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

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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
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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

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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 ≤ 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; $0.9 < \text{AUC} < 1.0$, excellent correlation; $0.8 < \text{AUC} \leq 0.9$, good correlation; $0.7 < \text{AUC} \leq 0.8$, fair correlation; $0.6 < \text{AUC} \leq 0.7$, poor correlation; $0.5 < \text{AUC} \leq 0.6$, 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: ≤ 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 ≤ 4 μg/mL, 100%

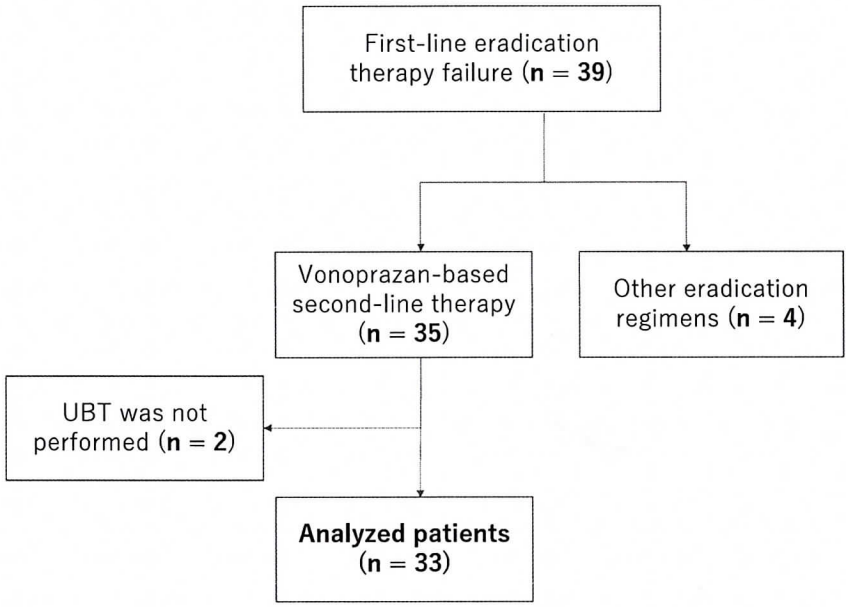

Fig. 1. Flowchart of the patient selection process.
First-line eradication therapy failed in a total of 39 patients. Of these, four patients underwent second-line eradication therapy other than vonoprazan-based second-line therapy, while the remaining 35 patients underwent vonoprazan-based second-line 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-

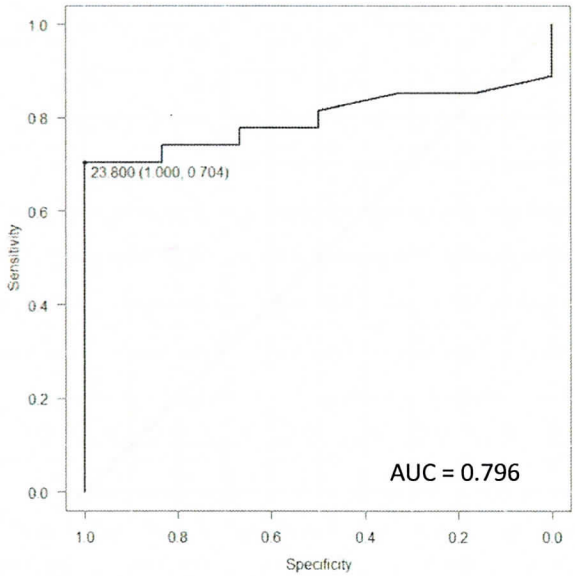

Fig. 2. ROC curve for body mass index.
The figure shows the ROC curve for BMI and the eradication success of vonoprazan-based second-line therapy. Data were analyzed for 33 patients who completed vonoprazan-based second-line therapy. The cutoff value of the ROC curve for BMI and eradication success was 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.

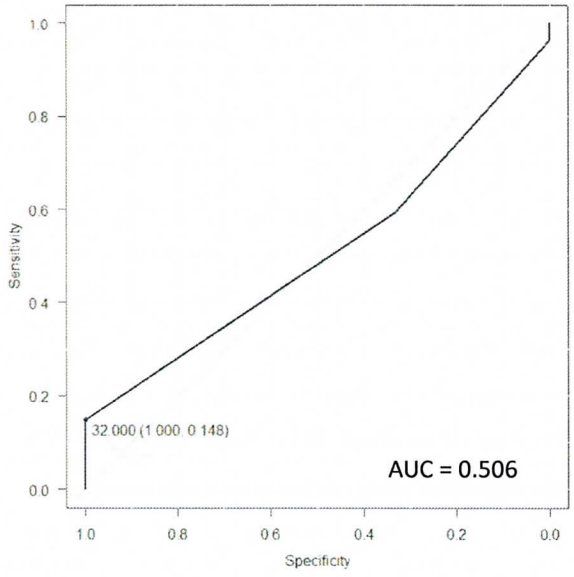

Fig. 3. ROC curve for metronidazole MIC values.
The figure shows the ROC curve for metronidazole MIC values and eradication success of vonoprazan-based second-line therapy. Data were analyzed for 33 patients who completed vonoprazan-based second-line therapy. The cutoff value of the ROC curve for metronidazole 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

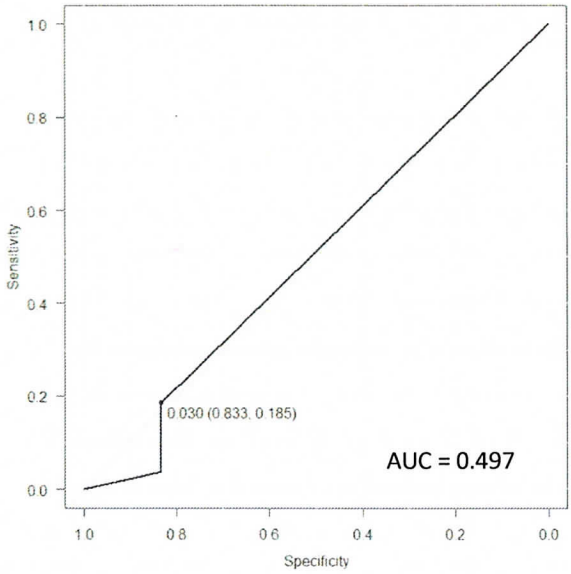

Fig. 4. ROC curve for amoxicillin MIC values.
The figure shows the ROC curve for amoxicillin MIC values and eradication success of vonoprazan-based second-line therapy. Data were analyzed for 33 patients who completed vonoprazan-based second-line therapy. The cutoff value of the ROC curve for amoxicillin 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.

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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.

Body Mass Index 高値は *Helicobacter pylori* 感染症に対する vonoprazan 二次除菌療法の成功と関連する

日本大学大学院医学研究科博士課程

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タイトル：

Body Mass Index 高値は *Helicobacter pylori* 感染症に対する vonoprazan 二次除菌療法の成功と関連する

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ア）概要：

Helicobacter pylori(Hp)の除菌は消化性潰瘍再発や胃癌発症の予防のために必要である。Potassium-competitive acid blocker：vonoprazan は胃酸分泌抑制薬であり、即効性および持続性のある高い酸分泌抑制効果により、Hp 除菌成功率の向上に寄与する。日本においては、一次除菌療法として vonoprazan, amoxicillin, clarithromycin 併用療法が、二次除菌療法として vonoprazan, amoxicillin, metronidazole 併用療法が使用可能である。しかし vonoprazan 一次除菌療法と異なり、vonoprazan 二次除菌療法の成否と関連する患者因子について検討した報告はない。本研究では vonoprazan 二次除菌療法の成否と関連する患者因子について検討した。Vonoprazan 二次除菌療法の成否と metronidazole, amoxicillin の最小発育阻止濃度値(minimal inhibitory concentration: MIC 値)を含めた患者因子の関連について解析した。MIC 値は個々の患者から分離培養された Hp を用いて測定した。連続変数と除菌成否の関連については Receiver operating characteristic(ROC)解析を用いて評価した。本研究では日本の 7 施設で 2018 年 10 月から 2019 年 6 月までの期間で、vonoprazan 一次除菌療法が不成功であり、vonoprazan 二次除菌療法を受けた 33 名を解析対象とした(年齢: 34～79 歳、男性 22 名/女性 11 名、body mass index(BMI): 16.1～28.8 kg/m^2)。除菌成功率は 81.8%だった(27/33)。BMI の ROC 解析では area under the curve 値は 0.796 であり、カットオフ値は 23.8kg/m^2 だった。BMI 高値群は BMI 低値群より除菌成功率が高かった(p 値 = 0.007)。本研究では BMI 高値が vonoprazan 二次除菌療法の成功と関連することが示された。

