mg twice per day with amoxicillin 750 mg twice per day, administered for 7 days) or three-drug therapy (vonoprazan 20 mg twice per day with amoxicillin 750 mg twice per day and clarithromycin 200 mg twice per day, administered for 7 days) as the first-line eradication therapy for 335 patients who were Hp-positive. The eradication success rates were compared between the two groups.

The present study identified 335 patients aged 20-79 years who were Hp-culture-positive in seven Japanese facilities and underwent eradication therapy between October 2018 and June 2019. Patients in whom first-line eradication therapy had failed who subsequently underwent vonoprazan-based second-line therapy (vonoprazan 20 mg twice per day with amoxicillin 750 mg twice per day and metronidazole 250 mg twice per day, administered for 7 days) were enrolled in the present study. The exclusion criteria included drug allergies, history of gastrectomy, pregnancy or nursing, regular PPI use, and use of antimicrobial agents or steroids and an inability to discontinue them. Furthermore, patients who underwent second-line eradication therapies other than vonoprazan-based second-line therapy were excluded. All patient data were obtained retrospectively from the primary study databases.

Definition of eradication success and failure

First-line eradication therapy failure was defined as having a value of $\geq 2.5\%$ in a urea breath test (UBT) conducted at least 4 weeks after first-line eradication therapy. Second-line eradication success was defined as having a

value of < 2.5% in a UBT conducted at least 4 weeks after second-line eradication therapy. We utilized the UBIT tablet (Otsuka Pharmaceutical, Tokyo, Japan) for the UBT.

Antimicrobial susceptibility test

Patients were confirmed to have Hp infection via gastric mucosal culture inspections. Antimicrobial susceptibility tests were simultaneously conducted with the microbroth dilution method using Eiken Chemical dry plates (Eiken Chemical, Tokyo, Japan). A solution using an antimicrobial agent against a fixed quantity of Hp was cultured. The minimum inhibitory concentration (MIC) was defined as the minimum antimicrobial concentration wherein cloudiness was not visually observed in the cultured solution. The MICs of metronidazole and amoxicillin were measured within the ranges of 4-16 and 0.015-1 μg/mL, respectively.

Study outcomes and statistical analyses

The study outcomes were the relationships between the following variables and the success of vonoprazan-based second-line therapy: age, sex, height, weight, body mass index (BMI), histories of smoking and alcohol consumption, regular PPI use prior to study participation, metronidazole/amoxicillin MICs, and the presence of Hp-related diseases. Hp induces peptic ulcers and stomach cancer (Asaka et al. 1996; Uemura et al. 2001; Malfertheiner et al. 2017). The presence of Hp-related diseases was defined as having a history of gastric ulcers, duodenal ulcers, or endoscopic resection for gastric neoplasia. Previous studies have reported that these factors are associated with the success of other Hp eradication regimens (Xia et al. 1994; Lopez-Brea et al. 1999; Hsu et al. 2005; Suzuki et al. 2006; Nishizawa et al. 2007; Abdullahi et al. 2008; Singh et al. 2008; Matsumoto et al. 2016; Chen et al. 2017; Costa et al. 2017; Shinozaki et al. 2018; Tan et al. 2018; Kusunoki et al. 2019; Lee et al. 2019). A receiver operating characteristic (ROC) analysis was used to evaluate the relationships between continuous variables (age, height, weight, BMI, metronidazole/amoxicillin MICs) and eradication success. ROC curves relating to each continuous variable and eradication success were created, and both the area under the curve (AUC) and cutoff values were calculated. The cutoff values were set as the threshold at which the sum of the sensitivity and specificity values was at a maximum. The ROC curves were created by setting the metronidazole MIC values that were $\leq$ 4 μg/mL as 4 μg/mL and those that were > 16 μg/mL as 32 μg/mL. The ROC curves were created by setting the amoxicillin MIC values that were $\leq$ 0.015 μg/mL as 0.015 μg/mL. The following standards were used to determine the eradication success relationships in terms of the AUC values: AUC = 1.0, perfect correlation; 0.9 < AUC < 1.0, excellent correlation; 0.8 < AUC $\leq$ 0.9, good correlation; 0.7 < AUC $\leq$ 0.8, fair correlation; 0.6 < AUC $\leq$ 0.7, poor correlation; 0.5 < AUC $\leq$ 0.6, failed correlation; and AUC $\leq$ 0.5, no correlation. Continuous variables were classified into categorical variables using the calculated cutoff values. Pearson's chi-square test was used to evaluate the relationships between the categorical variables (age, sex, height, weight, BMI, histories of smoking and alcohol consumption, regular PPI use prior to study participation, metronidazole/amoxicillin MICs, presence of Hp-related diseases) and eradication success. Statistical significance was determined as $p < 0.05$. EZR software (Jichi Medical University Saitama Medical Center, Saitama, Japan) was used for statistical analyses (Kanda 2013).

Results

Patients and Hp eradication success rate

Among the 335 patients who underwent first-line eradication therapy, the treatment failed in 39. Among them, four patients underwent second-line eradication therapy with regimens other than vonoprazan-based second-line therapy, while 35 patients underwent vonoprazan-based second-line therapy. Three patients received rabeprazole 10 mg twice per day with amoxicillin 750 mg twice per day and metronidazole 250 mg twice per day, administered for 7 days, due to the physicians' preference. One patient received vonoprazan 20 mg twice per day with clarithromycin 400 mg twice per day and metronidazole 250 mg twice per day, administered for 7 days, due to an allergy to amoxicillin. Among the 35 patients who underwent vonoprazan-based second-line therapy, 33 patients completed a UBT, whereas the remaining two patients did not. The 33 patients (age: 34-79 years; male/female: 22/11) who underwent vonoprazan-based second-line therapy and a UBT were selected for the present study. A flowchart of the patient selection process for the subgroup analysis is shown in Fig. 1. The vonoprazan-based second-line therapy success rate was 81.8% (95% confidence interval: 64.5-93.0%; 27/33).

