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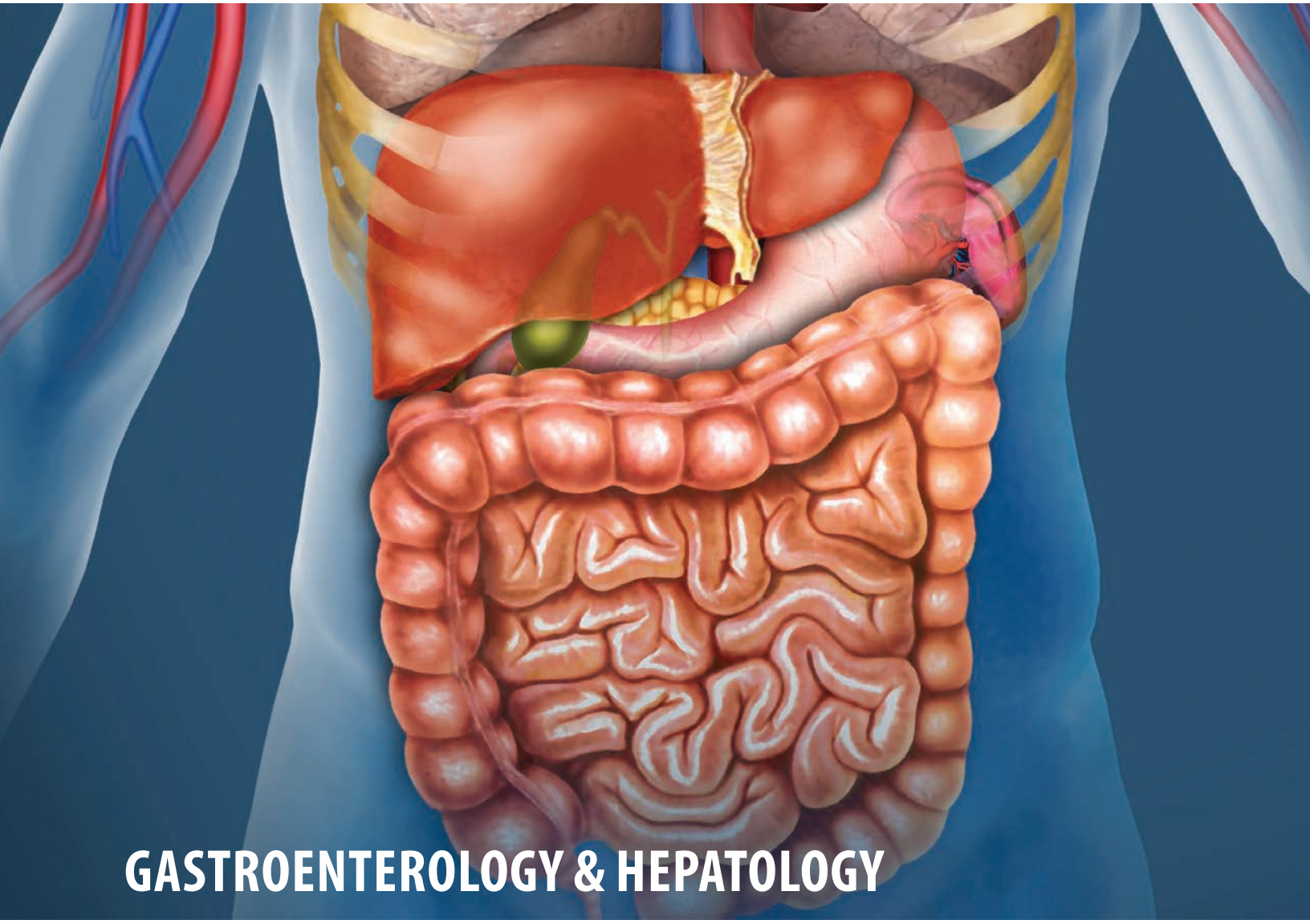

GASTROENTEROLOGY & HEPATOLOGY

In this issue:

- Gastrointestinal bleeding disorders
- Functional gastrointestinal disorders
- Inflammatory bowel disease
- *Helicobacter pylori* infection
- Barrett's esophagus
- Hepatitis
- Cirrhosis
- Non-alcoholic fatty liver disease

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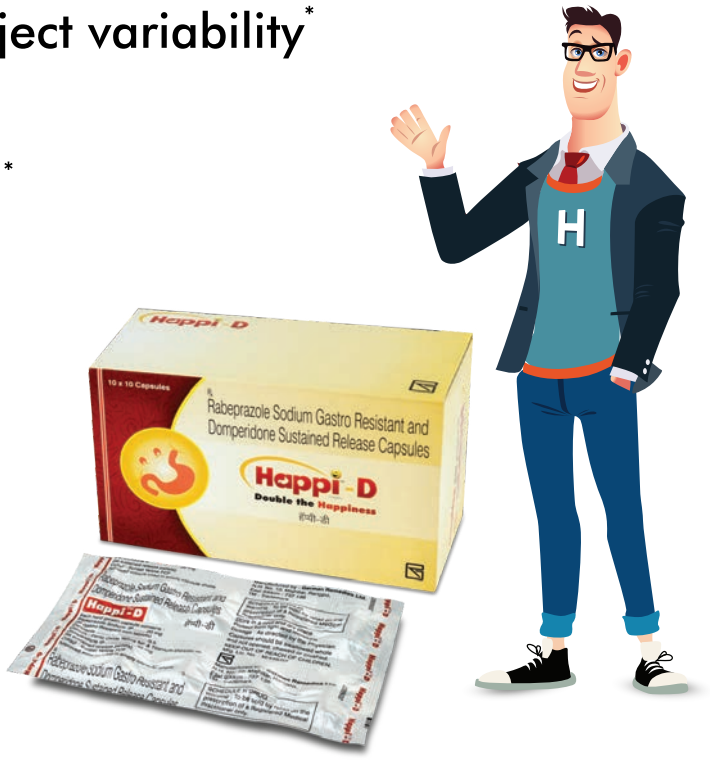

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ORIGINAL ARTICLE

Clinical Outcomes of Patients with Non-ulcer and Non-variceal Upper Gastrointestinal Bleeding: A Prospective Multicenter Study of Risk Prediction Using a Scoring System

Hyun Woo Park[1] · **Seong Woo Jeon**[1,2]

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Abstract

Background and Aims Compared with ulcer bleeding (UB) in non-variceal upper gastrointestinal bleeding (NVUGIB), non-ulcer bleeding (NUB) is often considered to have a low risk of poor outcomes and is treated less intensively without any risk stratification. We conducted this study to assess the predictability of scoring systems for NUB and compare the outcomes of NUB and UB.

Methods A total of 1831 UGIB patients were registered in the database during the period from February 2011 to December 2013. Among them, 1424 patients with NVUGIB were divided into two groups: Group UB (1101 patients with peptic ulcer bleeding) and Group NUB (323 patients with non-peptic ulcer-related bleeding).

Results The most common cause of bleeding in Group NUB was Mallory–Weiss tears (51.1%), followed by Dieulafoy lesions (18.9%). A receiver operating characteristic (ROC) analysis revealed that the pre-Rockall score [area under the ROC (AUROC) = 0.798; 95% CI 0.707–0.890] and full Rockall score (AUROC = 0.794; 95% CI 0.693–0.895) were relatively good at predicting overall mortality in NUB. Glasgow–Blatchford score (AUROC = 0.783; 95% CI 0.730–0.836) was the most closely correlated with the need for clinical intervention in NUB. Those who had Glasgow–Blatchford score of 0 did not require any interventions, including blood transfusions. There were no statistical differences in overall mortality ($p = 0.387$), bleeding-related mortality ($p = 0.447$), or the incidence of re-bleeding ($p = 0.117$) between the two groups.

Conclusions Scoring systems are useful to predict mortality and the need for clinical intervention in patients with NUB.

Keywords Gastrointestinal hemorrhages · Peptic ulcer hemorrhages · Etiology · Mortality

Introduction

Upper gastrointestinal bleeding (UGIB) remains a major cause of hospitalization, with substantial mortality, despite significant advances in its management [1]. To optimize the management of UGIB, many scoring systems have been suggested as tools for predicting outcomes and aiding early decision making for intervention. Among them, the Glasgow–Blatchford score (GBS) and the Rockall score (RS; "pre" or "full") systems are well known and widely utilized to predict mortality rates and the need for intervention.

As peptic ulcer disease is the most common cause of non-variceal UGIB (NVUGIB), many studies have reviewed the usefulness of scoring systems for predicting the prognosis and efficacy of interventions in patients with peptic ulcer bleeding [2–4]. Although the RS and GBS systems were derived by multicenter studies of unselected patients with UGIB, the applicability of these scoring systems to relatively small portion subgroups, such as patients with non-ulcer bleeding (NUB), has not been verified [5–7]. Furthermore, the features and appropriate management of non-ulcer and NVUGIB (NUNVUGIB) compared with those of peptic ulcer bleeding have not been identified.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10620-018-5255-5) contains supplementary material, which is available to authorized users.

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NUNVUGIB consists of Mallory–Weiss tear, gastric mucosal erosions, esophagitis, and others. A Dieulafoy lesion or aortoenteric fistula may lead to massive blood loss. However, because many cases of NUNVUGIB have an initial presentation of hemodynamically stable bleeding and minor lesions on endoscopy, they are often considered low risk with a low likelihood of poor outcomes in terms of mortality or re-bleeding. Owing to this perception, patients with NUNVUGIB might be treated less aggressively [8].

The aims of this study were to identify the characteristics and clinical outcomes of NUNVUGIB compared with those of peptic ulcer bleeding and assess the validity of the RS and GBS systems for predicting both outcomes and the need for clinical intervention in patients with NUNVUGIB.

Methods

Data of consecutive patients with UGIB at eight hospitals in Daegu-Gyeongsang, South Korea, between February 2011 and December 2013 were collected. We reviewed prospectively collected data from 1984 patients who underwent upper gastrointestinal endoscopy due to UGIB. We excluded in-patient bleeding episodes in cases that were admitted for another reason. This prospective observational cohort study is registered with the Clinical Research Information Service (clinical trial registration: cris.nih.go.kr/KCT0000514).