イ）緒言：

Helicobacter pylori(Hp)はWarrenとMarshallによって発見されたグラム陰性らせん状桿菌である[1)]。Hp感染症は胃炎、消化性潰瘍、胃癌、MALTリンパ腫を引き起こす慢性感染症として知られている[2~4)]。消化性潰瘍再発や胃癌発症の予防のため、Hp感染症に対してHp除菌療法が推奨されている[5~6)]。Hp除菌療法のレジメンは胃酸分泌抑制薬と抗菌薬で構成されており、90%以上の除菌成功率を有する除菌レジメンの使用が推奨されている[7)]。

全世界で様々な除菌レジメンにより、Hp除菌療法が行われている[8)]。海外では主なHp除菌療法として、3剤療法、ビスマス併用4剤療法、4剤コンコミタント療法、4剤シーケンシャル療法等が用いられる[9)]。日本ではproton pump inhibitors(PPIs), amoxicillin, clarithromycin併用療法(PPI一次除菌療法)が一次除菌療法として用いられる。しかし、抗菌薬耐性菌の増加により、Hp除菌成功率の低下が大きな問題となっている[8,10)]。最近の研究では、clarithromycin耐性菌の増加により、日本においてPPI一次除菌療法の除菌成功率が90%台から70%台まで低下したと報告されている[11,12)]。新規の胃酸分泌抑制薬であるpotassium-competitive acid blocker：vonoprazanはこの問題を解決する可能性がある。

PPIと同様に、vonoprazanは胃酸分泌経路の最終段階を触媒する酵素hydrogen/potassium-ATPaseを阻害する。PPIと異なる点は、vonoprazanはpotassiumと競合して可逆的にhydrogen/potassium-ATPaseを阻害することである[13)]。Vonoprazanは胃粘膜に対する高い蓄積性と遅いクリアランスにより、強力で持続的な酸分泌抑制作用を発揮する[14~16)]。

十分な胃酸分泌抑制は、Hp除菌療法の成功率に影響することが知られている。過去の研究では、胃酸分泌抑制により、Hpの抗菌薬に対する感受性が増加することや、胃粘膜での抗菌薬濃度が上昇することが報告されており、その結果として除菌成功率が向上する[17,18)]。PPIと比較して、vonoprazanを用いた除菌療法では即効性および持続性のある強力な酸分泌抑制効果により、除菌成功率が高いことが報告されている[19~21)]。Vonoprazan, amoxicillin, clarithromycin併用療法(vonoprazan一次除菌療法)は88%の除菌成功率を有し、PPI一次除菌療法より優れていることが報告されている[22)]。一次除菌療法が不成功だった患者には二次除菌療法が推奨されている。日本では従来PPI, amoxicillin, metronidazole併用療法(PPI二次除菌療法)が二次除菌療法として用いられていたが、PPIはvonoprazanに置き変わりつつある。最近の研究ではvonoprazan, amoxicillin, metronidazole併用療法(vonoprazan二次除菌療法)は90%の除菌成功率を有し、PPI二次除菌療法より優れていることが報告されている[23)]。以上の知見を踏まえ、日本のガイドラインではvonoprazanを用いた除菌療法が推奨されている[24)]。しかし、vonoprazanを用いた除菌療法を行っても、除菌不成功となる患者は存在する。

過去の研究でvonoprazan一次除菌療法の成否と関連する患者因子については報告がなされている。しかし、調べた限りにおいて、vonoprazan二次除菌療法の成否と関連する患者因子についての報告はない。従来のmetronidazoleやamoxicillinを用いた除菌療法では、

metronidazole 耐性群および amoxicillin 耐性群において、除菌成功率が低下することが報告されている [25~29]。しかし、vonoprazan 二次除菌療法において、metronidazole 耐性や amoxicillin 耐性と除菌成功率との関連についてはまだ評価されていない。本研究の目的は metronidazole と amoxicillin の薬剤感受性を含めた患者因子と vonoprazan 二次除菌療法の成否の関連について検討することである。

ウ）対象と方法：

患者：

本研究は「Seven-day vonoprazan and low-dose amoxicillin dual therapy as first-line *Helicobacter pylori* treatment: a multicenter randomized trial in Japan(主解析研究)」の副次解析研究である [30]。主解析研究は UMIN(University Hospital Medical Information Network)に 2018 年 9 月 14 日に登録されている(臨床試験登録番号 UMIN000034140)。副次解析研究は日本大学病院臨床研究審査委員会で 2020 年 10 月 8 日に承認されている(臨床研究審査委員会承認番号 20201003)。

主解析研究は、日本の 7 施設で 2018 年 10 月から 2019 年 6 月の期間に 20 歳～79 歳で Hp 培養検査を受けた 629 名のうち、Hp 培養陽性の 335 名に対し一次除菌療法として 2 剤療法(vonoprazan 20mg×2 回/日＋amoxicillin 750mg×2 回/日を 7 日間投与)または 3 剤療法(vonoprazan 20mg×2 回/日＋amoxicillin 750mg×2 回/日＋clarithromycin 200mg×2 回/日を 7 日間投与)を行い、2 群間の除菌成功率を比較した、多施設共同ランダム化比較試験である。除外基準は薬剤のアレルギーあり、胃切除歴あり、妊娠または授乳中、PPI・抗菌薬・ステロイドを常用しており中止不可である。335 名のうち 168 名が 2 剤療法群に、167 名が 3 剤療法群に割り付けられた。主解析研究のフローチャートを Fig. 1 に示した。2 剤療法群では 5 名が、3 剤療法群では 3 名が有害事象またはその他の理由で per-protocol(PP)解析から脱落した。Intent-to-treat 解析では 2 剤療法群の除菌成功率は 84.5%、3 剤療法群の除菌成功率は 89.2%で、除菌成功率の差の 95%信頼区間は-11.8%～2.4%だった。PP 解析では、2 剤療法群と 3 剤療法群の除菌成功率はそれぞれ 87.1%と 90.2%であり、除菌成功率の差の 95%信頼区間は-9.9%～3.7%となり事前に設定した非劣性マージン内だった [30]。

本研究では、上記の一次除菌療法を受けた患者のうち、一次除菌不成功で vonoprazan 二次除菌療法(vonoprazan 20mg×2 回/日＋amoxicillin 750mg×2 回/日＋metronidazole 250mg×2 回/日を 7 日間投与)を受けた患者を対象とした。Vonoprazan 二次除菌療法以外のレジメンで二次除菌療法を受けた患者は本研究から除外した。全ての患者から主解析研究参加の同意を書面により取得している。全ての患者データは、主解析研究のデータベースから後方視的に取得した。

除菌成否の定義：

一次除菌不成功の判定基準は、一次除菌から 4 週間以降に尿素呼気試験(urea breath

test: UBT)を受け、UBT 値 2.5‰以上とした。二次除菌成功の判定基準は、二次除菌から 4 週間以降に UBT を受け、UBT 値 2.5‰未満とした。UBT については「ユービット(大塚製薬、東京、日本)」を用いた。

薬剤感受性試験:

全ての適格患者は、胃粘膜組織培養検査で Hp 感染が確認され、同時に薬剤感受性試験が行われた。薬剤感受性試験は、「薬剤感受性キット:ドライプレート栄研(栄研化学株式会社、東京、日本)」を用いて微量液体希釈法により行った。最小発育阻止濃度値(minimal inhibitory concentration: MIC)は一定量の Hp に対し抗菌薬を作用させながら培養を行い、その培養液に目視で確認できる混濁が見られない最小の抗菌薬濃度と定義した。Hp の metronidazole および amoxicillin の MIC 値を、metronidazole は 4～16μg/mL の範囲で、amoxicillin は 0.015～1μg/mL の範囲で測定した。

評価項目と統計解析:

本研究の評価項目は、年齢、性別、身長、体重、body mass index(BMI)、喫煙の有無、飲酒の有無、研究参加前の PPI 常用の有無、metronidazole および amoxicillin の MIC 値、Hp 関連疾患の有無と vonoprazan 二次除菌療法の成否との関連である。Hp は消化性潰瘍および胃癌を引き起こす[2~4]。Hp 関連疾患有りの定義は、胃潰瘍、十二指腸潰瘍、胃腫瘍の内視鏡切除の既往があることとした。これらの患者因子は、Hp 除菌成否との関連が過去の研究で報告されている[25~29,31~39]。連続変数(年齢、身長、体重、BMI、metronidazole および amoxicillin の MIC 値)と除菌成否の関連の評価については、receiver operating characteristic(ROC)解析を用いた。各連続変数と除菌成否に関する ROC 曲線を作成し、曲線下面積:area under the curve(AUC)およびカットオフ値を算出した。カットオフ値は感度と特異度の和が最大になる閾値とした。Metronidazole の MIC 値≤4μg/mL は 4μg/mL、>16μg/mL は 32μg/mL として扱い ROC 解析を行った。Amoxicillin の MIC 値≤0.015μg/mL は 0.015μg/mL として扱い ROC 解析を行った。AUC の値について、下記の基準を用いて除菌成否との関連について判定した。AUC=1.0: perfect な関連、0.9<AUC<1.0: excellent な関連、0.8<AUC≤0.9: good な関連、0.7<AUC≤0.8: fair な関連、0.6<AUC≤0.7: poor な関連、0.5<AUC≤0.6: failed な関連、AUC≤0.5: 関連なし。算出されたカットオフ値で各連続変数をカテゴリー変数に分類した。全てのカテゴリー変数(年齢、性別、身長、体重、BMI、喫煙の有無、飲酒の有無、研究参加前の PPI 常用の有無、metronidazole および amoxicillin の MIC 値、Hp 関連疾患の有無)と除菌成否の関連について、ピアソンのカイ二乗検定を用いて評価した。p 値<0.05 で統計学的有意差ありと判定した。統計解析については「統計解析ソフト:EZR(自治医科大学付属さいたま医療センター、埼玉、日本)」を用いた[40]。

エ) 結果:

患者と除菌成功率:

一次除菌療法を受けた 335 名のうち 39 名が一次除菌不成功だった。一次除菌不成功だっ

た 39 名のうち 4 名は vonoprazan 二次除菌療法以外のレジメンで二次除菌療法を受け、35 名が vonoprazan 二次除菌療法を受けた。3 名の患者が医師の判断により、rabeprazole 10mg ×2 回/日＋amoxicillin 750mg×2 回/日＋metronidazole 250mg×2 回/日を 7 日間投与による二次除菌療法を受けた。1 名の患者が amoxicillin アレルギーのため、vonoprazan 20mg ×2 回/日＋clarithromycin 400mg×2 回/日＋metronidazole 250mg×2 回/日を 7 日間投与による二次除菌療法を受けた。Vonoprazan 二次除菌療法を受けた 35 名のうち 33 名が UBT を受けた。残りの 2 名は UBT 未施行だった。Vonoprazan 二次除菌療法および UBT を受けた 33 名が本研究の対象患者となった(年齢: 34～79 歳、男性 22 名/女性 11 名)。本研究の対象患者の選択に至るまでのフローチャートを Fig. 2 に示した。Vonoprazan 二次除菌療法の除菌成功率は 81.8%(95%CI: 64.5-93.0%; 27/33)だった。

患者身体因子の ROC 曲線とカットオフ値:

各患者身体因子と除菌成否についての ROC 解析の AUC 値は、年齢：0.537、身長：0.565、体重：0.673、BMI：0.796 であった。BMI の AUC 値は fair な関連と判定した。BMI と除菌成否との ROC 曲線を Fig. 3 に示した。各患者身体因子と除菌成否についての ROC 解析によるカットオフ値は、年齢：58 歳、身長：157cm、体重：56kg、BMI：23.8kg/m^2 だった。

Metronidazole/amoxicillin の MIC 値の分布と MIC 値別の除菌成功率:

Metronidazole の MIC 値の分布は、≤4μg/mL が 3.0%(1/33)、8μg/mL が 36.4%(12/33)、16μg/mL が 48.5%(16/33)、>16μg/mL が 12.1%(4/33)であった。Amoxicillin の MIC 値の分布は、≤0.015μg/mL が 81.8% (27/33)、0.03μg/mL が 12.1%(4/33)、0.06μg/mL が 6.1%(2/33)であった。

Metronidazole の MIC 値別の除菌率は≤4μg/mL: 100%(1/1)、8μg/mL: 83.3%(10/12)、16μg/mL: 75.0%(12/16)、>16μg/mL: 100%(4/4)だった。Amoxicillin の MIC 値別の除菌率は≤0.015μg/mL: 81.5%(22/27)、 0.03μg/mL: 100%(4/4)、0.06μg/mL: 50.0%(1/2)だった。

Metronidazole の MIC 値と除菌成否についての ROC 解析の AUC 値は 0.506 だった (Fig. 4)。Amoxicillin の MIC 値と除菌成否についての ROC 解析の AUC 値は 0.497 だった (Fig. 5)。Metronidazole/amoxicillin の MIC 値と除菌成否に関連を認めなかった。

患者因子と除菌成否との関連:

各連続変数は ROC 解析で算出されたカットオフ値(年齢:58 歳、身長:157cm、体重:56kg、BMI：23.8kg/m^2、metronidazole の MIC 値：32μg/mL、amoxicillin の MIC 値：0.03μg/mL)によりカテゴリー変数に分類された。全てのカテゴリー変数と除菌成否の関連について、ピアソンのカイ二乗検定を用いて評価した。解析結果を Table 1 に提示する。

Vonoprazan 二次除菌療法の除菌成功率は BMI 低値 (<23.8kg/m^2) の患者群と比較して、BMI 高値 (≥23.8kg/m^2) の患者群において高かった (p=0.007)。なお本研究の対象患者に重度の肥満患者 (BMI ≥30kg/m^2) はいなかった(Table 1)。その他の患者因子 (MIC 値を含む)と除菌成否に関連を認めなかった。

追加解析:

①除菌成否を目的変数、BMI、metronidazole/amoxicillin の MIC 値を説明変数としたロジスティック回帰分析を行った。BMI、metronidazole/amoxicillin の MIC 値のオッズ比は、1.18、0.000000112、1.05 だった。BMI、metronidazole/amoxicillin の MIC 値の p 値は 0.295、0.667、0.541 であり、除菌成否と関連を認めなかった。

②BMI 25kg/m^2 をカットオフ値にして 2 群に分けて、除菌成否との関連をカイ二乗検定で検討した。このカットオフ値では、BMI と除菌成否に関連は認めなかった(Table 1)。

オ）考察：

調べた限りにおいて、本研究は vonoprazan 二次除菌療法の成否と関連する患者因子について検討した初めての報告である。本研究では以下の 2 点が示された。第 1 に、患者背景因子の中で BMI のみ、vonoprazan 二次除菌療法による除菌成否と関連を認めた。第 2 に、metronidazole および amoxicillin の MIC 値と vonoprazan 二次除菌療法による除菌成否に関連を認めなかった。

Vonoprazan を用いた除菌療法に関する過去の研究は、PPI を用いた除菌療法との除菌成功率の比較に関するものが大半である。最新のメタ解析では vonoprazan 二次除菌療法は PPI 二次除菌療法と比較して、除菌成功率が高いことが報告されている [23]。PPI と比較して vonoprazan では、CYP2C19 の遺伝子多型に関わらず、即効性および持続性のある強力な酸分泌抑制効果を得られることが、除菌成功率向上の要因とされている [19~21]。Vonoprazan 一次除菌療法では除菌成否に関連する患者背景因子として、年齢、性別、PPI 常用の有無、clarithromycin 耐性の有無が報告されている [35,37,39]。Vonoprazan 一次除菌療法で、除菌成否と BMI との関連性を指摘している先行研究報告は認めない。本研究のデータベースを用いた解析では、vonoprazan 一次除菌療法において、2 剤療法を受けた患者群で除菌成功率は BMI 高値 (≥22.4kg/m^2) の患者群と比較して、BMI 低値 (<22.4kg/m^2) の患者群において高かった (83.9% vs 95.6%: p=0.047)。この結果については amoxicillin の薬物動態に着目して考察されている。BMI 高値の患者では、脂肪組織が多いため分布容積が増加し、amoxicillin の血中濃度が低下することにより、除菌成功率が低下すると推測されている [41]。本研究では、BMI 高値の患者群で vonoprazan 二次除菌療法の除菌成功率が高かった。BMI と除菌成否との関連は意見が分かれるところである。ある研究では BMI 高値の患者群で除菌成功率が高いことが報告されている [34,36]。一方で BMI 低値の患者群で除菌成功率が高いとする逆の報告もある [33,38]。この結果の不一致は、除菌レジメンの違いによるものと考えられる。本研究の結果については metronidazole の薬物動態に着目して考察した。