ROC curves and cutoff values for patient physical factors

The AUC values of the ROC curves for continuous variables of each patient physical factor and eradication success were as follows: age, 0.537; height, 0.565; weight, 0.673; and BMI, 0.796. The AUC value of BMI indicated fair correlation. The ROC curve for BMI and eradication success rate are shown in Fig. 2. The cutoff values of the ROC curves for each patient physical factor and eradication success were as follows: age, 58 years; height, 157 cm; weight, 56 kg; and BMI, 23.8 kg/m^2.

Distribution of the metronidazole/amoxicillin MIC values and eradication success rate according to metronidazole/amoxicillin MIC values

The distribution of metronidazole MIC values was as follows: $\leq$ 4 μg/mL, 3.0% (1/33); 8 μg/mL, 36.4% (12/33); 16 μg/mL, 48.5% (16/33); and > 16 μg/mL, 12.1% (4/33). The distribution of amoxicillin MIC values was as follows: $\leq$ 0.015 μg/mL, 81.8% (27/33); 0.03 μg/mL, 12.1% (4/33); and 0.06 μg/mL, 6.1% (2/33).

The Hp eradication success rate according to metronidazole MIC value was as follows: MIC $\leq$ 4 μg/mL, 100%

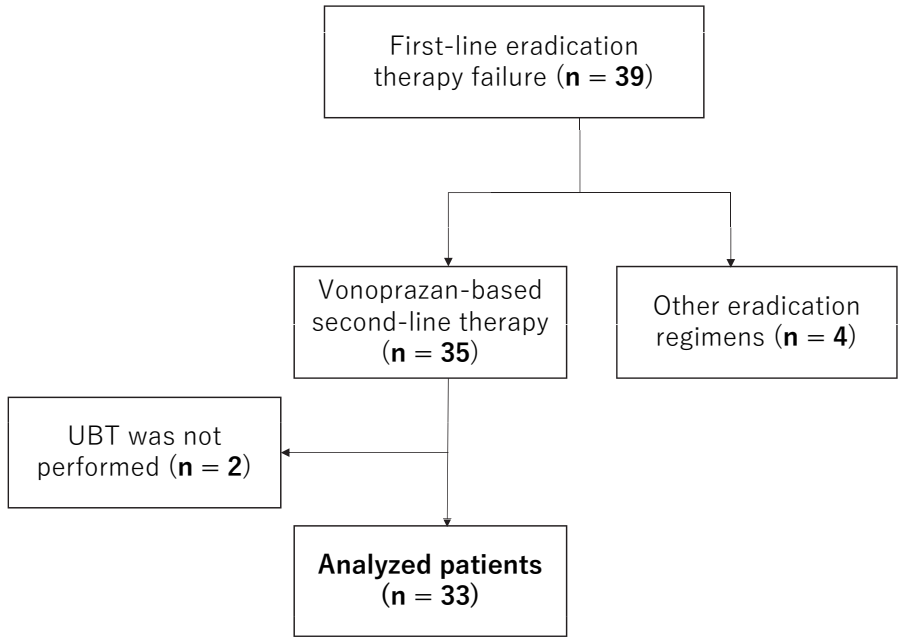

Fig. 1. Flowchart of the patient selection process.
First-line eradication therapy failed in a total of 39 patients. Of these, four patients underwent second-line eradication therapy other than vonoprazan-based second-line therapy, while the remaining 35 patients underwent vonoprazan-based second-line therapy. Of the 35 patients who underwent vonoprazan-based second-line therapy, 33 patients had completed a UBT, whereas the remaining two patients did not. The 33 patients who underwent vonoprazan-based second-line therapy and a UBT were selected for the present study.
UBT, urea breath test; vonoprazan-based second-line therapy, vonoprazan with amoxicillin and metronidazole therapy.

(1/1); MIC = 8 μg/mL, 83.3% (10/12); MIC = 16 μg/mL, 75.0% (12/16); and MIC > 16 μg/mL, 100% (4/4). The Hp eradication success rate according to amoxicillin MIC value was as follows: MIC ≤ 0.015 μg/mL, 81.5% (22/27); MIC = 0.03 μg/mL, 100% (4/4); and MIC = 0.06 μg/mL, 50.0% (1/2).

The AUC value of the ROC curve for the metronidazole value against eradication success was 0.506 (Fig. 3), indicating failed correlation. The AUC value of the ROC curve for the amoxicillin MIC value against eradication success was 0.497 (Fig. 4). Thus, there was no correlation between the amoxicillin MIC values and eradication success.

Relationship between patient factors and successful Hp eradication

Continuous variables were classified into categorical variables using the calculated cutoff values based on the ROC analyses (age, 58 years; height, 157 cm; weight, 56 kg; BMI, 23.8 kg/m^2; metronidazole MIC value, 32 μg/mL; amoxicillin MIC value, 0.03 μg/mL). Pearson's chi-square test was used to evaluate the relationships between each variable and eradication success. The results of these analyses are shown in Table 1.

The success rate of vonoprazan-based second-line therapy was greater in the high-BMI patient group (≥ 23.8 kg/m^2) than in the low-BMI patient group (< 23.8 kg/m^2; p = 0.007). The maximum BMI value was 28.8 kg/m^2 in the patients who achieved eradication success. There were no severe obesity patients (≥ 30 kg/m^2) in either eradication success or failure (Table 1). No correlations were observed between eradication success rates and other patient factors, including metronidazole/amoxicillin MICs.