Study Populations

A total of 1984 patients with overt UGIB symptoms such as hematemesis, melena, and hematochezia or a suspicious clinical presentation of UGIB such as syncope, epigastric pain, dyspnea, dizziness, altered mental status, or anemia who were aged over 16 years were considered for this study. After upper gastrointestinal endoscopy, 153 patients were excluded due to inconsistent endoscopic UGIB findings (lower gastrointestinal bleeding, obscure bleeding, gum bleeding, foreign body) or insufficient data; 347 patients with variceal (esophageal or gastric varices) bleeding were also excluded from this study. The final 1484 patients with NVUGIB, excluding patients with cancer bleeding due to their significantly high bleeding-unrelated mortality rates, were divided into two groups: Group UB (ulcer bleeding), consisting of 1101 patients with peptic ulcer bleeding, and Group NUB, consisting of 323 patients with NUNVUGIB (Fig. 1). We compared the characteristics, bleeding scores, and clinical outcomes between the two groups and investigated the predictive risk factors for Group NUB.

Patient Characteristics and Clinical Outcomes

We investigated the patients' demographic details, medical histories, presenting symptoms, comorbidities, and initial laboratory findings and vital signs. Overall mortality, bleeding-related mortality, worsening morbidity rates, incidence of re-bleeding, need for surgery or angioembolization, and transfusion requirements were compared to identify the clinical outcomes of the two groups.

Re-bleeding was defined as the development of overt bleeding such as hematemesis, hematochezia, or melena or a decrease in hemoglobin concentration of at least 2 g/dL after an initial successful treatment. Red blood cell transfusion was done when the hemoglobin level was less than 8 g/dL without significant comorbidities and less than 10 g/dL with significant comorbidities or anemia-related symptoms in patients with NVUGIB. Patients were followed up for at least 30 days after endoscopy for the assessment of re-bleeding or mortality by telephone survey or outpatient clinic visit. Comorbidity was defined as patients having one or more of the following: ischemic heart disease, chronic kidney disease, chronic liver disease, congestive heart failure, diabetes mellitus, hypertension, cerebral vascular accident, metastatic malignancy, peripheral vascular disease, or arrhythmia such as atrial fibrillation or flutter.

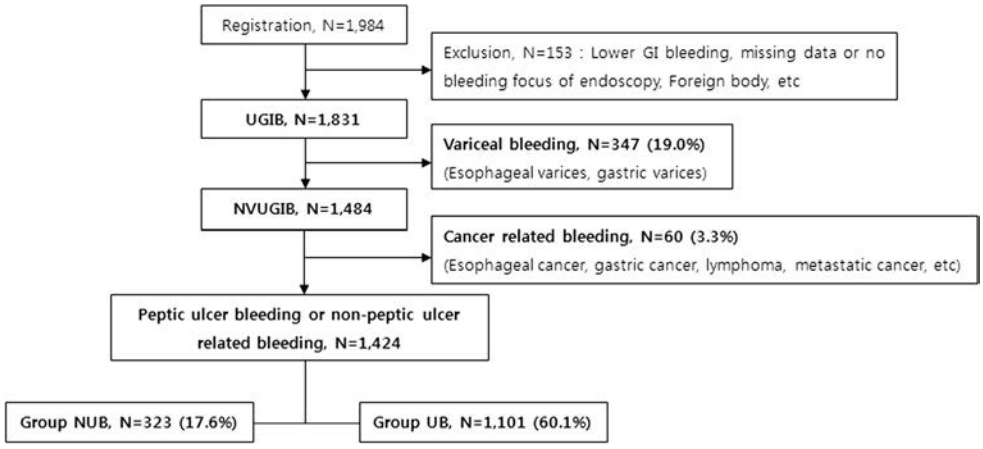


Fig. 1 Flow diagram showing the causes of upper gastrointestinal bleeding. UGIB, upper gastrointestinal bleeding; NVUGIB, non-variceal upper gastrointestinal bleeding. The numbers in parentheses are the proportions of each group relating to patients with UGIB

Risk Assessment

To identify high-risk patients with NUNVUGIB who were expected to have unfavorable outcomes, we analyzed the risk-predictive factors for overall mortality. The RS and GBS were calculated for all patients enrolled in this study (Supplemental Tables).

For the purpose of this analysis, bleeding scores were considered indicative of a high risk of overall mortality if pre-RS was ≥ 4 or full RS was ≥ 6 and a high-risk of the need for clinical intervention if GBS was ≥ 12. The other variables categorized as high-risk factors were patient age ≥ 60 years, hemoglobin level < 10 g/dL, systolic blood pressure < 100 mmHg, pulse rate ≥ 100 beats/min, blood urea nitrogen level ≥ 30 mg/dL, and prothrombin time ≥ 28 s.

Statistics

In a comparison of the clinical and demographic data between Group NUB and Group UB, continuous variables were analyzed using an independent sample *t* test, and the categorical variables were analyzed using the Chi-square test or Fisher's exact test. To compare the clinical outcomes between the two groups, each variable was analyzed using the Chi-square test and adjusted for confounders using multivariate logistic regression models.

We assessed the validity of the scoring systems in Group NUB by using plotting receiver operating characteristic (ROC) curves. Curves of the bleeding scores were plotted for overall mortality, the incidence of re-bleeding, and the need for clinical intervention. The discriminative ability was evaluated by using the area under the ROC curve (AUROC). An AUROC < 0.7 is usually considered to have a poor discriminative ability. An AUROC between 0.7 and 0.8 provides acceptable discrimination, and a test with an AUROC > 0.8 is considered to have an excellent discriminative ability [9].

It is possible to identify the optimal cutoff value for high-risk scores at which the scoring system is most predictable since the ROC curves are plotted over all possible threshold values. For each ROC curve of overall mortality and the need for clinical intervention, we identified the optimal threshold of the RS and GBS by calculating the Youden index for each score level. The cutoff level associated with the highest J coefficient is the one that minimizes the sum of false negatives and false positives.

In the univariate analysis, the associated factors for mortality in Group NUB were identified using the Chi-square test. Next, we analyzed the risk-predictive factors, including the high-risk bleeding scores for overall mortality, by using separate multivariate logistic regression models such as "including bleeding scores" and "excluding bleeding scores" to avoid confounders. All statistical analyses were performed using the SPSS statistical software version 18.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at values of $p < 0.05$.

Results

Characteristics and Clinical Outcomes of Group NUB and Group UB

The characteristics of the patients in each group are given in Table 1. The mean age of the patients was higher in Group UB than in Group NUB (63.7 ± 15.9 years versus 59.1 ± 16.7 years; $p < 0.001$). Mean hemoglobin levels (8.9 ± 2.9 g/dL versus 10.7 ± 7.7 g/dL; $p < 0{\cdot}001$) and systolic blood pressure (115.2 ± 23.7 mmHg versus 120.1 ± 26.7 mmHg; $p = 0.002$) on admission were significantly lower in Group UB than in Group NUB. GBS (11.2 ± 3.6 versus 9.8 ± 4.2; $p < 0.001$) and full RS (4.7 ± 2.1 versus 3.9 ± 2.3; $p < 0.001$) were higher in Group UB than in Group NUB. Similarly, a greater number of patients required endoscopic hemostasis in Group UB than in Group NUB (669, 60.8% versus 176, 54.5%; $p = 0.044$) (Table 1). Nevertheless, there were no statistical differences in overall mortality ($p = 0.387$), bleeding-related mortality ($p = 0.447$), worsening of morbidity ($p = 0.446$), or incidence of re-bleeding ($p = 0.117$) between the two groups. In addition, the proportion of patients undergoing surgical or radiological intervention ($p = 0.193$) or requiring blood transfusion ($p = 0.345$) did not differ significantly between the groups (Table 2).

Group NUB Details by Subgroup

Group NUB was divided into several subgroups according to the causes of bleeding (Fig. 2), including Mallory–Weiss tears, Dieulafoy lesions, acute gastric mucosal lesion/erosion, angiodysplasia, esophagitis, portal hypertensive gastropathy, and aortoenteric fistula. The most common cause of bleeding in Group NUB was Mallory–Weiss tears (51.1%), followed by Dieulafoy lesions (18.9%). Mallory–Weiss tears showed a relatively high re-bleeding rate (10.6%). Dieulafoy lesions showed the highest rates of bleeding-related mortality (6.6%) and need for clinical intervention (73.8%), with the exception of patients with aortoenteric fistula in Group NUB. Dieulafoy lesions were also responsible for the majority of overall mortality, and affected patients had a relatively high re-bleeding rate (11.9%) after portal hypertensive gastropathy (13.3%).

Table 1 Comparison of clinical and demographic data between Group NUB and Group UB

Variable	Group NUB (n = 323)	Group UB (n = 1101)	*p* value
Age, median(range)	59(16-90)	65(18–96)	<0.001
Male, n(%)	248(77.7)	807(73.9)	0.165
Current or ex-smoker, n(%)	151(46.9)	512(46.8)	0.976
Heavy alcoholics, n(%)	106(32.8)	236(21.4)	<0.001
Medication history, n(%)			
Anti-platelet drug	64(19.8)	240(21.8)	0.444
Anticoagulants	14(4.3)	44(4.0)	0.787
NSAIDs	14(4.3)	122(11.1)	<0.001
Comorbidity, n(%)	207(64.1)	688(62.5)	0.601
Presenting symptom, n(%)			
Syncope	7(2.2)	13(1.2)	0.185
Melena	90(27.9)	581(52.8)	<0.001
Initial vital sign (mean ± SD)			
Systolic blood pressure(mmHg)	120.12 ± 26.73	115.21 ± 23.68	0.002
Heart rate(/min)	91.93 ± 21.06	90.52 ± 19.16	0.257
Initial laboratory finding (mean ± SD)			
Hemoglobin(g/dL)	10.65 ± 7.70	8.92 ± 2.86	<0.001
Platelet(k)	214.51 ± 109.15	246.86 ± 109.33	<0.001
Blood urea nitrogen	33.08 ± 23.10	38.67 ± 24.62	<0.001
Prothrombin time(sec)	12.92 ± 11.41	13.64 ± 13.26	0.374
Bleeding scores, median (range)			
Glasgow–Blatchford score	10(0–19)	12(0–23)	<0.001
Pre-Rockall score	2(0–7)	2(0–10)	0.594
Full Rockall score	4(0–10)	5(0–11)	<0.001
Endoscopic hemostasis, n(%)	176(54.5)	669(60.8)	0.044