Metronidazole の薬物動態の研究では、静脈または経口投与された metronidazole は筋肉や脂肪組織にも分布し、その metronidazole 濃度の上昇は血液と比較して緩やかであることが報告されている [42~44]。そのため、BMI 高値の患者群では metronidazole が体内に残留する時間が長いことにより、除菌成功率が向上している可能性が考えられる。この点を踏まえると、BMI 低値の患者群においては、投与期間の延長や metronidazole 投与量を増やすこ

とで除菌成功率の向上が得られる可能性がある。具体的には、BMI 低値の患者群において、vonoprazan 二次除菌療法の投与期間を 7 日間から 14 日間に延長することや、metronidazole の投与回数を 250mg×2 回/日から 250mg×3 回/日に増やす方法が考えられる。しかし、この点に関してはさらなる研究を要する。一方、metronidazole は肝疾患や糖尿病があると代謝が低下して、血中や組織中の濃度が上昇することが知られている [45]。本研究の解析対象患者では肝硬変の患者は認めなかった。糖尿病の有無についてはデータを収集していない。BMI 高値の患者は NASH による肝障害や糖尿病を基礎疾患として有している可能性が考えられる。肝疾患や糖尿病は BMI と除菌成否の交絡因子となる可能性があるため、肝疾患や糖尿病の詳細なデータを収集した上での解析が望ましかったと考える。

尚、日本人における Hp 陽性者の BMI と最大胃酸分泌量の関係を検討した先行研究では、BMI と最大胃酸分泌量に正の相関を認めたと報告されている [46]。この結果を踏まえると、BMI 低値の患者群の方が、最大胃酸分泌能が低値のため、より高い除菌成功率が得られる可能性が考えられる。しかし、本研究においては vonoprazan により、BMI の高低にかかわらず十分な胃酸分泌抑制効果が得られたため、BMI 低値の患者群において BMI 高値の患者群よりも除菌成功率が高い結果にならなかったと推測する。

喫煙・飲酒と vonoprazan 二次除菌療法の除菌成否に関連を認めなかった。メタ解析において喫煙は PPI を用いた除菌療法の除菌成功率を低下させることが報告されている。喫煙により胃酸分泌の刺激、胃粘膜血流の低下、胃粘液分泌の低下が引き起こされる [32,47,48]。このため胃粘膜へ到達する抗菌薬量が減少するため、除菌成功率が低下すると考えられている。飲酒も PPI を用いた除菌療法の除菌成功率を低下させることが報告されている [31]。飲酒による胃酸分泌の刺激がその理由とされている [49,50]。一方、vonoprazan を用いた除菌療法では、喫煙・飲酒が除菌成功率に影響しないことが報告されている [51,52]。この理由は PPI と比較して vonoprazan のより強力な胃酸分泌抑制効果によるものと考えられている。本研究においても、喫煙・飲酒による vonoprazan 二次除菌療法の除菌成功率の低下は認めなかった。

Metronidazole の MIC 値と vonoprazan 二次除菌療法の除菌成否には関連を認めなかった。MIC 値に関して論じる際に、どの薬剤感受性試験を用いたかは重要である。本研究において、薬剤感受性試験は微量液体希釈法を用いた。Hp の薬剤感受性試験は、他の研究においては、ディスク拡散法・E テスト・寒天平板希釈法が主に用いられている [8]。微量液体希釈法は Clinical and Laboratory Standard Institute で設定された標準法であり、感受性検査の基になる最も精密で正確な方法とされている [53]。ディスク拡散法は微量液体希釈法を基に設定された定性的な方法であり、MIC 値を直接測定することはできない。E テストはスウェーデンで開発された測定法であり、寒天平板希釈法を基に設定されている [54]。寒天平板希釈法は、微量液体希釈法と同じく感受性検査の基となる方法のひとつである。しかし、寒天平板希釈法は操作が煩雑であるため、日常検査には使用されていない。微量液体希釈法は寒天平板希釈法に比べ、(Ⅰ) 抗菌薬、培地が節約できる、(Ⅱ) 操作が簡便、(Ⅲ) 長期保存に

耐える、(Ⅳ)自動機器にも応用できるなどの利点があり、現在の薬剤感受性試験の標準法とされている[53]。Metronidazoleを含む除菌レジメンを用いた、metronidazole耐性とHp除菌成否に関する既報をTable 2に示す[25~28]。European Committee on Antimicrobial Susceptibility Testing (EUCAST)では、in vitroでmetronidazole耐性と判定するMIC値を>8μg/mLと定めている[55]。このカットオフ値に基づくと、metronidazole耐性率はclarithromycin耐性率と同等かそれ以上であることが知られている[8]。Maastricht Ⅴ/Florence Consensus Reportにおいて、薬剤感受性試験に基づきHp除菌療法のレジメンを決定することが推奨されている[4]。Table 2に示されるように、vonoprazanを用いないmetronidazoleレジメンではmetronidazole感受性群と比較して、metronidazole耐性群において除菌成功率の低下を認めた。しかし、vonoprazan二次除菌療法ではmetronidazole耐性群においても、metronidazole感受性群と同等の除菌成功率が得られた。これは本研究のmetronidazoleのMIC値が除菌の成否と関連しない結果と合致する。

AmoxicillinのMIC値とvonoprazan二次除菌療法の除菌成否にも関連を認めなかった。EUCASTでは、in vitroでamoxicillin耐性と判定するMIC値を>0.125μg/mLと定めている[55]。このカットオフ値に基づくと、本研究では対象患者にamoxicillin耐性を認めなかった。Amoxicillin耐性率については、アジア太平洋地域では3%と低率であることが報告されており、本研究の結果と矛盾しない[56]。Amoxicillinを含む除菌レジメンでは、amoxicillin耐性が除菌不成功の因子であることが知られている[29]。Vonoprazan一次除菌療法においても、amoxicillin耐性が除菌不成功の因子であることが報告されている[12,30,57]。Vonoprazan二次除菌療法とamoxicillin耐性の関係についての報告はない。本研究ではamoxicillinのMIC値とvonoprazan二次除菌療法の除菌成否に関連を認めなかった。しかし、MIC ≥ 0.06μg/mlを耐性基準に設定しても、本研究ではamoxicillin耐性率は6.1%(2/33)と低値である。この症例数でamoxicillin耐性についての統計学的評価を行うことは難しい。アフリカ地域ではamoxicillin耐性率は38%と報告されている[58]。Amoxicillin耐性率が高値の地域において、vonoprazan二次除菌療法が行われることにより、vonoprazan二次除菌療法とamoxicillin耐性についてのさらなる検討が望まれる。

本研究のLimitationとしては以下が挙げられる。(Ⅰ)後ろ向きの副次解析研究であること、(Ⅱ)解析症例数が少ないこと、(Ⅲ)対象患者が全て日本人であること、(Ⅳ)二次除菌療法レジメンの選択バイアス、(Ⅴ)二次除菌療法の服薬遵守率のデータ収集ができていないこと、(Ⅵ)肝疾患、糖尿病がBMIと除菌成否の交絡因子として関与している可能性があることである。(Ⅱ)については、追加解析のロジスティック回帰分析ではBMI、metronidazole/amoxicillinのMIC値と除菌成否と有意な関連を認めなかった。しかし回帰モデルに入れられる説明変数の個数の目安として、「イベントありとなしの小さい方の数を10で割った数まで」との考え方がある[59]。3項目を説明変数として解析する場合には、除菌不成功患者が少なくとも30名いることが望ましい。本研究の除菌不成功患者6名では、ロジスティック回帰分析のための症例数としては不十分と考えられる。約5倍の症例数が