Discussion

To the best of our knowledge, the present study is the first study to investigate patient factors related to the success of vonoprazan-based second-line therapy for Hp infection. Our findings indicated that, among the patient factors examined, only BMI was correlated with the success of vonoprazan-based second-line therapy. In addition, metronidazole/amoxicillin MIC values were not correlated with the success of vonoprazan-based second-line therapy.

Previous reports have mostly focused on comparing eradication success rates between vonoprazan-based second-line therapy and PPI-based second-line therapy. One of the latest meta-analyses has reported the superiority of vonoprazan-based second-line therapy in terms of eradication success rate relative to PPI-based second-line therapy (Shinozaki et al. 2020). Relative to PPIs, vonoprazan is associated with potent, immediate, and persistent inhibition of acid excretion regardless of the genetic polymorphisms of CYP2C19, potentially explaining increases in the eradication success rate (Suzuki et al. 2016; Kusano et al. 2018; Lyu et al. 2019). Patient factors such as age, sex, regular PPI use, and clarithromycin resistance have also been asso-

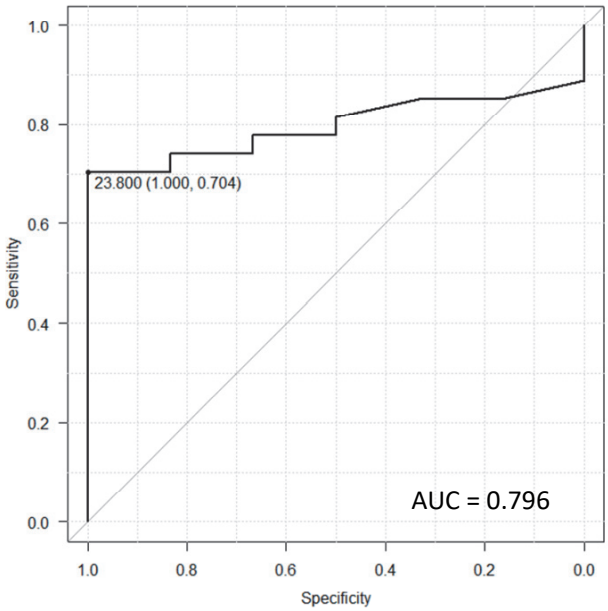

Fig. 2. ROC curve for body mass index.
The figure shows the ROC curve for BMI and the eradication success of vonoprazan-based second-line therapy. Data were analyzed for 33 patients who completed vonoprazan-based second-line therapy. The cutoff value of the ROC curve for BMI and eradication success was 23.8 kg/m^2. The AUC value for BMI and eradication success was 0.796, indicating fair correlation.
AUC, area under the curve; BMI, body mass index; ROC, receiver operating characteristic; vonoprazan-based second-line therapy, vonoprazan with amoxicillin and metronidazole therapy.

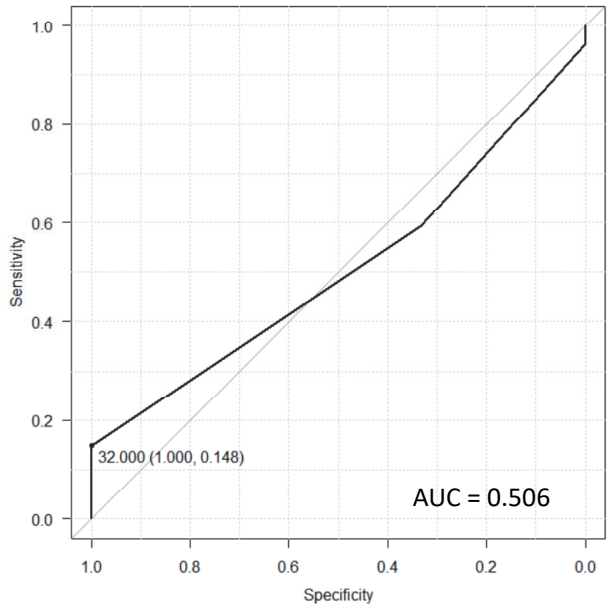

Fig. 3. ROC curve for metronidazole MIC values.
The figure shows the ROC curve for metronidazole MIC values and eradication success of vonoprazan-based second-line therapy. Data were analyzed for 33 patients who completed vonoprazan-based second-line therapy. The cutoff value of the ROC curve for metronidazole MIC values and eradication success was 32 μg/mL. The AUC value for metronidazole MIC values and eradication success was 0.506, indicating failed correlation.
AUC, area under the curve; MIC, minimal inhibitory concentration; ROC, receiver operating characteristic; vonoprazan-based second-line therapy, vonoprazan with amoxicillin and metronidazole therapy.

ciated with eradication success in individuals undergoing vonoprazan-based first-line therapy (Matsumoto et al. 2016; Shinozaki et al. 2018; Kusunoki et al. 2019). In the present study, the success rate of vonoprazan-based second-line therapy for Hp infection was higher in the high-BMI patient group than in the low-BMI patient group. The relationship between BMI and the success of Hp eradication is controversial for other eradication regimens. Some studies have also reported that higher BMI is correlated with a higher eradication success rate than low BMI (Singh et al. 2008; Costa et al. 2017), while other studies have reported the reverse (Abdullahi et al. 2008; Tan et al. 2018). These discrepancies may be explained by differences in eradication regimens and regions.

Previous studies on metronidazole pharmacokinetics have reported that intravenously or intraorally administered metronidazole is distributed in the muscle and fat tissues. In the muscle and fat, the increase in metronidazole concentration is gradual relative to that in the blood (Li and Qu 1992; Badia et al. 1995; Karjagin et al. 2005). The residence time of metronidazole in the body may have been higher in the high-BMI group, which may have in turn improved the eradication success rate. Thus, extending the duration of administration or increasing the metronidazole administration dose in the low-BMI patient group may improve the eradication success rate. However, further research is required to verify this hypothesis.