SD, standard deviation; NSAID, nonsteroidal anti-inflammatory drugs

Table 2 The clinical outcomes between Group NUB and Group UB

Variable	Group NUB N = 323	Group UB N = 1101	OR (Unadjusted)	*p* value (Unadjusted)	OR (Adjusted*)	*p* value (Adjusted*)
Overall mortality	12(3.7)	37(3.4)	1.112	0.753	1.348	0.387
Bleeding-related mortality	6(1.9)	17(1.5)	1.619	0.694	1.447	0.447
Worsening of morbidity	18(6.5)	56(5.9)	1.097	0.742	1.242	0.446
Re-bleeding	30(9.7)	80(7.6)	1.317	0.220	1.430	0.117
Surgery/angioembolization	3(0.9)	22(2.0)	1.297	0.198	0.444	0.193
Blood transfusion	178(60.3)	683(65.7)	1.240	0.115	0.876	0.345

All values are presented as number (%)

OR, odds ratio

*Adjusted for confounder such as age, heavy alcoholics, and endoscopic hemostasis

Validation of Scoring Systems for NUNVUGIB

According to the ROC analysis for assessing discriminative ability, both pre-RS (AUROC = 0.798; 95% CI 0.707–0.890) and full RS (AUROC = 0.794; 95% CI 0.693–0.895) were better at predicting overall mortality than GBS (AUROC = 0.675; 95% CI 0.505–0.845). Of the three scores, GBS (AUROC = 0.783; 95% CI 0.730–0.836) was the most closely correlated with the need for clinical intervention than the other two scores (pre-RS: AUROC = 0.727, full RS: AUROC = 0.682). The discriminative ability of the three bleeding scores was relatively poor when considering re-bleeding. Among the three scoring systems, the maximum AUROC of re-bleeding was obtained using full RS (AUROC = 0.668; 95% CI 0.576–0.760). Based on the optimal cutoff value calculated

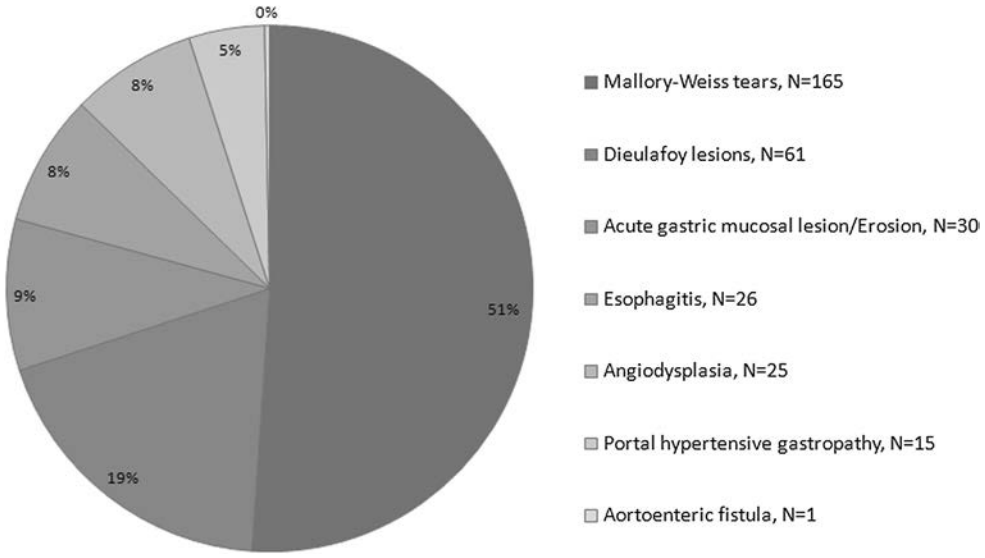
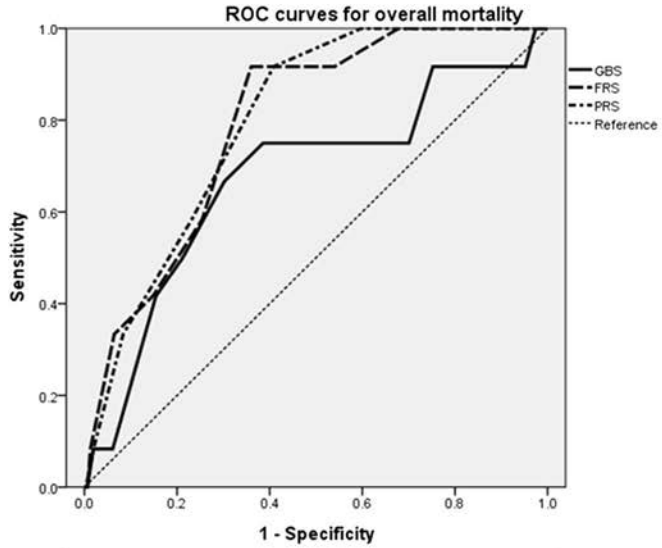
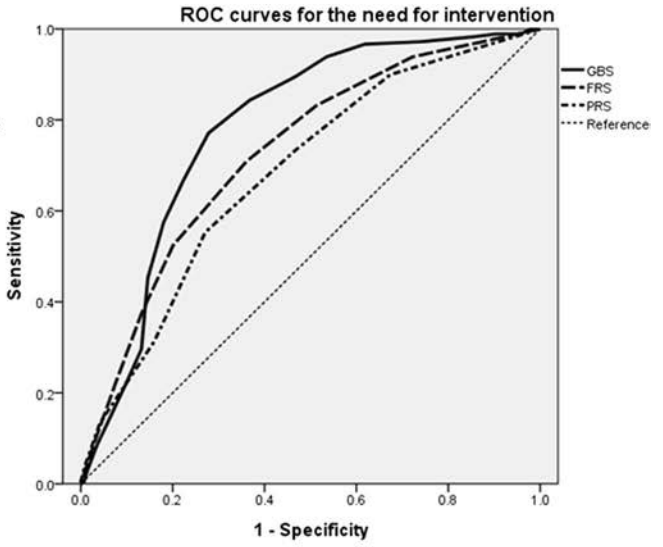


Fig. 2 Subgroups of Group NUB

by the Youden index with ROC curves, the classification of high-risk scores of overall mortality with pre-RS at cutoff ≥ 4 showed 58.3% sensitivity and 76.8% specificity; with full RS at cutoff ≥ 6, a sensitivity of 58.3% and a specificity of 74.6% were observed. High-risk GBS for the need for clinical intervention at cutoff ≥ 12 showed a 57.5% sensitivity and 81.9% specificity (Fig. 3). According to the distribution of bleeding scores, those who had GBS of 0 did not require any interventions, including blood transfusions. Endoscopic hemostasis was not performed in patients with GBS < 2. No patient with pre-RS < 2 points or full RS < 3 points died during the study (Fig. 4).



	AUROC (95% CI)	*p*-value
GBS	0.675(0.505-0.845)	0.04
PRS	0.798(0.707-0.890)	<0.001
FRS	0.794(0.693-0.895)	0.001



	AUROC (95% CI)	*p*-value
GBS	0.783(0.730-0.836)	<0.001
PRS	0.727(0.671-0.782)	<0.001
FRS	0.682(0.624-0.741)	<0.001

Fig. 3 Comparison of the pre-Rockall, full Rockall and Glasgow–Blatchford scores with AUROC figures for the prediction of overall mortality (PRS = 0.798, FRS = 0.794, GBS = 0.675) and the need for clinical intervention (PRS = 0.727, FRS = 0.682, GBS = 0.783). GBS, Glasgow–Blatchford score; PRS, pre-Rockall score; FRS, full Rockall score