必要となるが、その症例数により解析できた場合には、ロジスティック回帰分析でもBMIと除菌成否に関連を認める結果となる可能性がある。(IV)については、本研究では一次除菌不成功の患者が二次除菌療法を受ける際に、二次除菌療法のレジメンの内容が個々の医師の判断により選択されていた。そのため解析対象（vonoprazan 二次除菌療法を受けた患者）を決める際に、選択バイアスが生じている。本研究で除外された患者がvonoprazan二次除菌療法を受けた場合には、患者因子と除菌成否の解析結果が異なる結果になった可能性が考えられる。最後にHp除菌成功に関する肥満の影響に注意する必要がある。本研究の解析対象患者に重度の肥満患者は含まれていない。Vonoprazan二次除菌療法において、BMI高値群で除菌成功率が高いことは、肥満であることがHp除菌療法において有利であることを意味するわけではない。

カ）まとめ：

結論として、本研究では患者因子の中ではBMIのみvonoprazan二次除菌療法の除菌成否と関連を認めることが示された。また metronidazole および amoxicillin の MIC 値とvonoprazan 二次除菌療法の除菌成否に関連を認めないことが示された。本結論を検証するため、解析症例数を増やして、さらなる検討が望まれる。

Table 1. 患者因子と vonoprazan 二次除菌療法の成否との関連

	除菌成功群 (n = 27)	除菌不成功群 (n = 6)	*P* value
年齢 (歳, mean±SD)	63.0±11.1	62.1±9.4	
範囲	34-79	49-73	
年齢 (≥ 58 歳/< 58 歳)	22/5	3/3	0.271
性別 (男性/女性)	18/9	4/2	1.000
身長 (cm, mean±SD)	163.6±10.3	161.0±10.7	
範囲	142-188	151-180	
身長 (≥ 157 cm/< 157 cm)	21/6	2/4	0.099
体重 (kg, mean±SD)	65.4±12.4	58.9±9.4	
範囲	37.8-90.0	47.0-76.0	
体重 (≥ 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	
範囲	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
Body mass index (≥ 25.0 kg/m^2/< 25.0 kg/m^2)	12/15	0/6	0.115
喫煙 (有/無)	5/22	3/3	0.271
飲酒 (有/無)	5/22	1/5	1.000
試験参加前の PPI 常用 (有/無)	1/26	0/6	1.000
Metronidazole の MIC 値 (≥ 32 μg/mL/< 32 μg/mL)	4/23	0/6	0.753
Amoxicillin の MIC 値 (≥ 0.03 μg/mL/< 0.03 μg/mL)	5/22	1/5	1.000

Helicobacter pylori 関連疾患

合計 (有/無)	8/19	0/6	0.315
胃潰瘍 (有/無)	2/25	0/6	1.000
十二指腸潰瘍 (有/無)	5/22	0/6	0.607
胃腫瘍内視鏡切除歴 (有/無)	1/26	0/6	1.000

略語: MIC, minimal inhibitory concentration; PPI, proton pump inhibitor; SD, standard deviation.

Legend: カテゴリー変数に対して、ピアソンのカイ二乗検定を用いて解析した。*Helicobacter pylori* 関連疾患は胃潰瘍、十二指腸潰瘍、胃腫瘍内視鏡切除歴と定義した。Vonoprazan 二次除菌療法の除菌成功率は BMI 低値 ($<23.8 kg/m^2$) の患者群と比較して、BMI 高値 ($\geq 23.8 kg/m^2$) の患者群において高かった ($p=0.007$)。その他の患者因子 (MIC 値を含む) と除菌成否に関連を認めなかった。

Table 2. Metronidazole 耐性と *Helicobacter pylori* 除菌成否の関連についての研究

研究	症例数	薬剤感受性試験	MNZ 耐性の定義	MNZ 耐性率	除菌レジメン	除菌成功率 (全体)	除菌成功率 (MNZ 耐性)
Xia et al. 1994	76	ディスク拡散法	ZD < 20 mm	25%	BI + MNZ + TET	82%	53%
Lopez-Brea et al. 1999	57	寒天平板希釈法	MIC > 8 μg/mL	14%	MNZ + AMO + BI	79%	50%
Nishizawa et al. 2007	107	寒天平板希釈法	MIC ≥ 8 μg/mL	3.7%	LPZ + AMO + MNZ	90%	50%
Lee et al. 2019	54	寒天平板希釈法	MIC > 32 μg/mL	19%	EPZ + BI + MNZ + TET	89%	60%
自験例	33	微量液体希釈法	MIC > 8 μg/mL	61%	vonoprazan + MNZ + AMO	82%	80%

略語: AMO, amoxicillin; BI, bismuth; EPZ, esomeprazole; LPZ, lansoprazole; MIC, minimal inhibitory concentration; MNZ, metronidazole; TET, tetracycline; ZD, zone diameter.

Legend: Vonoprazan を用いない metronidazole レジメンでは metronidazole 感受性群と比較して、metronidazole 耐性群において除菌成功率の低下を認めた。しかし、vonoprazan 二次除菌療法では metronidazole 耐性群においても、metronidazole 感受性群と同等の除菌成功率が得られた。

Figure 1

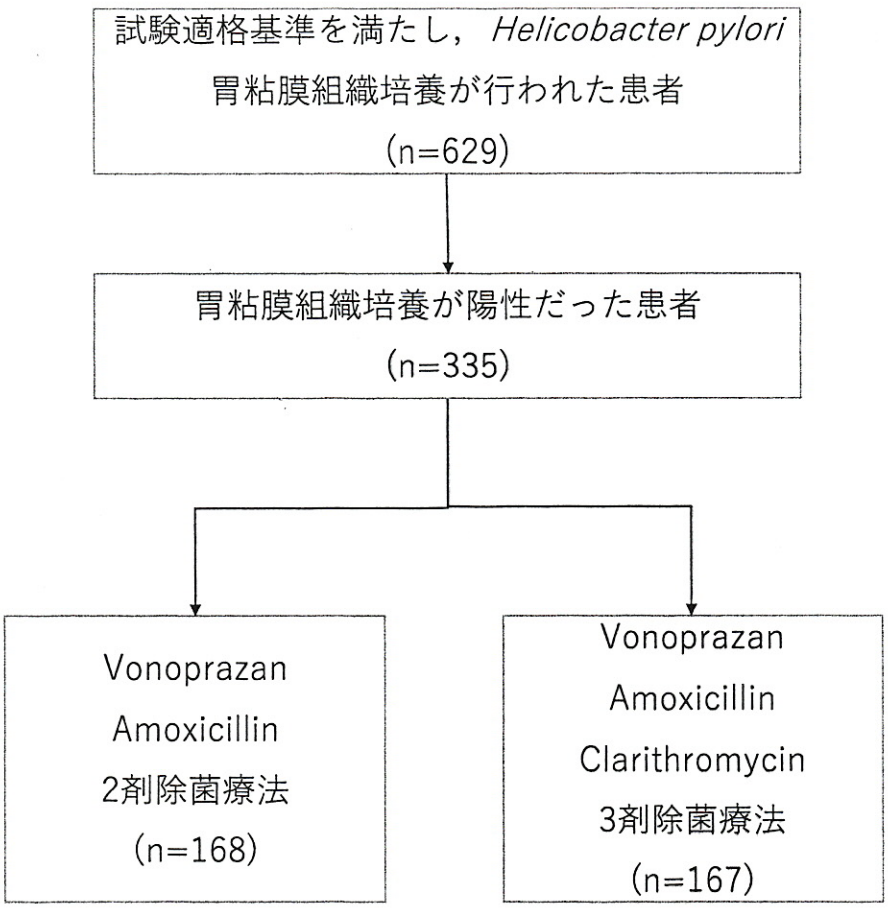
試験適格基準を満たし，*Helicobacter pylori*
胃粘膜組織培養が行われた患者
(n=629)
胃粘膜組織培養が陽性だった患者
(n=335)
Vonoprazan
Amoxicillin
2剤除菌療法
(n=168)
Vonoprazan
Amoxicillin
Clarithromycin
3剤除菌療法
(n=167)