Our findings indicated that cigarette smoking and alcohol consumption were not related to the success of vonoprazan-based second-line therapy. Meta-analyses have reported that smoking decreases the eradication success rate of PPI-based therapy by stimulating gastric acid secretion and decreasing gastric blood flow and mucous secretion (Lanas and Hirschowitz 1992; Iwao et al. 1993; Suzuki et al. 2006). For this reason, the delivery of antibiotics to the gastric mucosa is reduced. Other studies have reported that alcohol consumption also decreases the eradication success rate of PPI-based therapy (Hsu et al. 2005) by stimulating gastric acid secretion (Tsukimi et al. 2001; Matsuno et al. 2002). In contrast, recent studies have reported that neither smoking nor alcohol consumption decreases the eradication success rate of vonoprazan-based first-line therapy (Sakurai et al. 2017; Takara et al. 2019), which can be explained by the more potent inhibition of acid excretion by vonoprazan than by PPI. In accordance with these findings, smoking and alcohol consumption did not decrease the eradication success rate of vonoprazan-based second-line therapy in the present study.

The metronidazole MIC value was not correlated with

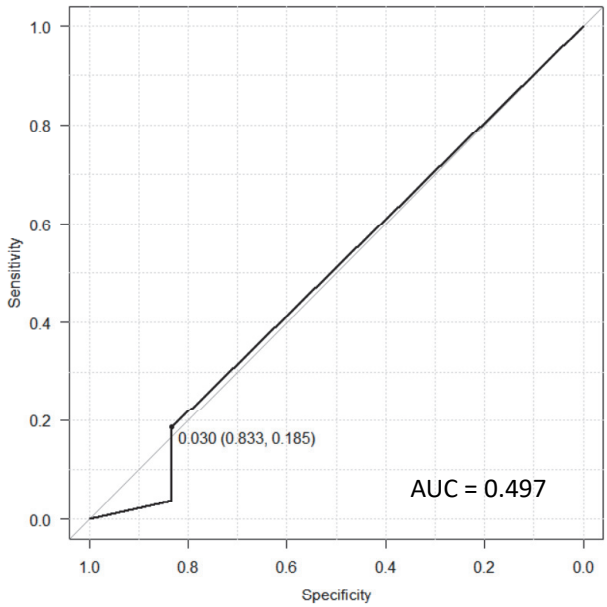

Fig. 4. ROC curve for amoxicillin MIC values.
The figure shows the ROC curve for amoxicillin MIC values and eradication success of vonoprazan-based second-line therapy. Data were analyzed for 33 patients who completed vonoprazan-based second-line therapy. The cutoff value of the ROC curve for amoxicillin MIC values and eradication success was 0.03 μg/mL. The AUC value for amoxicillin MIC values and eradication success was 0.497. There was no correlation between the amoxicillin MIC values and eradication success.
AUC, area under the curve; MIC, minimal inhibitory concentration; ROC, receiver operating characteristic; vonoprazan-based second-line therapy, vonoprazan with amoxicillin and metronidazole therapy.

the success of vonoprazan-based second-line therapy. Notably, the method used for antimicrobial susceptibility testing is important. The present study used the microbroth dilution method. The disk diffusion method, epsilometer test (E-test), and agar plate dilution method have also been used for antimicrobial susceptibility testing in other Hp studies (Hu et al. 2017). The microbroth dilution method is a standard method established by the Clinical and Laboratory Standards Institute and is considered the most accurate and precise method for antimicrobial susceptibility testing (Jorgensen and Ferraro 2009). The disk diffusion method is a qualitative method based on the microbroth dilution method and cannot measure MIC values directly. The E-test is a method developed in Sweden that was based on the agar plate dilution method (Baker et al. 1991). Similar to the microbroth dilution method, the agar plate dilution method is a fundamental antimicrobial susceptibility test. However, it is not used in everyday investigations due to its technical complexity. Compared with the agar plate dilution method, the microbroth dilution method is advantageous for the following reasons: (I) It conserves antimicrobial agents and culture media; (II) it is simple to perform; (III) the materials can endure long-term storage; and (IV) the process can be automated (Jorgensen and Ferraro 2009). The microbroth dilution method is currently the standard method of antimicrobial susceptibility testing. Previous studies that have investigated the correlations between metronidazole resistance and the success of Hp eradication therapy under various regimens utilizing metronidazole are summarized in Table 2 (Xia et al. 1994; Lopez-Brea et al. 1999; Nishizawa et al. 2007; Lee et al. 2019). EUCAST, The European Committee on Antimicrobial Susceptibility Testing (2020) determined that in vitro metronidazole resistance should be set at an MIC value of > 8 μg/mL. Based on this cutoff value, the metronidazole resistance rate was either equal to or above the clarithromycin resistance rate (Hu et al. 2017). The Maastricht V/Florence Consensus Report recommended that Hp eradication therapy regimens should be determined based on the results of antimicrobial susceptibility tests (Malfertheiner et al. 2017). As shown in Table 2, relative to the metronidazole-susceptible group, previous regimens without vonoprazan had a decreased eradication success rate in the metronidazole-resistant group. However, the metronidazole-resistant group had an eradication success rate equivalent to that of the metronidazole-susceptible group with vonoprazan-based second-line therapy. This is consistent with our finding that the metronidazole MIC value was not correlated with the success of vonoprazan-based second-line therapy.