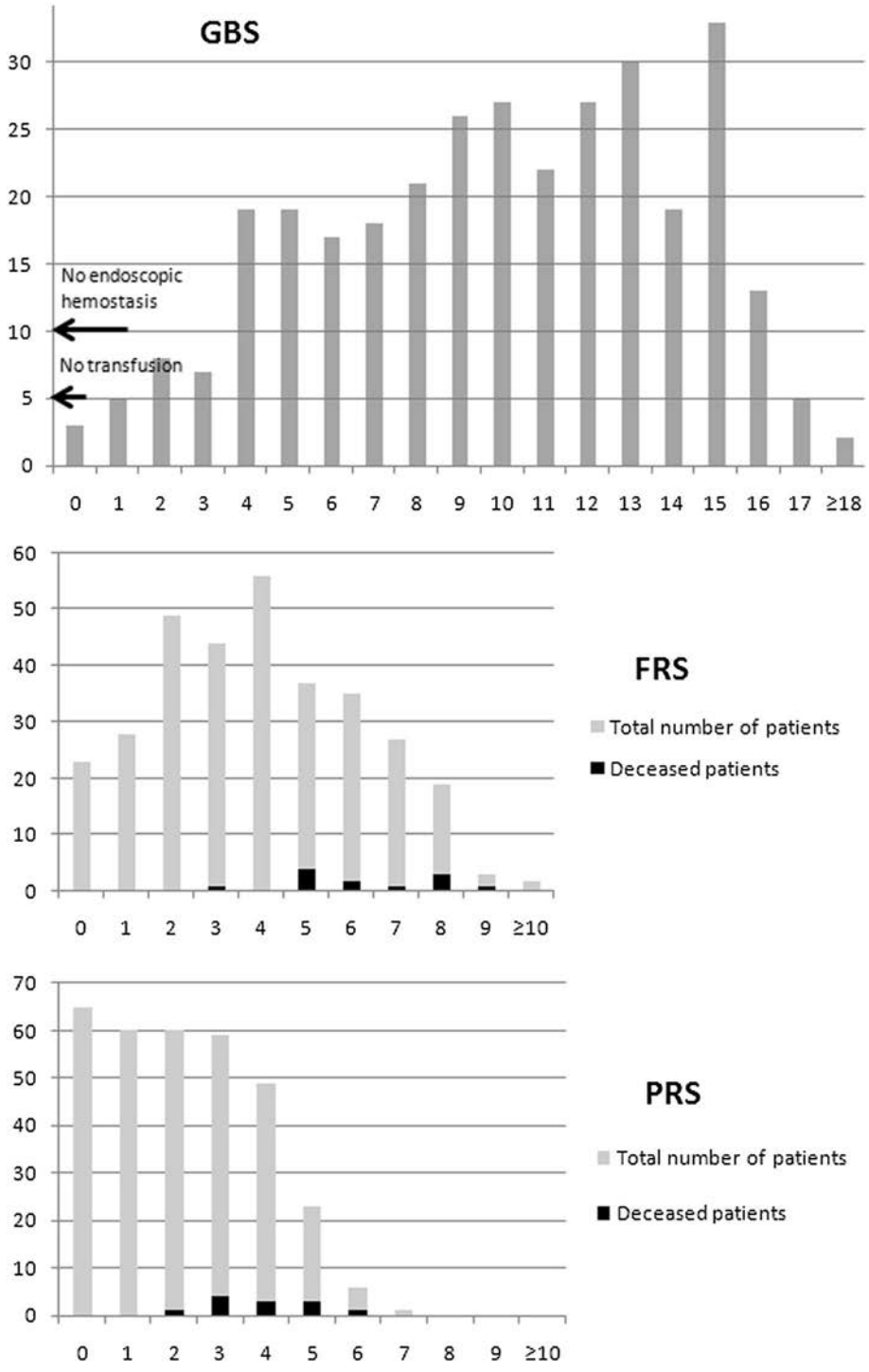


Fig. 4 Distribution of bleeding scores. GBS of 0 did not require any interventions including blood transfusions. Endoscopic hemostasis was not performed in patients with GBS < 2. No patient with a pre-RS < 2 or a full RS < 3 died during 30 days. GBS, Glasgow–Blatchford score; PRS, pre-Rockall score; FRS, full Rockall score

Risk Assessment for 30-Day Mortality in Patients with NUNVUGIB

To identify high-risk patients who were expected to have unfavorable outcomes due to NUNVUGIB, we analyzed the risk-predictive factors for overall mortality. To compare RS with other predictors, pre-RS ≥ 4 and full RS ≥ 6 were included in the analysis of overall mortality. The mortality rate was 3.7% (12 patients). In terms of patients with NUNVUGIB in the univariate analysis, deceased patients had an advanced age ($p < 0.017$) and were more likely to have more than one comorbidity ($p = 0.008$) compared with surviving patients. Surviving patients had evidence of higher hemoglobin levels ($p = 0.012$), normal prothrombin time ($p = 0.023$), and lower pre-RS ($p = 0.003$) and full RS ($p = 0.012$) compared with the

deceased patients. In the multivariate analysis, pre-RS ≥ 4 (OR = 4.971; 95% CI 1.332–18.556; $p = 0.017$) and full RS ≥ 6 (OR = 3.582; 95% CI 0.972–13.199; $p = 0{\cdot}045$) were significantly associated with overall mortality in patients with NUNVUGIB. Among all other variables except for bleeding scores, age > 60 years showed an independently significant association with overall mortality (Table 3).

Discussion

Recently, many updated guidelines for NVUGIB have enabled a more cost-effective use of medical resources and helped improve patient outcomes [10, 11]. However, most attention to date has been focused on the management of peptic ulcer bleeding [3, 12, 13]. In this study, based on prospectively collected data from a multicenter database, we identified the characteristics and clinical outcomes of patients with NUNVUGIB compared with those of peptic

Table 3 Independent predictors of overall mortality from the logistic regression analysis in NUNVUGIB

Variable	Number of patients	Univariate analysis		Multivariate logistic regression analysis					
		Overall mortality, N = 12		Excluding bleeding scores†		Including bleeding scores†			
		p value	OR(95% CI)	*p* value	OR(95% CI)	*p* value‡	OR(95% CI)‡	*p* value†	OR(95% CI)‡
Age > 60 yrs	160	0.017	5.367(1.157–24.893)	0.025	10.600(1.341–83.810)				
Male	248	0.816	1.172(0.309–4.448)						
Comorbidity	207	0.008	1.062(1.026–1.098)	§	§				
Syncope	7	0.559	0.962(0.941–0.983)						
Hematemesis	202	0.363	1.934(0.487–6.911)						
Melena	90	0.124	0.227(0.029–1.782)						
Hematochezia	12	0.389	2.479(0.294–2.941)						
SBP < 100 mmHg	53	0.416	1.733(0.453–6.628)						
HR ≥ 100/min	106	0.199	2.100(0.661–6.675)						
Hb < 10 g/dL	154	0.012	5.799(1.250–26.899)	0.171	3.044(0.619–14.978)	0.064	4.402(0.918–21.113)	0.123	3.546(0.710–17.712)
Plt < 100 k	49	0.333	1.920(0.501–7.360)						
BUN ≥ 30 mg/dL	142	0.311	1.815(0.564–5.844)						
PT ≥ 28 s	14	0.023	5.500(1.070–28.276)	0.185	3.128(0.0.578–16.905)	0.136	3.755(0.661–21.335)	0.121	3.885(0.698–21.638)
Pre-RS ≥ 4	79	0.005	4.647(1.432–15.086)			0.017	4.971(1.332–18.556)		
Full RS ≥ 6	86	0.011	4.115(1.269–13.323)					0.045	3.582(0.972–13.199)

OR odds ratio, *CI* confidence interval, *GBS* Glasgow–Blatchford score, *RS* Rockall score, *SBP* systolic blood pressure, *HR* Heart rate, *Hb* hemoglobin, *Plt* platelet, *BUN* blood urea nitrogen, *PT* prothrombin time, *NUNVUGIB* non-ulcer and non-variceal upper gastrointestinal bleeding

†The predictors that were obtained by univariate analysis were divided into two categories: Those "excluding bleeding scores" and those "including bleeding scores." They were performed the logistic regression analysis as the variable such as age was part of the Rockall score

‡The pre-Rockall scores and full Rockall scores were analyzed separately through logistic regression analysis as the full Rockall scores included some data of the pre-Rockall score

§Comorbidity was excluded as all of deceased patients were more than 60 years old

ulcer bleeding. We performed an analysis of the predictive factors for overall mortality and assessed the validity of the RS and GBS systems in predicting both overall mortality and the need for clinical intervention in patients with NUNVUGIB. Although patients with peptic ulcer bleeding exhibited relatively severe clinical signs such as lower mean hemoglobin level, decreased systolic blood pressure at admission, and older age, as well as increased need for endoscopic hemostasis and higher bleeding scores, there was no significant difference in clinical outcomes, such as overall mortality, bleeding-related mortality, worsening of morbidity, incidence of re-bleeding, surgery, or angioembolization, or transfusion needs between patients with NUNVUGIB and those with peptic ulcer bleeding. The findings of this study are consistent with those of a study from Italy [8]. These outcomes can be attributed to the possibility that patients with NUNVUGIB are less intensively managed because of their less serious clinical presentation.

It is essential that patients at high risk of death be identified early upon admission so that they can be intensively managed. It is also useful to accurately predict patients at high risk of death in order to decide whether they should be treated in a general ward or an intensive care unit. Many studies have verified the usefulness of the RS system in predicting outcomes such as death or re-bleeding in patients with NVUGIB. In our study, several variables such as age $\geq$ 60 years, the presence of more than one comorbidity, and a hemoglobin level $<$ 10 g/dL were associated with an increased risk of overall mortality in patients with NUNVUGIB along with peptic ulcer-related bleeding. As expected in NVUGIB, "high-risk" RS, such as pre-RS $\geq$ 4 and full RS $\geq$ 6, was able to predict which patients with NUNVUGIB had a high risk of death.

Identifying patients with a need for clinical intervention, including blood transfusion, endoscopic hemostasis, surgical treatment, or interventional radiology, assists with early decision making and the timely management of bleeding [14]. Other factors were also associated with the need for clinical intervention; age $\geq$ 60 years, hemoglobin level $<$ 10 g/dL, or blood urea nitrogen level $\geq$ 30 mg/dL at admission were independently associated with an increased risk of the need for clinical intervention. GBS $\geq$ 12 is an effective predictor of the need for early clinical intervention in patients with NUNVUGIB. The results of GBS validation achieved in this study were nearly the same as the widely identified results of previous studies [15–18].

Using the data in this study, we can recommend that patients with NUNVUGIB and pre-RS $\geq$ 4 and full RS $\geq$ 6 be managed at an earlier stage with intensive monitoring. Moreover, elderly patients with NUNVUGIB and GBS $\geq$ 12 should be treated with aggressive hemostatic strategies including endoscopic, radiological, or surgical interventions as well as blood transfusions. Furthermore, this study's findings indicate that such scoring systems are better at identifying low-risk patients. In many studies, patients with full RS $\leq$ 2 have been generally accepted as being at low risk of unfavorable outcomes, while GBS of 0 has been reported to have high sensitivity in identifying those who do not require clinical intervention [15, 19–23]. In our study, patients with both GBS of 0 and full RS $<$ 3 could be considered for management on an outpatient basis after endoscopic diagnosis. Among the three bleeding scores, full RS and pre-RS were superior to GBS in predicting a high risk of death in patients with NUNVUGIB, and GBS showed the best correlation with the need for blood transfusion, endoscopic hemostasis, angioembolization, or surgery; GBS is useful for predicting the need for clinical intervention prior to endoscopy as it does not consider endoscopic data [24, 25]. These study findings are in line with many recently reported studies on the bleeding scores of patients with UGIB [16].

Most cases of NUB have been considered to have minor lesions on endoscopy, which has led to the misconception of a low probability of poor outcomes in terms of mortality. However, in our study, the risk of a poor outcome in NUB was comparable to that in UB. It is important to systemically assess these patients, with consideration of their global health status. RS and GBS can indicate the presence of comorbidities in the absence of endoscopic findings and may be useful in clinical practice. Even in the case of a known bleeding source on endoscopy, especially a minor lesion such as a Mallory–Weiss tear, we should not neglect the lesion but should focus on the patient's general condition if their RS and GBS are high.