Figure 2

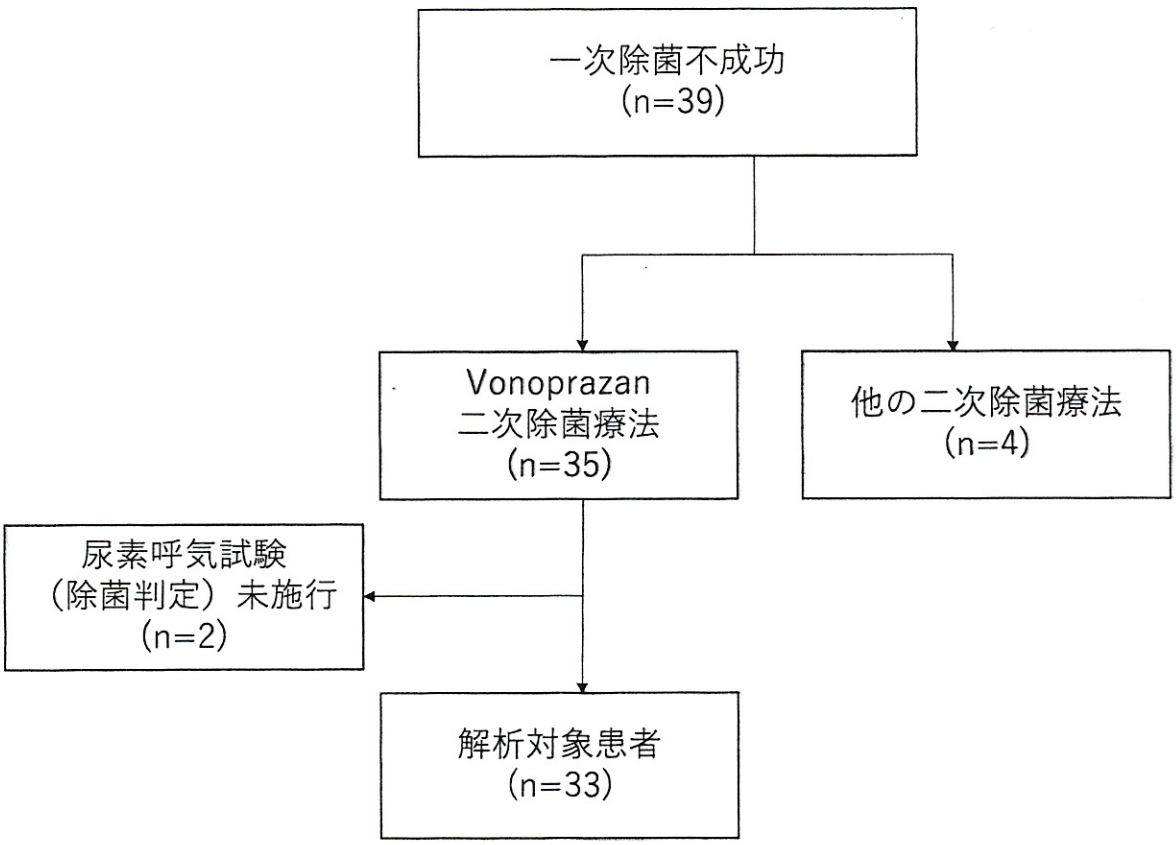
一次除菌不成功
(n=39)
Vonoprazan
二次除菌療法
(n=35)
他の二次除菌療法
(n=4)
尿素呼気試験
(除菌判定) 未施行
(n=2)
解析対象患者
(n=33)