The amoxicillin MIC value was also not correlated with the success of vonoprazan-based second-line therapy. The EUCAST determined that in vitro amoxicillin resistance should be set at an MIC value of > 0.125 μg/mL (The European Committee on Antimicrobial Susceptibility Testing 2020). Based on this cutoff value, amoxicillin resistance was not observed in the patients in the present study. A previous study demonstrated that the rate of amoxicillin resistance is low in Asian-Pacific populations (3%), consistent with our findings (Kuo et al. 2017). Amoxicillin resistance has been reported to influence eradication failure in regimens utilizing amoxicillin (Chen et al. 2017), and in vonoprazan-based first-line therapy (Murakami et al. 2016; Shinmura et al. 2019; Suzuki et al. 2020). No studies have examined the relationships between vonoprazan-based second-line therapy and amoxicillin resistance. In the present study, we observed no correlations between the amoxicillin MIC and the success of vonoprazan-based second-line therapy. However, the rate of amoxicillin resistance was low (6.1%, 2/33), even when the MIC value of ≥ 0.06 μg/mL was set as the resistance standard. It is difficult to conduct statistical analyses on amoxicillin resistance with this small sample size. In contrast, the reported rate of amoxicillin resistance in African populations is 38% (Savoldi et al. 2018). Further studies may wish to examine the relationships between vonoprazan-based second-line therapy and amoxicillin resistance by administering vonoprazan-based second-line therapy in populations with high amoxicillin resistance rates.

The present study has the following limitations: (I) This was a retrospective subgroup analysis of a primary

Table 1. Relationship between patient factors and the success or failure of vonoprazan-based second-line therapy for Hp eradication.

	Eradication success group (n = 27)	Eradication failure group (n = 6)	*p* value
Age (years, mean ± SD)	63.0 ± 11.1	62.1 ± 9.4	
(years, range)	34-79	49-73	
Age (≥ 58 years/< 58 years)	22/5	3/3	0.271
Height (cm, mean ± SD)	163.6 ± 10.3	161.0 ± 10.7	
(cm, range)	142-188	151-180	
Height (≥ 157 cm/< 157 cm)	21/6	2/4	0.099
Body weight (kg, mean ± SD)	65.4 ± 12.4	58.9 ± 9.4	
(kg, range)	37.8-90.0	47.0-76.0	
Body weight (≥ 56 kg/< 56 kg)	21/6	2/4	0.099
Body mass index (kg/m^2, mean ± SD)	24.3 ± 3.2	22.6 ± 0.9	
(kg/m^2, range)	16.1-28.8	20.6-23.5	
Body mass index (≥ 23.8 kg/m^2/< 23.8 kg/m^2)	19/8	0/6	0.007
Cigarette smoking (+/−)	5/22	3/3	0.271
Alcohol consumption (+/−)	5/22	1/5	1.000
Daily PPI use before the trial (+/−)	1/26	0/6	1.000
MIC value for metronidazole (≥ 32 μg/mL/< 32 μg/mL)	4/23	0/6	0.753
MIC value for amoxicillin (≥ 0.03 μg/mL/< 0.03 μg/mL)	5/22	1/5	1.000
Presence of *Helicobacter pylori*-related disease			
Total (+/−)	8/19	0/6	0.315
Gastric ulcer (+/−)	2/25	0/6	1.000
Duodenal ulcer (+/−)	5/22	0/6	0.607
ER for gastric neoplasia (+/−)	1/26	0/6	1.000

The chi-square test was used for categorical variables. The presence of *Helicobacter pylori*-related diseases was defined as having a history of gastric ulcers, duodenal ulcers, or ER for gastric neoplasia. The success rate of vonoprazan-based second-line therapy was greater in the high-BMI patient group (≥ 23.8 kg/m^2) than in the low-BMI patient group (< 23.8 kg/m^2; $p = 0.007$). No correlations were observed between eradication success and other patient factors, including the metronidazole/amoxicillin MICs.
BMI, body mass index; ER, endoscopic resection; Hp, *Helicobacter pylori*; MIC, minimal inhibitory concentration; PPI, proton pump inhibitor; SD, standard deviation; vonoprazan-based second-line therapy, vonoprazan with amoxicillin and metronidazole therapy.

Table 2. Studies that investigated the correlations between metronidazole resistance and the success of Hp eradication therapy.

Study	Sample size	AST	Definition of MNZ resistance	MNZ resistance rate	Eradication regimen	Eradication success (all)	Eradication success (MNZ resistance)
Xia et al. 1994	76	Disc diffusion method	ZD < 20 mm	25%	BI + MNZ + TET	82%	53%
Lopez-Brea et al. 1999	57	Agar dilution method	MIC > 8 μg/mL	14%	MNZ + AMO + BI	79%	50%
Nishizawa et al. 2007	107	Agar dilution method	MIC ≥ 8 μg/mL	3.7%	LPZ + AMO + MNZ	90%	50%
Lee et al. 2019	54	Agar dilution method	MIC > 32 μg/mL	19%	EPZ + BI + MNZ + TET	89%	60%
Present study	33	Microbroth dilution method	MIC > 8 μg/mL	61%	vonoprazan + MNZ + AMO	82%	80%

Eradication success rates for previous regimens without vonoprazan were lower in the metronidazole-resistant group than in the metronidazole-susceptible group. However, the metronidazole-resistant group had an eradication success rate equivalent to that of the metronidazole-susceptible group with vonoprazan-based second-line therapy.
AMO, amoxicillin; AST, antimicrobial susceptibility test; BI, bismuth; EPZ, esomeprazole; Hp, *Helicobacter pylori;* LPZ, lansoprazole; MIC, minimal inhibitory concentration; MNZ, metronidazole; TET, tetracycline; vonoprazan-based second-line therapy, vonoprazan with amoxicillin and metronidazole therapy; ZD, zone diameter.

study; (II) this was an analysis with a small sample size; (III) the subjects were all Japanese; and (IV) there was potential selection bias for second-line eradication therapy regimens. Finally, we should be aware of the effect of obesity on Hp eradication success. As there were no patients with severe obesity in the present study, the impact of higher BMI on Hp eradication success does not mean that obesity has a beneficial effect for vonoprazan-based second-line therapy.