Despite the clinical significance of the present study, it has some limitations. First, it was performed in academic or teaching hospitals; therefore, patients with less severe symptoms, such as anemia, as opposed to hematemesis, and minor endoscopically documented lesions, such as erosions, might have been excluded. Indeed, the proportion of peptic ulcer bleeding related to UGIB was higher compared with the average reported in the West [26], which could be result of the highly prevalent *Helicobacter pylori* infection rates in South Korea [27]. Secondly, NUNVUGIB consisted of heterogeneous subgroups with different causes of bleeding; therefore, the need for endoscopic intervention and the modality of endoscopic hemostasis was subjectively determined, and there may have been variability between different endoscopists in terms of their perceptions of high-risk stigmata. In addition, we could not analyze risk factors in subgroup disease entities like Mallory–Weiss tears or Dieulafoy lesions. The heterogeneity of the NUNVUGIB group may have precluded any statistically significant differences in clinical outcomes. Lastly, although more than 1800 patients were enrolled in this study, the number of deceased patients with NUNVUGIB was small. Thus, further prospective

studies using more data are warranted to confirm our results and identify the optimal scoring system for NUNVUGIB.

In conclusion, patients with NUNVUGIB have a similar clinical course as those with peptic ulcer bleeding. RS and GBS are useful for the risk stratification of a small proportion of patient subgroups with NUNVUGIB as well as those with NVUGIB. Thus, the use of these scoring systems for the systematic management of NUNVUGIB should be prioritized.

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Compliance with ethical standards

Conflicts of interest There are no financial or other conflicts of interest to disclose.

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Endoscopy (P Siersema, Section Editor)

What Is the Best Endoscopic Strategy in Acute Non-variceal Gastrointestinal Bleeding?

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Opinion statement

Purpose of review Upper non-variceal gastrointestinal bleeding (UNVGIB) remains an important clinical challenge for endoscopists, requiring skill and expertise for correct management. In this paper, we suggest the best strategy for an effective treatment of this complex category of patients.
Recent findings Early endoscopic examination, the increasingly widespread use of endoscopic hemostasis methods, and the most powerful antisecretory agents that induce clot stabilization have radically modified the clinical scenario for treating this pathology. While hospitalization for digestive hemorrhage is decreasing, the incidence of bleeding seems to be increasing, especially in the elderly for whom a greater use of gastrolesive drugs and the presence of comorbidities are more common.
Summary A multidisciplinary approach for initial patient evaluation and hemodynamic resuscitation prior to endoscopic treatment is crucial for correct management, prevention of rebleeding, and reduction of morbidity and mortality rates and hospital stays**.** Appropriate operator technical expertise, together with the availability of a wide range of endoscopes and devices, is mandatory. Newer endoscopic techniques may improve patient

outcomes for difficult-to-treat lesions. Today, endoscopic hemostasis can be achieved in over 95% of patients.

Introduction

Acute upper non-variceal gastrointestinal bleeding (UNVGIB) is a global phenomenon. Its estimated annual incidence ranges between 50 and 160 cases per 100,000 and commonly requires hospitalization [1].

UNVGIB has significantly high rates of rebleeding, need for surgery, and mortality. This is particularly true for patients at a high risk for ulcer stigmata or with major comorbidities [2, 3] compared to patients with minor lesions or with no significant comorbidities. The hospital mortality rate has decreased over the last 20 years, yet ranges from 2.1 to 2.5% in American nationwide database studies, and from 3.4 to 10% in prospective European observational studies [4]. The role of comorbidities is crucial, affecting the outcome of patients with non-variceal bleedings [5••, 6].

Risk stratification is an important aspect to consider in order to correctly assess timing of endoscopy and hospital discharge. Many scoring systems are available for predicting outcomes following acute UNVGIB. The Glasgow–Blatchford score and Rockall score are the most commonly used [7–9]. Integrated into risk assessment scores, endoscopic findings of a bleeding ulcer have prognostic implications in terms of rebleeding, need for surgery, and mortality. Endoscopy is the treatment of choice for patients with high-risk stigmata for rebleeding based on the Forrest classification: peptic ulcers spurting blood (Forrest Ia) or oozing blood (Ib) and a non-bleeding visible vessel (Forrest IIa). Ulcers affected with an overlying clot (Forrest IIb) should undergo irrigation in order to evaluate the underlying stigmata, followed by appropriate treatment [4, 10, 11]. Patients with low-risk stigmata, e.g., ulcers with pigmented spots of hematin (Forrest IIc) or with fibrin-covered clean base (Forrest III), do not warrant any endoscopic intervention.

The most common causes of acute UNVGIB are non-variceal [2, 3]. These include peptic ulcers in 30–60% of cases (gastric, duodenal, or anastomotic), non-ulcer etiology in 30%, and neoplasia (esophagus, cardia, stomach, and duodenum) in 2–5% [3, 4]. Among the non-ulcer causes, the most frequent are esophageal, gastric, or duodenal mucosal erosive diseases, Mallory–Weiss syndrome, Dieulafoy's, or other vascular lesions, such as hemobilia, angiodysplasia, vascular-enteric fistula, and gastric antral vascular ectasia (GAVE); in very few cases (5–7%), no exact cause can be determined.

Gastrointestinal bleeding prompts an unstable balance of the patient's comorbidities [12].

A true multidisciplinary approach (endoscopist, endoscopy nurse technician, and anesthesiologist) for initial patient evaluation and hemodynamic resuscitation prior to endoscopic treatment is required. Furthermore, the early involvement of other professional specialists (interventional radiologist and surgeon) is important for a tailored operative flowchart taking into consideration complexity of management in this setting of patients due to multiple comorbidities. Sharing information is indeed the key point in order to further reduce the risk of death from non-variceal bleeding [4, 11].

Appropriate resuscitation and stabilization of hemodynamic parameters are essential. Anesthesiology support is particularly required for patients suffering from severe hematemesis, and in these cases, orotracheal intubation should be considered in order to prevent aspiration [4].

Endoscopic procedures should not be done at the expense of adequate resuscitation.

The endoscopy nurse technician plays an important role in the gastroenterology bleeding team. This technician ensures that all equipment is functioning properly before use and, in fact, has to prepare the endoscopy room in order to put all the devices in their correct places. The endoscopy nurse technician must be familiar with rules, standards, practices, and procedures and must have the experience to accomplish the goals of the procedure. The endoscopy nurse technician helps by assisting the procedure, managing staff, and troubleshooting any problems that may arise. Endoscopy technicians must learn how to work fast in a busy environment and must

understand the importance of bleeding-control teamwork.

Upper endoscopy in patients with GI bleeding should be performed within 12–24 h in an adequately equipped setting by qualified teams and operators. All the devices needed for endoscopic hemostasis (injection needles and solutions, monopolar or bipolar thermal probes, mechanical devices such as hemoclips, over-the-scope clips, powders, and suturing systems) must be available and ready for use.

Every patient presenting with UNVGIB at admission should be administered high-dose intravenous bolus of proton pump inhibitors (PPI), followed by continuous infusion (80 mg then 8 mg/h), as recommended. However, PPI infusion should not postpone early endoscopy (within 24 h) [13].

Pre-endoscopic intravenous erythromycin (single dose, 250 mg 30–120 min prior to upper GI endoscopy) can be administered in patients with UNVGIB, with the advantage, in select patients, of improving endoscopic visualization. This results in a reduced need for second-look endoscopy, a decrease in the number of units of blood transfused, and a reduction of hospital stay [14–16].

Endoscopic hemostasis

Endoscopic treatment can be delivered using injection, as well as thermal and mechanical modalities. Some of the more recent endoscopic techniques, such as hemostatic sprays and endoscopic suturing methods, represent an alternative treatment and can potentially improve outcomes for difficult-to-treat lesions. Endoscopic therapy of any kind has been reported to be a more effective treatment compared with pharmacotherapy in patients with FIa, FIb, and FIIa ulcers [17, 18].

When severe non-variceal bleeding presents, there is an endoscopic attempt to rapidly arrive at hemostasis in an approach that is simple, permanent, and safe. There can be a compromise of quality in endoscopic images caused by inadequate visibility due to blood in the gastric lumen. In some cases, a bleeding lesion is not visualized due to the presence of food or hematic debris, which impedes proper endoscopic visualization (this is particularly true in the presence of awkwardly positioned lesions) or due to lesions that are difficult to locate when they are not actually bleeding, in particular, vascular lesions. The therapeutic endoscope of choice in these cases is one with a 3.7-mm operative channel, though on occasion, scopes with 6-mm channel scopes can be needed.

In addition to water-jet pikes for irrigating, and appropriate suction power in the endoscope, in some cases, the patient will have to be placed in positions other than the left lateral decubitus in order to dislocate the blood pool and render the lesion visible.

Endoscopic hemostasis: injection therapy

Endoscopic injection is widely used to stop active ulcer bleeding and to prevent rebleeding from ulcers with visible vessels. The simultaneous uses of hydrostatic pressure, tissue edema, vasoconstriction, and inflammatory changes around the ulcer are the principles at the basis of injection therapy [19, 20]. Injection therapy alone is effective at achieving primary hemostasis. However, in terms of prevention of ulcer rebleeding, it has been reported inferior to other endoscopic hemostasis monotherapies or combination therapy [17, 18, 21].

It might be useful to clean the field of view for a better visualization of the bleeding site in order to proceed precisely with other hemostatic modalities. The most commonly used injectates [19, 22] for controlling bleeding are epinephrine, sclerosants, and tissue adhesives or glues (acrylates and fibrin glue).

Epinephrine is the most commonly used injective therapy because it is widely available, costs little, and is simple. Epinephrine should be diluted 1:10,000 or 1:20,000 saline solution with a standard needle. Normally, four injections, each 1–2 ml in volume, are injected around the target lesions. Higher doses (> 20–30 ml) are more likely to induce cardiovascular side effects, particularly when injected in the area of the gastroesophageal junction and the distal esophagus. However, some endoscopists favor a solution that is more diluted (1: 100,000) to avoid complications [23]. The injections are tangential and made directly into the bleeding source and the surrounding areas until there is halting or slowing of the bleeding, with the surface paling. The mechanisms of epinephrine injection are a local tamponade effect with vessel squeezing, vasoconstriction without vessel thrombosis, and direct effects in the clotting on the arterial defect (platelet aggregation) [19].