Figure 3

Body mass indexと除菌成否のROC曲線

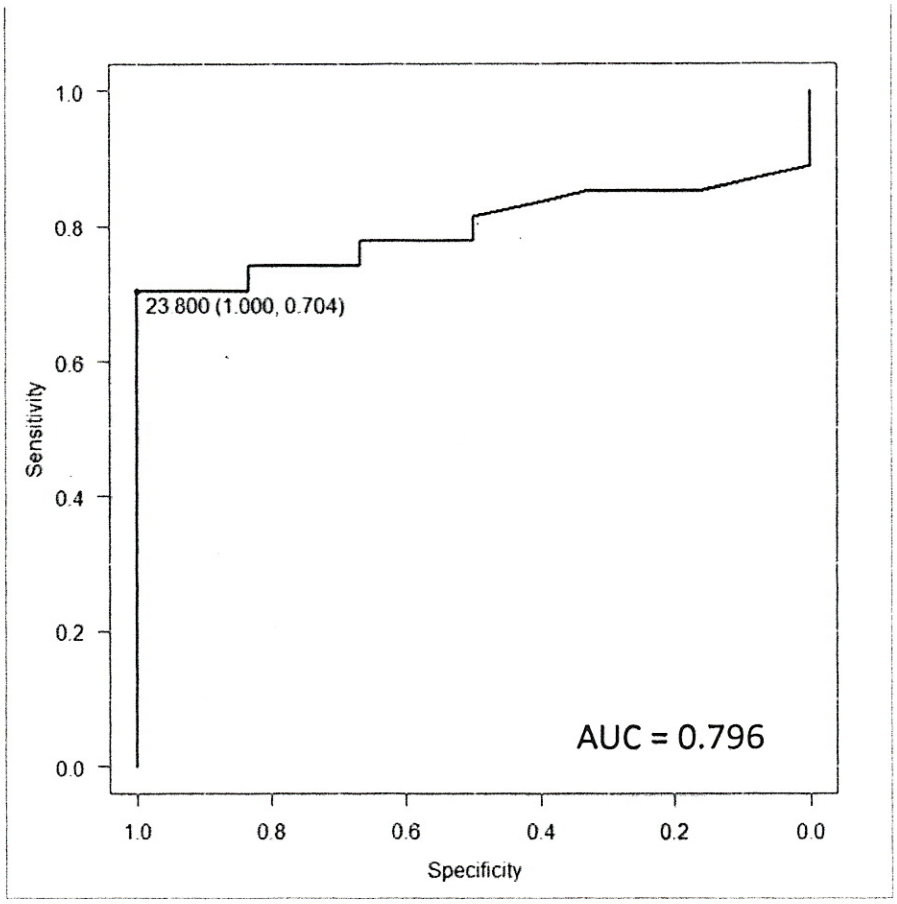

Figure 4

MetonidazoleのMIC値と除菌成否のROC曲線

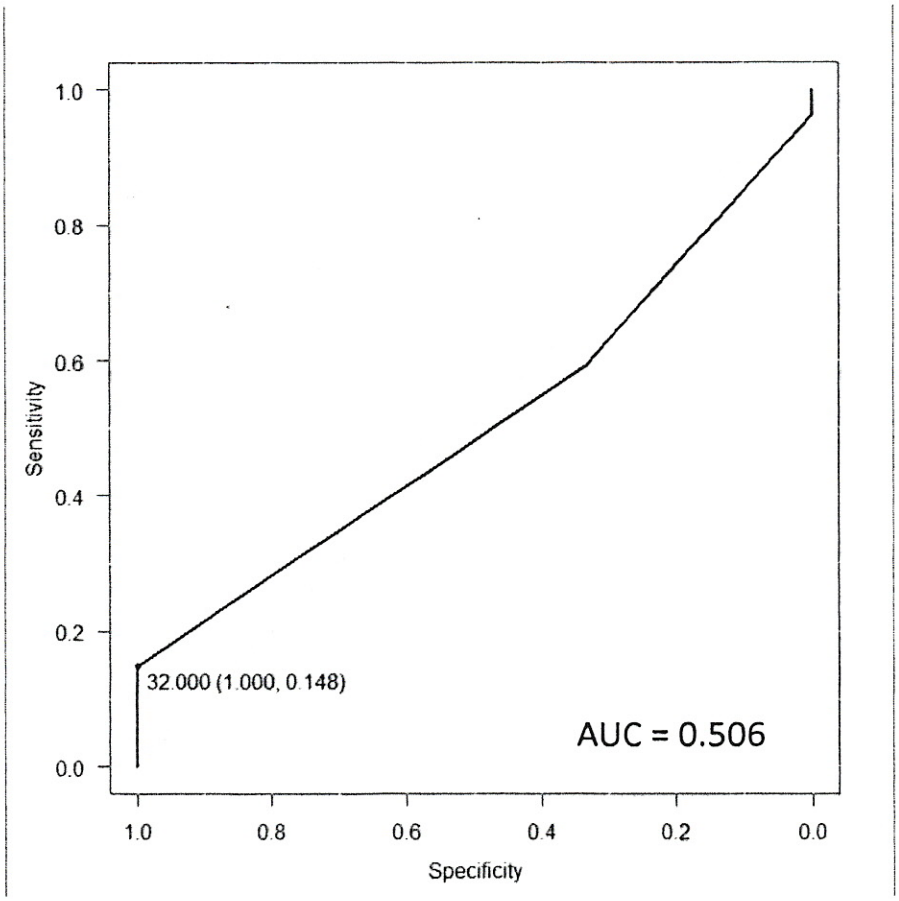

Figure 5

AmoxicillinのMIC値と除菌成否のROC曲線

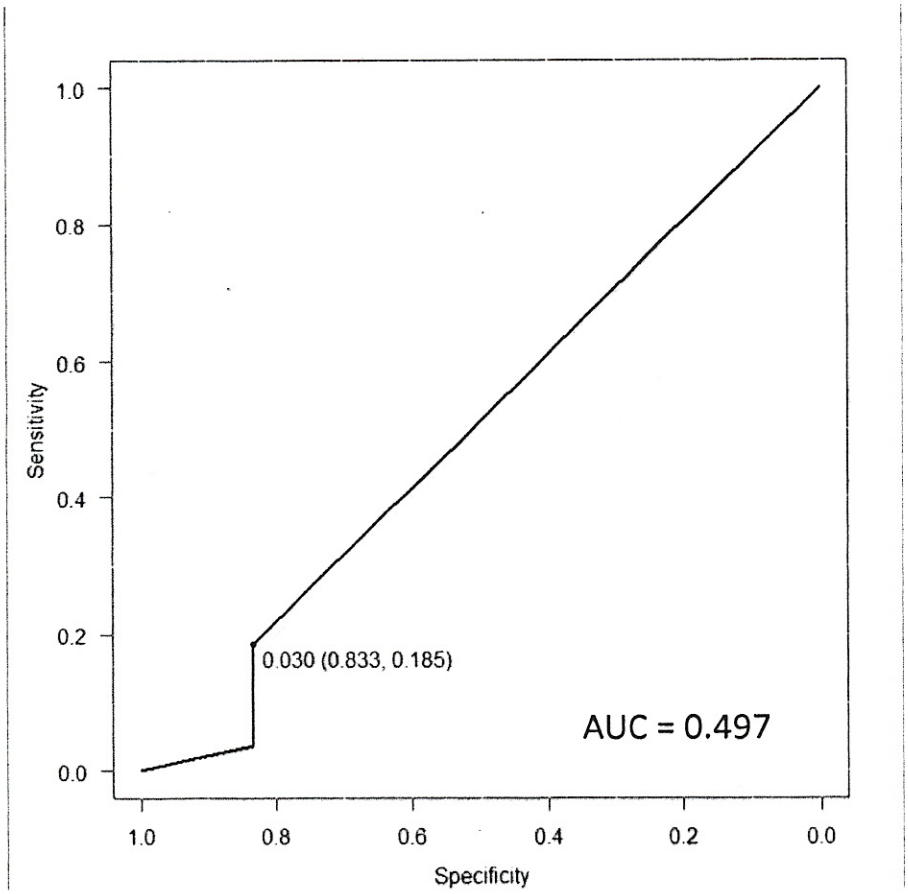

Figure legends

Figure 1. 主解析研究のフローチャート

試験適格基準を満たし、*Helicobacter pylori* 培養検査を受けた629名のうち、培養陽性の335名に一次除菌療法を施行した。168名に対して2剤療法(vonoprazan 20mg×2回/日＋amoxicillin 750mg×2回/日を7日間投与)、167名に対して3剤療法(vonoprazan 20mg×2回/日＋amoxicillin 750mg×2回/日＋clarithromycin 200mg×2回/日を7日間投与)を施行した。

Figure 2. 解析対象患者選択のフローチャート

一次除菌療法を受けた335名のうち39名が一次除菌不成功だった。一次除菌不成功だった39名のうち4名はvonoprazan二次除菌療法以外のレジメンで二次除菌療法を受け、35名がvonoprazan二次除菌療法を受けた。Vonoprazan二次除菌療法を受けた35名のうち33名が尿素呼気試験を受けた。残りの2名は尿素呼気試験未施行だった。Vonoprazan二次除菌療法および尿素呼気試験を受けた33名が本研究の対象患者となった。

Figure 3. Body mass indexと除菌成否のROC曲線

BMIとvonoprazan二次除菌療法の除菌成否のROC曲線を示した。カットオフ値は23.8 kg/m^2だった。AUC値は0.796であり、fairな関連と判定した。

略語: AUC, area under the curve; BMI, body mass index.

Figure 4. Metronidazoleの MIC値と除菌成否のROC曲線

Metronidazoleの MIC値とvonoprazan二次除菌療法の除菌成否のROC曲線を示した。カットオフ値は32 μg/mLだった。AUC値は0.506であり、failedな関連と判定した。

略語: AUC, area under the curve; MIC, minimal inhibitory concentration; ROC, receiver operating characteristic.

Figure 5. Amoxicillinの MIC値と除菌成否のROC曲線

Amoxicillinの MIC値とvonoprazan二次除菌療法の除菌成否のROC曲線を示した。カットオフ値は0.03 μg/mLだった。AUC値は0.497であり、関連となし判定した。

略語: AUC, area under the curve; MIC, minimal inhibitory concentration; ROC, receiver operating characteristic.

引用文献：

1) Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; **1**: 1273-1275.

2) Asaka M, Kato M, Kudo M, et al. Atrophic changes of gastric mucosa are caused by *Helicobacter pylori* infection rather than aging: studies in asymptomatic Japanese adults. *Helicobacter* 1996; **1**: 52-56.

3) Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789.

4) Malfertheiner P, Megraud F, O'Morain CA, et al. Management of *Helicobacter pylori* infection-The Maastricht Ⅴ/Florence Consensus Report. *Gut* 2017; **66**: 6-30.

5) Leodolter A, Kulig M, Brasch H, et al. A meta-analysis comparing eradication, healing and relapse rates in patients with *Helicobacter pylori*-associated gastric or duodenal ulcer. *Aliment Pharmacol Ther* 2001; **15**: 1949-1958.

6) Lee YC, Chiang TH, Chou CK, et al. Association between *Helicobacter pylori* eradication and gastric cancer incidence: A systematic review and meta-analysis. *Gastroenterology* 2016; **150**: 1113-1124.

7) Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. *Gut* 2010; **59**: 1143-1153.

8) Hu Y, Zhu Y, Lu NH. Novel and effective therapeutic regimens for *Helicobacter pylori* in an era of increasing antibiotic resistance. *Front Cell Infect Microbiol* 2017; **7**: 168.

9) 鈴木翔, 後藤田卓志. *Helicobacter pylori* 除菌の問題と２剤療法の可能性. 日大医誌, 2022; **81**: 53-57.

10) Thung I, Aramin H, Vavinskaya V, et al. Review article: the global emergence of *Helicobacter pylori* antibiotic resistance. *Aliment Pharmacol Ther* 2016; **43**: 514-533.

11) Nishizawa T, Maekawa T, Watanabe N, et al. Clarithromycin versus metronidazole as first-line *helicobacter pylori* eradication: A multicenter, prospective, randomized controlled study in Japan. *J Clin Gastroenterol* 2015; **49**: 468-471.

12) Murakami K, Sakurai Y, Shiino M, et al. 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, randomized, double-blind study. *Gut* 2016; **65**: 1439-1446.

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

14) Hori Y, Imanishi A, Matsukawa J, et al. 1-[5-(2-Fluorophenyl)-1-(pyridin-3-

ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine monofumarate (TAK-438), a novel and potent potassium-competitive acid blocker for the treatment of acid-related diseases. *J Pharmacol Exp Ther* 2010; **335**: 231-238.

15) Shin JM, Inatomi N, Munson K, et al. 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-methylmethanamine monofumarate (TAK-438). *J Pharmacol Exp Ther* 2011; **339**: 412-420.

16) Hori Y, Matsukawa J, Takeuchi T, et al. A study comparing the antisecretory effect of TAK-438, a novel potassium-competitive acid blocker, with lansoprazole in animals. *J Pharmacol Exp Ther* 2011; **337**: 797-804.