In conclusion, the present study has demonstrated that, among patient factors examined, only BMI is correlated with the success of vonoprazan-based second-line therapy. Furthermore, metronidazole/amoxicillin MIC values were not correlated with the success of vonoprazan-based second-line therapy. Further studies involving larger sample sizes are required to verify our findings.

Acknowledgments

This study was funded by Nihon University School of Medicine.

Conflict of Interest

The authors declare no conflict of interest. On the other hand, Takuji Gotoda received an honorarium from Takeda Pharmaceutical Company Limited, the manufacturer of vonoprazan; however, Takeda Pharmaceutical Company Limited did not influence the data analysis and was not involved in this study.

References

Abdullahi, M., Annibale, B., Capoccia, D., Tari, R., Lahner, E., Osborn, J., Leonetti, F. & Severi, C. (2008) The eradication of Helicobacter pylori is affected by body mass index (BMI). *Obes. Surg.*, **18**, 1450-1454.

Andersson, K. & Carlsson, E. (2005) Potassium-competitive acid blockade: a new therapeutic strategy in acid-related diseases. *Pharmacol. Ther.*, **108**, 294-307.

Asaka, M., Kato, M., Kudo, M., Katagiri, M., Nishikawa, K., Koshiyama, H., Takeda, H., Yoshida, J. & Graham, D.Y. (1996) Atrophic changes of gastric mucosa are caused by Helicobacter pylori infection rather than aging: studies in asymptomatic Japanese adults. *Helicobacter*, **1**, 52-56.

Badia, J.M., de la Torre, R., Farré, M., Gaya, R., Martínez-Ródenas, F., Sancho, J.J. & Sitges-Serra, A. (1995) Inadequate levels of metronidazole in subcutaneous fat after standard prophylaxis. *Br. J. Surg.*, **82**, 479-482.

Baker, C.N., Stocker, S.A., Culver, D.H. & Thornsberry, C. (1991) Comparison of the E Test to agar dilution, broth microdilution, and agar diffusion susceptibility testing techniques by using a special challenge set of bacteria. *J. Clin. Microbiol.*, **29**, 533-538.

Chen, M.J., Wu, M.S., Chen, C.C., Chen, C.C., Fang, Y.J., Bair, M.J., Chang, C.Y., Lee, J.Y., Hsu, W.F., Luo, J.C., Lin, J.T. & Liou, J.M.; Taiwan Gastrointestinal Disease and Helicobacter Consortium (2017) Impact of amoxicillin resistance on the efficacy of amoxicillin-containing regimens for Helicobacter pylori eradication: analysis of five randomized trials. *J. Antimicrob. Chemother.*, **72**, 3481-3489.

Costa, S., Soares, J.B. & Gonçalves, R. (2017) Efficacy and tolerability of culture-guided treatment for Helicobacter pylori infection. *Eur. J. Gastroenterol. Hepatol.*, **29**, 1258-1263.

Graham, D.Y. & Fischbach, L. (2010) Helicobacter pylori treatment in the era of increasing antibiotic resistance. *Gut*, **59**, 1143-1153.

Hori, Y., Imanishi, A., Matsukawa, J., Tsukimi, Y., Nishida, H., Arikawa, Y., Hirase, K., Kajino, M. & Inatomi, N. (2010) 1-[5-(2-Fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamin e monofumarate (TAK-438), a novel and potent potassium-competitive acid blocker for the treatment of acid-related diseases. *J. Pharmacol. Exp. Ther.*, **335**, 231-238.

Hori, Y., Matsukawa, J., Takeuchi, T., Nishida, H., Kajino, M. & Inatomi, N. (2011) A study comparing the antisecretory effect of TAK-438, a novel potassium-competitive acid blocker, with lansoprazole in animals. *J. Pharmacol. Exp. Ther.*, **337**, 797-804.

Hsu, P.I., Lai, K.H., Lin, C.K., Chen, W.C., Yu, H.C., Cheng, J.S., Tsay, F.W., Wu, C.J., Lo, C.C., Tseng, H.H., Yamaoka, Y., Chen, J.L. & Lo, G.H. (2005) A prospective randomized trial of esomeprazole- versus pantoprazole-based triple therapy for Helicobacter pylori eradication. *Am. J. Gastroenterol.*, **100**, 2387-2392.

Hu, Y., Zhu, Y. & Lu, N.H. (2017) Novel and effective therapeutic regimens for Helicobacter pylori in an era of increasing antibiotic resistance. *Front. Cell. Infect. Microbiol.*, **7**, 168.

Iwao, T., Toyonaga, A., Ikegami, M., Oho, K., Sumino, M., Sakaki, M., Shigemori, H., Harada, H., Sasaki, E. & Tanikawa, K. (1993) Gastric mucosal blood flow after smoking in healthy human beings assessed by laser Doppler flowmetry. *Gastrointest. Endosc.*, **39**, 400-403.

Jorgensen, J.H. & Ferraro, M.J. (2009) Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin. Infect. Dis.*, **49**, 1749-1755.

Jung, Y.S., Kim, E.H. & Park, C.H. (2017) Systematic review with meta-analysis: the efficacy of vonoprazan-based triple therapy on Helicobacter pylori eradication. *Aliment. Pharmacol. Ther.*, **46**, 106-114.

Kanda, Y. (2013) Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant.*, **48**, 452-458.

Karjagin, J., Pähkla, R., Karki, T. & Starkopf, J. (2005) Distribution of metronidazole in muscle tissue of patients with septic shock and its efficacy against Bacteroides fragilis in vitro. *J. Antimicrob. Chemother.*, **55**, 341-346.

Kato, M., Ota, H., Okuda, M., Kikuchi, S., Satoh, K., Shimoyama, T., Suzuki, H., Handa, O., Furuta, T., Mabe, K., Murakami, K., Sugiyama, T., Uemura, N. & Takahashi, S. (2019) Guidelines for the management of Helicobacter pylori infection in Japan: 2016 revised edition. *Helicobacter*, **24**, e12597.