International consensus recommendations advocate that epinephrine alone should not be employed. Rather, a second modality of endoscopic treatment should also be used [4, 24]. Epinephrine monotherapy is not as effective as other monotherapies or combination therapy that uses two or more methods to prevent further bleeding in patients at a high risk of stigmata [21, 25].

Sclerosants

Polidocanol, ethanol, ethanolamine, and other sclerosing agents, prompt local inflammation and subsequent fibrosis and lead to obliteration of the vessel's lumen. The effect is much like that of epinephrine, but the volumes are greatly reduced because of the risk of ulceration, necrosis, and perforation [19].

Cyanoacrylate

N-butyl-2-cyanoacrylate is a tissue adhesive. Upon contact with blood, it polymerizes into a firm clot. It can be used to treat refractory peptic ulcer bleeding. It can be undiluted or mixed with cyanoacrylate and lipiodol, which are oily contrast agents used to delay the action of polymerization. The injection technique is not as easy as the one for epinephrine because the glue hardens rapidly and can damage the endoscope, the operator, and the patient if it is accidentally dispersed. When the injection is complete, the glue forms as a hard plug, clotting the bleeding point.

Reports in the literature indicate that cyanoacrylate is effective in the treatment of bleeding Dieulafoy's lesions and, in its spray form, for treatment of difficult-to-control malignant and non-malignant GI bleeding [26, 27].

Use of acrylate glue injection should be restricted to select cases of refractory non-variceal bleeding as a form of rescue therapy when conventional endoscopic therapies fail.

Fibrin glue

Fibrin glue is composed of concentrated fibrinogen and factor XIII. These are then mixed with thrombin and calcium, thus simulating the final stage of the

cascade of clotting. These can be injected subsequently with a standard 23-G injector needle or mixed as two through a special dual-channel needle, thus mixing and then activating the clotting cascade only when injected.

The precise role for fibrin sealant has yet to be defined.

Approach to high-risk lesions with injection therapy alone should be avoided.

Thermal therapy: contact probe

The thermal bipolar contact probe, such as Gold Probe (Boston Scientific), Quicksilver (Cook Endoscopy), and BiCOAG Probe (Olympus), takes advantage of a compression effect and of coagulation induced by vascular protein denaturation and vessel sealing (also known as coaptation).

Bipolar coagulation therapy is more effective than injection therapy alone in high-risk bleeding ulcers [28]. There is no difference between contact vs. non-contact probe [29].

When choosing a device, endoscopists should take into account that the bipolar catheter probe can be applied either perpendicularly or tangentially (Fig. 1).

Non-contact probe: argon plasma coagulation

The argon plasma coagulation (APC) probe emits an ionized argon gas (plasma) that conducts high-frequency monopolar current to the tissue.

Argon gas is sprayed from the tip of the probe toward the targeted tissue. The dispersal of thermal energy is both linear and tangential and with a maximum depth of penetration of 3 mm for applications of up to 5 s. The distance between the tip of the probe and the tissue should be approximately 2 mm.

Since tissue is desiccated and not carbonized, coagulation does not result in smoke production Furthermore, APC is a multidirectional coagulation modality. In fact, it is the electrical field created between the ionized gas and the tissue that determines the direction of the current and does not depend on the direction of the gas flow or the positioning of the probe (Fig. 2).

APC is an easy technical approach and can treat lesions that are awkwardly positioned. It has a reduced depth of penetration and has been shown to be effective in the treatment of extended superficial vascular lesions, such as watermelon stomach and vascular ectasias. It also helps control NVUGIB.

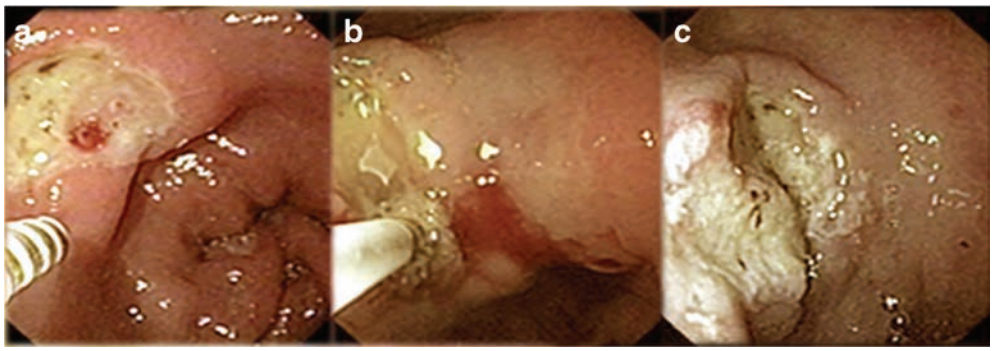

Fig. 1. a Gastric ulcer with NBVV (Forrest IIa). **b** Thermic therapy with contact probe. **c** After treatment.

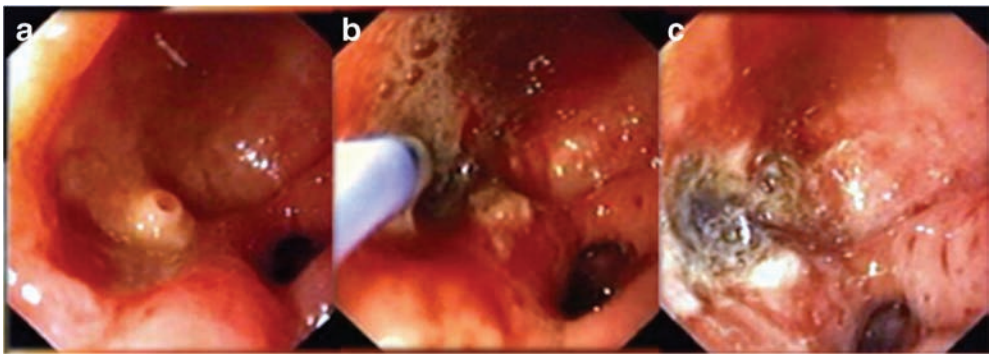

Fig. 2. a Gastric ulcer with NBVV (Forrest IIa). **b** Thermic non-contact therapy with argon plasma coagulation (APC). **c** After treatment.

Nonetheless, due to the superficial coagulation this non-contact device provides, it can miss some deeper vessels with insufficient hemostasis. As a result, it should be used only for non-actively bleeding lesions, within the protocols of combined therapy.

Comparative studies have reported that the rebleeding rate after APC in ulcers is comparable to injective therapy, to hemoclips, and other contact thermal devices, e.g., the heater probe [30, 31].

All cautery methods are equally effective tools, and the choice essentially depends on the experience of the operator and the type and site of the bleeding lesion [17, 18].

Mechanical modalities

Wide varieties of hemostatic mechanical therapies have been developed over the years.

Hemostasis can be achieved by clips placement by means of direct compression or tamponade of vessels and tissue approximation of bleeding stigmata, with minimal to no tissue damage. This ideal combination of safety and efficacy notwithstanding, the use of clips is highly dependent on the location and size of the bleeding lesion and the operator's expertise.

Through-the-scope endoclips are made by various companies and come in many different sizes, lengths, and shapes, and with different grasping and rotational abilities, and deployment mechanisms (Fig. 3).

OTSC Ovesco (Ovesco Endoscopy AG, Tübingen, Germany) is a fairly new endoscopic device. It is mounted onto the endoscope's distal tip. Much like rubber-band ligation or mucosal resection devices, it is designed for tissue approximation [32]. It has been used to close perforations and fistulas, and in the treatment of post-polypectomy bleeding. There are several case series reporting its use as second-line therapy following failure of standard endoscopic treatment of bleeding peptic ulcers [32–36].

Mechanical therapy with through-the-scope clips substantially reduces the risk of re-bleeding and the need for surgery (78%) compared with injections alone. However, there is no indication that there is a statistically significant difference in mortality [17].

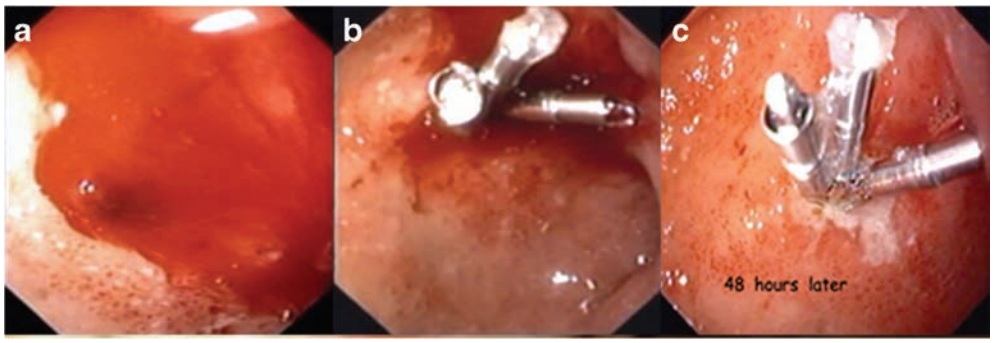

Fig. 3. **a** Active bleeding (Forrest Ib) from duodenal ulcer. **b** Mechanical therapy with hemoclips. **c** Two days later.

Dual therapy, i.e., injection of epinephrine and another therapy, has been definitively proven to be better than injection therapy alone, substantially reducing the risk of re-bleeding and need for surgery, and with a trend toward reduced mortality [21]. The combination of injection therapy and mechanical treatment does not seem to offer further clinical benefits. Clips compared with thermo-coagulation demonstrated no improvement in definitively controlling hemostasis, reducing the need for surgery, or mortality, alone or in combination with injection therapy.

Meta-analyses have revealed that combined endoscopic hemostasis therapy (diluted epinephrine injection and a second hemostasis modality, e.g., injectable, thermal contact probe, or clips) is preferable to injection therapy alone, though not to clips or contact thermal therapy alone [18, 21]. Mechanical therapy with clips is safe, and induces only limited tissue injury, and may be a first choice option in patients at high risk of stigmata and elevated INR [1.5–2.0] [4].

The use of hemoclips might be limited because of the difficulty of achieving a frontal position in the posterior walls of the duodenal bulb and posterior wall of gastric body, in addition to the lesser curve of the stomach. An additional limitation is a scarring ulcer that can limit the possibility of drawing near the tissues.