17) Villoria A, Garcia P, Calvet X, et al. Meta-analysis: high-dose proton pump inhibitors vs. standard dose in triple therapy for *Helicobacter pylori* eradication. *Aliment Pharmacol Ther* 2008; **28**: 868-877.

18) Marcus EA, Inatomi N, Nagami GT, et al. The effects of varying acidity on *Helicobacter pylori* growth and the bactericidal efficacy of ampicillin. *Aliment Pharmacol Ther* 2012; **36**: 972-979.

19) Suzuki S, Gotoda T, Kusano C, et al. 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* 2016; **111**: 949-956.

20) Kusano C, Gotoda T, Suzuki S, et al. Safety of first-line therapy with a potassium-competitive acid blocker for *Helicobacter pylori* eradication in children. *J Gastroenterol* 2018; **53**: 718-724.

21) Lyu QJ, Pu QH, Zhong XF, et al. 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; **2019**: 9781212.

22) Jung YS, Kim EH, Park CH. Systematic review with meta-analysis: the efficacy of vonoprazan-based triple therapy on *Helicobacter pylori* eradication. *Aliment Pharmacol Ther* 2017; **46**: 106-114.

23) Shinozaki S, Kobayashi Y, Osawa H, et al. Effectiveness and safety of vonoprazan versus proton pump inhibitors for second-line *Helicobacter pylori* eradication therapy: systematic review and meta-analysis. *Digestion* 2021; **102**: 319-325.

24) Kato M, Ota H, Okuda M, et al. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2016 Revised Edition. *Helicobacter* 2019; **24**: e12597.

25) Xia H, Keane CT, Beattie S, et al. Standardization of disk diffusion test and its clinical significance for susceptibility testing of metronidazole against

Helicobacter pylori. *Antimicrob Agents Chemother* 1994; **38**: 2357-2361.
26) Lopez-Brea M, Martinez MJ, Domingo D, et al. Metronidazole resistance and virulence factors in *Helicobacter pylori* as markers for treatment failure in a paediatric population. *FEMS Immunol Med Microbiol* 1999; **24**: 183-188.
27) Nishizawa T, Suzuki H, Masaoka T, et al. A new eradication resistance index as a predictor of metronidazole-containing second-line treatment of *Helicobacter pylori*. *Digestion* 2007; **76**: 215-220.
28) Lee JW, Kim N, Nam RH, et al. Risk factors of rescue bismuth quadruple therapy failure for *Helicobacter pylori* eradication. *J Gastroenterol Hepatol* 2019; **34**: 666-672.
29) Chen MJ, Wu MS, Chen CC, et al. Impact of amoxicillin resistance on the efficacy of amoxicillin-containing regimens for *Helicobacter pylori* eradication: analysis of five randomized trials. *J Antimicrob Chemother* 2017; **72**: 3481-3489.
30) Suzuki S, Gotoda T, Kusano C, et al. Seven-day vonoprazan and low-dose amoxicillin dual therapy as first-line *Helicobacter pylori* treatment: a multicentre randomised trial in Japan. *Gut* 2020; **69**: 1019-1026.
31) Hsu PI, Lai KH, Lin CK, et al. A prospective randomized trial of esomeprazole-versus pantoprazole-based triple therapy for *Helicobacter pylori* eradication. *Am J Gastroenterol* 2005; **100**: 2387-2392.
32) Suzuki T, Matsuo K, Ito H, et al. Smoking increases the treatment failure for *Helicobacter pylori* eradication. *Am J Med* 2006; **119**: 217-224.
33) Abdullahi M, Annibale B, Capoccia D, et al. The eradication of *Helicobacter pylori* is affected by body mass index (BMI). *Obes Surg* 2008; **18**: 1450-1454.
34) Singh N, Deb R, Kashyap PC, et al. Body mass index and per capita income influence duodenal ulcer healing and *H. pylori* eradication whilst dietary factors play no part. *Trop Gastroenterol* 2008; **29**: 26-31.
35) Matsumoto H, Shiotani A, Katsumata R, et al. *Helicobacter pylori* eradication with proton pump inhibitors or potassium-competitive acid blockers: the effect of clarithromycin resistance. *Dig Dis Sci* 2016; **61**: 3215-3220.
36) Costa S, Soares JB, Goncalves R. Efficacy and tolerability of culture-guided treatment for *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 2017; **29**: 1258-1263.
37) Shinozaki S, Osawa H, Sakamoto H, et al. Pre-treatment with proton pump inhibitors decreases the success of primary *Helicobacter pylori* eradication using a vonoprazan-based regimen. *Kaohsiung J Med Sci* 2018; **34**: 456-460.
38) Tan B, Yang JC, Young CL, et al. *Helicobacter pylori* antimicrobial

susceptibility testing-guided salvage therapy in the USA: a real life experience. *Dig Dis Sci* 2018; **63**: 437-445.

39) Kusunoki M, Yuki M, Ishitobi H, et al. Effect of age on effectiveness of vonoprazan in triple therapy for *Helicobacter pylori* eradication. *Intern Med* 2019; **58**: 1549-1555.

40) Kanda Y. Investigation of the freely-available easy-to-use software "EZR" for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452-458.

41) Eto H, Suzuki S, Kusano C, et al. Impact of body size on first-line *Helicobacter pylori* eradication success using vonoprazan and amoxicillin dual therapy. *Helicobacter* 2021; **26**: e12788.

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

43) Badia JM, de la Torre R, Farre M, et al. Inadequate levels of metronidazole in subcutaneous fat after standard prophylaxis. *Br J Surg* 1995; **82**: 479-482.

44) Karjagin J, Pahkla R, Karki T et al. Distribution of metronidazole in muscle tissue of patients with septic shock and its efficacy against Bacteroides fragilis in vitro. *J Antimicrob Chemother* 2005; **55**: 341-346.

45) Muscara MN, Pedrazzoli Jr J, Miranda EL, et al. Plasma hydroxy-metronidazole/metronidazole ratio in patients with liver disease and in healthy volunteers. *Br J Clin Pharmacol* 1995; **40**: 477-480.

46) Ishimura N, Owada Y, Aimi M, et al. No increase in gastric acid secretion in healthy Japanese over the past two decades. *J Gastroenterol* 2015; **50**: 844-852.

47) Lanas A, Hirschowitz BI. Influence of smoking on basal and on vagally and maximally stimulated gastric acid and pepsin secretion. *Scand J Gastroenterol* 1992; **27**: 208-212.

48) Iwao T, Toyonaga A, Ikegami M, et al. Gastric mucosal blood flow after smoking in healthy human beings assessed by laser Doppler flowmetry. *Gastrointest Endosc* 1993; **39**: 400-403.

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

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

51) Sakurai K, Suda H, Ido Y, et al. Comparative study: vonoprazan and proton pump inhibitors in *Helicobacter pylori* eradication therapy. *World J Gastroenterol* 2017;

23: 668-675.

52) Takara Y, Endo H, Nakano R, et al. Smoking and drinking did not increase the failure of therapeutic *Helicobacter pylori* eradication by vonoprazan, clarithromycin, and amoxicillin. *Digestion* 2019; **99**: 172-178.

53) Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis* 2009; **49**: 1749-1755.

54) Baker CN, Stocker SA, Culver DH et al. 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* 1991; **29**: 533-538.

55) European Committee on Antimicrobial Susceptibility Testing. Clinical Breakpoints for Bacteria. https://www.eucast.org/clinical_breakpoints/ [*Accessed*: September 26, 2020].

56) Kuo YT, Liou JM, El-Omar EM, et al. Primary antibiotic resistance in *Helicobacter pylori* in the Asia-Pacific region: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2017; **2**: 707-715.

57) Shinmura T, Adachi K, Yamaguchi Y, et al. Vonoprazan-based triple-therapy could improve efficacy of the tailored therapy of *Helicobacter pylori* infection. *J Gastrointestin Liver Dis* 2019; **28**: 389-395.

58) Savoldi A, Carrara E, Graham DY, et al. Prevalence of antibiotic resistance in *Helicobacter pylori*: a systematic review and meta-analysis in World Health Organization regions. *Gastroenterology* 2018; **155**: 1372-1382.

59) 新谷歩. 多変量解析－説明変数の選び方. 今日から使える医療統計. 医学書院, 東京, 2015; 101-113.