Kuo, Y.T., Liou, J.M., El-Omar, E.M., Wu, J.Y., Leow, A.H.R., Goh, K.L., Das, R., Lu, H., Lin, J.T., Tu, Y.K., Yamaoka, Y. & Wu, M.S.; Asian Pacific Alliance on Helicobacter and Microbiota (2017) Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. *Lancet Gastroenterol. Hepatol.*, **2**, 707-715.

Kusano, C., Gotoda, T., Suzuki, S., Ikehara, H. & Moriyama, M. (2018) Safety of first-line triple therapy with a potassium-competitive acid blocker for Helicobacter pylori eradication in children. *J. Gastroenterol.*, **53**, 718-724.

Kusunoki, M., Yuki, M., Ishitobi, H., Kobayashi, Y., Nagaoka, M., Takahashi, Y., Fukuba, N., Komazawa, Y., Shizuku, T. & Kinoshita, Y. (2019) Effect of age on effectiveness of vonoprazan in triple therapy for Helicobacter pylori eradication. *Intern. Med.*, **58**, 1549-1555.

Lanas, A. & Hirschowitz, B.I. (1992) Influence of smoking on basal and on vagally and maximally stimulated gastric acid and pepsin secretion. *Scand. J. Gastroenterol.*, **27**, 208-212.

Lee, J.W., Kim, N., Nam, R.H., Lee, S.M., Soo In, C., Kim, J.M. & Lee, D.H. (2019) Risk factors of rescue bismuth quadruple therapy failure for Helicobacter pylori eradication. *J. Gastroenterol. Hepatol.*, **34**, 666-672.

Lee, Y.C., Chiang, T.H., Chou, C.K., Tu, Y.K., Liao, W.C., Wu, M.S. & Graham, D.Y. (2016) Association between Helicobacter pylori eradication and gastric cancer incidence: a systematic review and meta-analysis. *Gastroenterology*, **150**, 1113-1124. e1115.

Leodolter, A., Kulig, M., Brasch, H., Meyer-Sabellek, W., Willich, S.N. & Malfertheiner, P. (2001) A meta-analysis comparing eradication, healing and relapse rates in patients with Helicobacter pylori-associated gastric or duodenal ulcer. *Aliment. Pharmacol. Ther.*, **15**, 1949-1958.

Li, T. & Qu, S. (1992) A comparison of metronidazole distribution following intravenous metronidazole and metronidazole phosphate disodium in mice. *Drug Metabol. Drug Interact.*, **10**, 229-236.

Lopez-Brea, M., Martinez, M.J., Domingo, D., Sanchez, I. & Alarcon, T. (1999) Metronidazole resistance and virulence factors in Helicobacter pylori as markers for treatment failure in a paediatric population. *FEMS Immunol. Med. Microbiol.*, **24**, 183-188.

Lyu, Q.J., Pu, Q.H., Zhong, X.F. & Zhang, J. (2019) Efficacy and safety of vonoprazan-based versus proton pump inhibitor-based triple therapy for Helicobacter pylori eradication: a meta-analysis of randomized clinical trials. *Biomed Res. Int.*, **2019**, 9781212.

Malfertheiner, P., Megraud, F., O'Morain, C.A., Gisbert, J.P., Kuipers, E.J., Axon, A.T., Bazzoli, F., Gasbarrini, A., Atherton, J., Graham, D.Y., Hunt, R., Moayyedi, P., Rokkas, T., Rugge, M., Selgrad, M., et al. (2017) Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut*, **66**, 6-30.

Marcus, E.A., Inatomi, N., Nagami, G.T., Sachs, G. & Scott, D.R. (2012) The effects of varying acidity on Helicobacter pylori growth and the bactericidal efficacy of ampicillin. *Aliment. Pharmacol. Ther.*, **36**, 972-979.

Matsumoto, H., Shiotani, A., Katsumata, R., Fujita, M., Nakato, R., Murao, T., Ishii, M., Kamada, T., Haruma, K. & Graham, D.Y. (2016) Helicobacter pylori eradication with proton pump inhibitors or potassium-competitive acid blockers: the effect of clarithromycin resistance. *Dig. Dis. Sci.*, **61**, 3215-3220.

Matsuno, K., Tomita, K. & Okabe, S. (2002) Wine stimulates gastric acid secretion in isolated rabbit gastric glands via two different pathways. *Aliment. Pharmacol. Ther.*, **16** Suppl 2, 107-114.

Murakami, K., Sakurai, Y., Shiino, M., Funao, N., Nishimura, A. & Asaka, M. (2016) Vonoprazan, a novel potassium-competitive acid blocker, as a component of first-line and second-line triple therapy for Helicobacter pylori eradication: a phase III, randomised, double-blind study. *Gut*, **65**, 1439-1446.

Nishizawa, T., Maekawa, T., Watanabe, N., Harada, N., Hosoda, Y., Yoshinaga, M., Yoshio, T., Ohta, H., Inoue, S., Toyokawa, T., Yamashita, H., Saito, H., Kuwai, T., Katayama, S., Masuda, E., et al. (2015) Clarithromycin versus metronidazole as first-line Helicobacter pylori eradication: a multicenter, prospective, randomized controlled study in Japan. *J. Clin. Gastroenterol.*, **49**, 468-471.

Nishizawa, T., Suzuki, H., Masaoka, T., Iwasaki, E. & Hibi, T. (2007) A new eradication resistance index as a predictor of metronidazole-containing second-line treatment of Helicobacter pylori. *Digestion*, **76**, 215-220.

Sakurai, K., Suda, H., Ido, Y., Takeichi, T., Okuda, A., Hasuda, K. & Hattori, M. (2017) Comparative study: vonoprazan and proton pump inhibitors in Helicobacter pylori eradication therapy. *World J. Gastroenterol.*, **23**, 668-675.