Hemostatic powders

Hemospray

Hemospray (Cook Medical, Ireland) is an easy-to-use hemostatic mineral powder [37] that has been described as an effective treatment option in gastrointestinal active bleeding lesions. The possibility to control a diffuse bleeding from a large area (like in neoplastic bleeding lesions) represents one of the main advantages of its use. In fact, in this setting of lesions, current endoscopic modalities (such as clip placement or other targeted techniques) are often unfeasible [38•, 39, 40, 41]. Thus, application of Hemospray does not require any *en-face* positioning of the scope, making easier the treatment of such lesions in a cumbersome position. Furthermore, since it is a non-contact technique, Hemospray potentially reduces the risk of perforation arising from other standard modalities (Fig. 4).

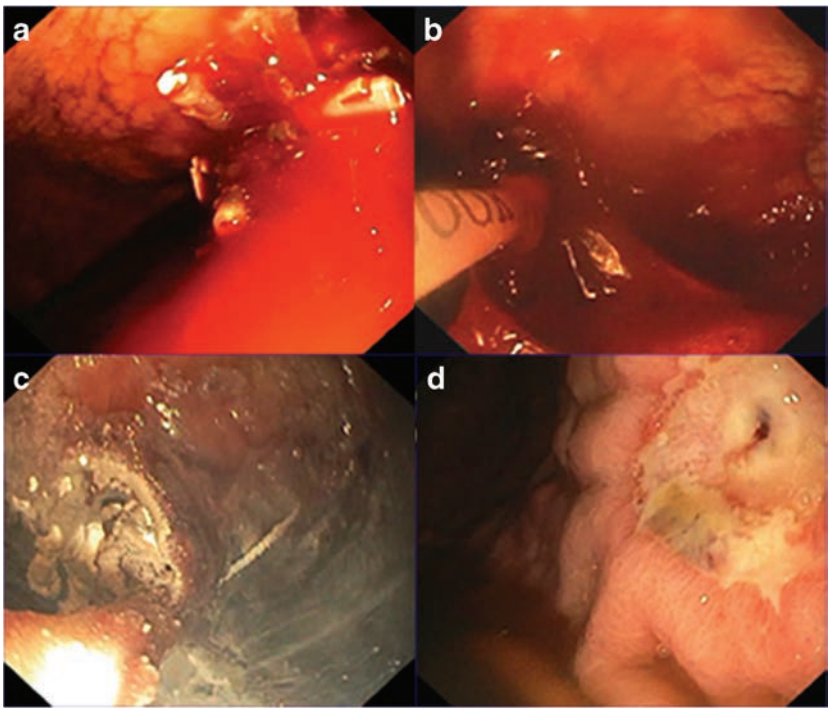

Fig. 4. **a** Active severe bleeding (Forrest Ia) from ulcer at proximal gastric body after unsuccessful hemostasis with Thermal therapy and hemoclips. **b** Catheter for rescue therapy with hemostatic powder (Hemospray). **c** Final result. **d** 72 h later.

During the entire procedure (insertion of the catheter, release of the powder), the endoscopist needs to avoid the suction. The contact between the powder and the water inside the working channel and, consequentially, in the catheter effects the activation and the hardening of the powder, causing the occlusion of both the catheter and channel [41–43].

Hemospray provides a safe hemostasis of actively bleeding lesions and should be used as a bridge modality toward more definitive therapy [44].

EndoClot (polysaccharide hemostatic system)

EndoClot particles have a molecular structure that, similarly to Hemospray, absorbs water from the blood. This results in a concentration of platelets, red blood cells, and coagulating proteins at the site of the bleeding. This results in both an acceleration of the physiological clotting cascade and in a rapid production of a matrix that is gelled and adheres to and seals the bleeding tissue. However, in theory, a high flow of arterial bleeding may represent a limitation in the use of EndoClot. In fact, high pressure from arterial spurting can produce a sort of "cleaning" effect, causing some difficulty for the hemostatic layer to form [45–47].

Endoscopic suturing

The endoscopic suturing device (OverStitch, Apollo Endosurgery Inc., Austin, TX) requires the use of a dual channel endoscope to mount the device.

The device has three components: an end cap with a hinged, hollow, curved needle, a needle driver handle that opens and closes the arm of the suture, and an anchor exchange catheter [48]. Some additional components are required, e.g., the suture attached to a tissue anchor (acting as a T tag), a device for cinching, and a customized corkscrew-like retracting device (Helix, Apollo Endosurgery Inc., Austin, TX). Patients with large chronic marginal ulcers for whom conventional medical management failed were treated with the suturing device with complete ulcer healing [49, 50].

The process of suturing is technically complex, resulting in limitations in the use of the device, particularly in terms of limited maneuverability, and the impossibility of accessing certain sites. The process also calls for a dual channel endoscope, and visibility during active bleeding can be impaired. In addition, the endoscopic suturing device may be limited in managing active bleeding and may well be preferable for treating chronic ulcers or for preventing bleeding after endoscopic submucosal dissection [51].

Conclusion

International guidelines have well defined the correct approach in cases of UNVGIB.

Endoscopy should be performed within 24 h; only major-risk lesions should be treated, injection therapy with epinephrine alone is not recommended, thermic or mechanical therapy alone or in combination with injection therapy are the most effective treatment, and mechanical or thermic monotherapy have the same effectiveness.

Endoscopists must bear in mind not to care only about the source of bleeding, but particularly about the patient. An episode of gastrointestinal bleeding prompts an unstable balance of the patient's comorbidities. This is a condition that can occur both with major peptic ulcer bleeding and from "minor" lesions, such as vascular lesions, Mallory–Weiss tears, or gastroduodenal erosions [12]. Careful evaluation of the patient's illness and early and aggressive endoscopic and pharmacologic treatment has contributed to improving outcomes in the management of UNVGIB [52, 53].

A "winning strategy" begins long before the patient is positioned in the endoscopy room.

Before starting the procedure, invite your nursing staff to verify that all endoscopic instruments and devices are handy and ready to use and that the surgical unit is properly functioning.

At this point, the technical abilities and the experience of the operator are crucial.

Peptic ulcers are the most frequent cause of bleeding in UNVGIB, and a significant part of the literature on endoscopic therapy refers to treatment of these lesions. Vascular anomalies that most commonly cause UGIB include angiodysplasia, Dieulafoy's lesion, and gastric antral vascular ectasia. Other vascular lesions, such as hemobilia and vascular-enteric fistula, can occur. Neoplastic lesions (both primary and metastatic) of the upper GI tract account for 2 to 8% of the cases of UNVGIB.

Knowledge of different sources of bleeding is crucial for the most effective and adequate endoscopic therapy.

Faced with a bleeding vessel, it is always recommended to quickly elaborate a working strategy. Choose the best approach after *considering the type of lesion, the characteristics of the surrounding tissue, its location, and the possibility of maneuvering the endoscope and hemostatic devices.*

A proper and successful endoscopic strategy includes the use of the correct device at the right time, and for the right locations.

The choice of a different approach may depend on the type of lesion, particularly when located in difficult-to-access sites (lesser gastric curvature, posterior duodenal wall, gastric fundus). In some cases, a transparent cap mounted on the tip of the endoscope can be helpful.

It is important, whenever possible, to consider the possibility of a second (combined) therapy; some procedures (e.g., mechanical therapy), if ineffective, can render difficult or impossible use of other therapeutic modalities.

Before proceeding to hemostasis of a difficult lesion due to its location, especially if not actively bleeding at the moment, consider the real possibilities of an approach to the lesions and the therapeutic feasibility in case of major bleeding. A well-visible lesion at first is not always well approachable later.

In case of tricky lesions, do not forget the therapeutic effectiveness of radiological vascular procedures and surgery.

The effectiveness of the endoscopic hemostatic modalities is currently well known, as witnessed by studies in the literature. Several meta-analyses and international guidelines have identified and certified the validity of each of them, alone, or in combination. The experience, expertise, and technical abilities of the operators (endoscopists, anesthesiologists, nursing staff) play a crucial role and can make a difference in the successful endoscopic strategy (Fig. 5).

THE ENDOSCOPIST'S RULEBOOK FOR A WINNING STRATEGY

1. *A "winning strategy" begins long before the patient is positioned in the endoscopy room*
2. *Endoscopy should be performed only when the patient is haemodynamically stable*
3. *Nursing staff have to verify properly functioning and ready to use all instruments and devices before starting the procedures*
4. *Remember that you have to treat both the bleeding lesion and the Patient.*
5. *Elaborate a "working strategy" and choose the best approach based on evaluation of the type of lesion, surrounding tissue, location, maneuverability of the endoscope and hemostatic devices.*
6. *Use the correct device at the right time and for the right locations*
7. *In case of combination therapy consider that some techniques (such as mechanical therapy), if used first, can hamper following techniques.*
8. *A well visible lesion at first is not always well approachable later*
9. *The experience, expertise and technical abilities of the operators (endoscopists, anesthesiologists, nursing staff) can make a difference in the successful endoscopic strategy.*

Do not forget the therapeutic effectiveness of radiological vascular procedures and surgery.

Fig. 5. Conclusions.

Compliance with Ethical Standards

Conflict of Interest

Livio Cipolletta declares that he has no conflict of interest.
Fabio Cipolletta declares that he has no conflict of interest.
Granata Antonio declares that he has no conflict of interest.
Ligresti Dario declares that he has no conflict of interest.
Barresi Luca declares that he has no conflict of interest.
Tarantino Ilaria declares that she has no conflict of interest.
Mario Traina declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

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ORIGINAL ARTICLE

A Positive Correlation Between Gastric and Esophageal Dysmotility Suggests Common Causality

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Abstract

Background Gastric and esophageal dysmotility syndromes are some of the most common motility diagnoses, but little is known about their interrelationship.

Aims The aim of our study was to determine if a correlation exists between gastric and esophageal dysmotility syndromes.

Methods We reviewed the records of all patients who underwent both solid gastric emptying scintigraphy (GES) and high-resolution esophageal manometry (HRM) within a 2 year period, with both done between August 2012 and August 2017. All GESs were classified as either rapid, normal, or delayed. All HRMs were classified according to the Chicago Classification 3.0. Correlations were assessed using Fisher's exact test and multiple logistic regression.