Savoldi, A., Carrara, E., Graham, D.Y., Conti, M. & Tacconelli, E. (2018) Prevalence of antibiotic resistance in Helicobacter pylori: a systematic review and meta-analysis in World Health Organization regions. *Gastroenterology*, **155**, 1372-1382. e1317.

Shin, J.M., Inatomi, N., Munson, K., Strugatsky, D., Tokhtaeva, E., Vagin, O. & Sachs, G. (2011) Characterization of a novel potassium-competitive acid blocker of the gastric H,K-ATPase, 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamin e monofumarate (TAK-438). *J. Pharmacol. Exp. Ther.*, **339**, 412-420.

Shinmura, T., Adachi, K., Yamaguchi, Y., Izawa, S., Hijikata, Y., Ebi, M., Funaki, Y., Ogasawara, N., Sasaki, M. & Kasugai, K. (2019) Vonoprazan-based triple-therapy could improve efficacy of the tailored therapy of Helicobacter pylori infection. *J. Gastrointestin. Liver Dis.*, **28**, 389-395.

Shinozaki, S., Kobayashi, Y., Osawa, H., Sakamoto, H., Hayashi, Y., Lefor, A.K. & Yamamoto, H. (2020) Effectiveness and safety of vonoprazan versus proton pump inhibitors for second-line helicobacter pylori eradication therapy: systematic review and meta-analysis. *Digestion*, 1-7.

Shinozaki, S., Osawa, H., Sakamoto, H., Hayashi, Y., Kobayashi, Y., Miura, Y., Lefor, A.K. & Yamamoto, H. (2018) Pre-treatment with proton pump inhibitors decreases the success of primary Helicobacter pylori eradication using a vonoprazan-based regimen. *Kaohsiung J. Med. Sci.*, **34**, 456-460.

Singh, N., Deb, R., Kashyap, P.C., Bhatia, V., Ahuja, V. & Sharma, M.P. (2008) Body mass index and per capita income influence duodenal ulcer healing and H. pylori eradication whilst dietary factors play no part. *Trop. Gastroenterol.*, **29**, 26-31.

Suzuki, S., Gotoda, T., Kusano, C., Ikehara, H., Ichijima, R., Ohyauchi, M., Ito, H., Kawamura, M., Ogata, Y., Ohtaka, M., Nakahara, M. & Kawabe, K. (2020) Seven-day vonoprazan and low-dose amoxicillin dual therapy as first-line Helicobacter pylori treatment: a multicentre randomised trial in Japan. *Gut*, **69**, 1019-1026.

Suzuki, S., Gotoda, T., Kusano, C., Iwatsuka, K. & Moriyama, M. (2016) The efficacy and tolerability of a triple therapy containing a potassium-competitive acid blocker compared with a 7-day PPI-based low-dose clarithromycin triple therapy. *Am. J. Gastroenterol.*, **111**, 949-956.

Suzuki, T., Matsuo, K., Ito, H., Sawaki, A., Hirose, K., Wakai, K., Sato, S., Nakamura, T., Yamao, K., Ueda, R. & Tajima, K. (2006) Smoking increases the treatment failure for Helicobacter pylori eradication. *Am. J. Med.*, **119**, 217-224.

Takara, Y., Endo, H., Nakano, R., Kawachi, K., Hidaka, H., Matsunaga, T., Tsuruoka, N., Sakata, Y., Shimoda, R., Hara, M. & Fujimoto, K. (2019) Smoking and drinking did not increase the failure of therapeutic Helicobacter pylori eradication by vonoprazan, clarithromycin, and amoxicillin. *Digestion*, **99**, 172-178.

Tan, B., Yang, J.C., Young, C.L., Bishu, S., Owyang, S.Y., El-Zaatari, M., Zhang, M., Grasberger, H., Qian, J.M. & Kao, J.Y. (2018) Helicobacter pylori antimicrobial susceptibility testing-guided salvage therapy in the USA: a real life experience. *Dig. Dis. Sci.*, **63**, 437-445.

The European Committee on Antimicrobial Susceptibility Testing (2020) Clinical breakpoints: bacteria. https://www.eucast.org/clinical_breakpoints/ [*Accessed*: September 26, 2020].

Thung, I., Aramin, H., Vavinskaya, V., Gupta, S., Park, J.Y., Crowe, S.E. & Valasek, M.A. (2016) Review article: the global emergence of Helicobacter pylori antibiotic resistance. *Aliment. Pharmacol. Ther.*, **43**, 514-533.

Tsukimi, Y., Ogawa, T. & Okabe, S. (2001) Pharmacological analysis of wine-stimulated gastric acid secretion in dogs. *J. Physiol. Paris*, **95**, 221-228.

Uemura, N., Okamoto, S., Yamamoto, S., Matsumura, N., Yamaguchi, S., Yamakido, M., Taniyama, K., Sasaki, N. & Schlemper, R.J. (2001) Helicobacter pylori infection and the development of gastric cancer. *N. Engl. J. Med.*, **345**, 784-789.

Villoria, A., Garcia, P., Calvet, X., Gisbert, J.P. & Vergara, M. (2008) Meta-analysis: high-dose proton pump inhibitors vs. standard dose in triple therapy for Helicobacter pylori eradica-

tion. *Aliment. Pharmacol. Ther.*, **28**, 868-877.
Warren, J.R. & Marshall, B. (1983) Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet*, **1**, 1273-1275.
Xia, H., Keane, C.T., Beattie, S. & O'Morain, C.A. (1994) Standardization of disk diffusion test and its clinical significance for susceptibility testing of metronidazole against Helicobacter pylori. *Antimicrob. Agents Chemother.*, **38**, 2357-2361.