Results In total, 482 patients met inclusion criteria. Of patients with a normal, delayed, and rapid GES, 53.1, 64.5, and 77.3% had an abnormal HRM, respectively ($p < 0.05$ vs. normal GES). Likewise, patients with an abnormal HRM were more likely to have an abnormal GES (54.9 vs. 41.8%, $p = 0.005$). Multiple logistic regression showed abnormal GES [odds ratio (OR) 2.14], age (OR 1.013), scleroderma (OR 6.29), and dysphagia (OR 2.63) were independent predictors of an abnormal HRM. Likewise, an abnormal HRM (OR 2.11), diabetes (OR 1.85), heart or lung transplantation (OR 2.61), and autonomic dysfunction (OR 2.37) were independent predictors of an abnormal GES.

Conclusions The correlation between an abnormal GES and HRM argues for common pathogenic mechanisms of these motility disorders, and possibly common future treatment options. Clinicians should have a high index of suspicion for another motility disorder if one is present.

Keywords Gastroparesis · Gastric emptying · Esophageal motility disorder · Diabetes mellitus · Lung transplantation · Enteric nervous system

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Introduction

Some of the most commonly encountered disorders of gastrointestinal motility are esophageal and gastric. Gastroparesis is estimated to affect 33.4 per 100,000 people as measured in a Minnesota population-based study [1]. The epidemiology of established esophageal motility disorders is less well defined, but in one center, 65% of patients presenting for esophageal manometry had a motility disorder [2]. Multiple overlapping risk factors have been identified for gastroparesis and esophageal dysmotility which include diabetes, prior lung or heart transplantation, Parkinson's disease, scleroderma, and amyloidosis [3–10]. These disorders represent systemic disorders, and presumably effects on gastrointestinal motility would be diffuse and affect multiple sites in the gastrointestinal tract.

There have been a few smaller studies evaluating the correlation between esophageal and gastric dysmotility in specific patient populations. These studies have been somewhat conflicting, finding a correlation in patients with scleroderma [4], but not in patients with diabetes [6, 11, 12].

To date there are no larger studies evaluating the relationship between gastric and esophageal dysmotility. This study sought to address this deficiency by evaluating a large and diverse population of patients presenting to a tertiary care referral center.

Methods

Cohort Acquisition

The STRIDE interface (Stanford Translational Research Integrated Database Environment) was used to search the electronic medical records to return an appropriate cohort for our study [13]. We specifically searched for all patients who had both a gastric emptying study (GES) and high-resolution esophageal manometry (HRM) within 2 years of each other, with both occurring between 8/31/12 and 8/31/17. All records were reviewed with the aid of the STRIDE interface. In addition to basic demographic and symptom information at the time of the studies, GES and HRM records were reviewed in detail. Special care was taken to ensure that these studies did not occur on opposite sides of a major procedure such as a transplantation or other major gastrointestinal surgery. If so, such patients were excluded from the analysis. Patients were also excluded if their studies were incomplete, or if they did not have a solid GES. The study was approved by the institutional review board at Stanford University.

Gastric Emptying Study Interpretation

Only patients with solid GES were included. These studies were conducted according to standard protocol. A standard meal consisting of eggbeater, toast, jam, and water was labeled with Tc-99 sulfur colloid 0.528 mCi. Subsequent static images were obtained at 1, 2, 3, and 4 h. Occasionally, the 4-h time point was not acquired if the 3-h time point had emptying > 90%. All images were interpreted by experienced staff radiologists. Normal ranges for gastric emptying were defined according to the consensus recommendations of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine [14] and were as follows: at 1 h 10–70%, at 2 h 40–100%, at 3 h 70–100%, and at 4 h 90–100%. Gastric emptying studies were classified as "delayed" if any time point was below the normal range and "rapid" if the 1-h time point was above the normal range. If both delayed and rapid components present, they were classified as rapid for the purposes of this analysis. Otherwise the studies were classified as normal.

High-Resolution Esophageal Manometry Interpretation

All HRM studies were performed according to standard protocol. Catheters were either placed during endoscopy or by blind passage. At least 10 supine 5-ml swallows were acquired for interpretation. All HRM studies were read by experienced gastroenterologists. For all HRM studies, the raw numbers were reviewed, and in specific cases, the individual topography plots were examined. All studies were re-classified according to the Chicago 3.0 classification scheme, if this was not done on the initial manometry reading [15]. Having any Chicago Classification 3.0 motility disorder was considered abnormal, otherwise the studies were considered normal.

Statistical and Data Analysis

The main goal of our analysis was to determine if there was a correlation between abnormal HRM and abnormal GES. The percentage of abnormal HRM studies among patients with delayed and rapid gastric emptying were compared to normal gastric emptying using Fisher's exact test. Confidence intervals were calculated using the binomial distribution. Conversely, the percentage of abnormal GESs among different HRM abnormalities were compared to normal HRM using Fisher's exact test, and confidence intervals were calculated using the binomial distribution.

Logistic regression was used to calculate both adjusted and unadjusted odds ratios for predictors of interest. Separate models were run to determine predictors of an

abnormal HRM, with the main variable of interest being abnormal GES, gastric emptying at 1, 2, 3, 4 h, and gastric emptying at the final time point (either gastric emptying at 4 h, or 3 h if the 4-h time point was not acquired). A model to determine predictors of an abnormal GES was also run and included abnormal HRM as the main variable of interest. All models contained the following variables: age, gender, race, body mass index, diabetes, prior heart or lung transplantation, scleroderma, autonomic dysfunction (based on either clinical impression or formal autonomic testing), the presence of nausea or vomiting, the presence of gastroesophageal reflux symptoms, the presence of atypical chest pain, the presence of dyspepsia, and the presence of dysphagia. Several subgroups were analyzed including heart or lung transplantation patients, diabetics, patients with autonomic dysfunction, patients with scleroderma, patients with no known risk factors for dysmotility, patients who had GES and HRM done within 3 months of each other versus greater than 3 months, and patients who had GES done first versus HRM first.

Results

Demographics

Of the total 512 patients identified by the STRIDE cohort discovery tool, 482 were eligible for inclusion in our study. The remaining 30 were excluded either because they did not undergo the appropriate testing, or because testing was performed on opposite sides of transplant or foregut surgery. The basic demographics of this cohort are seen in Table 1. The mean age was 49.8 years (range 18–85), 33.4% were male, 35.7% had diabetes, and 27.2% had a heart or lung transplantation. Overall 50.4% had a normal GES, 45.5% had a delayed GES, and 4.6% had a rapid GES. In total, 59.3% had an abnormal HRM.

Table 1 Basic cohort demographics

Variable	Value
Mean age (years, range)	49.8 (18–85)
Male gender (%)	33.4
Race (%)	
White	60.8
Black	3.1
Hispanic (any race)	16.5
Asian	10.2
Other	9.4
Mean BMI (SD)	25.8 (6.1)
Diabetes (%)	35.7
Type I	3.1
Type II	27.8
Cystic fibrosis related	4.8
Parkinson's disease (%)	0.6
Amyloidosis (%)	0.8
Autonomic dysfunction (%)	12.2
Scleroderma (%)	6.9
Heart or lung transplant (%)	27.2
Lung transplant only	24.9
Heart/lung transplant	2.1
Heart transplant only	0.2
Symptoms (%)	
Nausea or emesis	36.9
Typical GERD	67.8
Atypical chest pain	6.6
Dyspepsia	54.2
Dysphagia	36.1
Diarrhea	1.6
Gastric emptying (%)	
Normal	50.4
Delayed	45.0
Rapid	4.6
HRM abnormal (%)	59.3
Achalasia	6.2
EGJOO	12.5
Absent contractility	7.7
Distal esophageal spasm	11.4
Jackhammer esophagus	5.8
Ineffective esophageal motility	21.0
Fragmented peristalsis	2.1
2+ HRM abnormalities	7.1

BMI body mass index, *GERD* gastroesophageal reflux disease, *HRM* high-resolution esophageal manometry, *EGJOO* esophagogastric junction outflow obstruction

Prevalence of Abnormal High-Resolution Manometry in Patients with Normal, Delayed, and Rapid Gastric Emptying

Among all patients with a normal GES, the rate of an abnormal HRM was 53.1%. Patients with a delayed GES had an abnormal HRM a higher percentage of the time at 64.5% ($p = 0.014$ vs. normal GES), and patients with rapid GES (classified as such even if delayed components were present) had an even higher abnormal HRM rate at 77.3% ($p = 0.042$ vs. normal GES) (Fig. 1). When comparing rapid gastric emptying to delayed gastric emptying, there was no statistical difference in abnormal HRM rate. A separate analysis of GESs with only rapid emptying ($n = 16$) and no delayed component demonstrated a similar abnormal HRM rate of 75%, but there was no statistical difference from normal GES given smaller sample size.

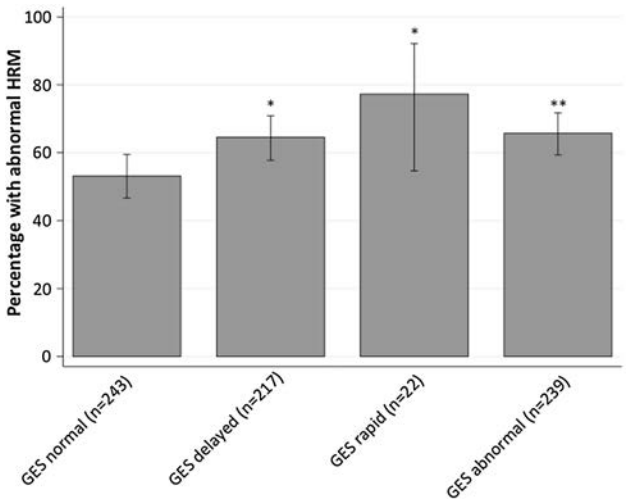

Fig. 1 Prevalence of abnormal high-resolution manometry in patients with normal, delayed, and rapid gastric emptying. Error bars represent 95% confidence intervals calculated by the binomial distribution. $*p<0.05$; $**p<0.01$ compared to normal gastric emptying (Fisher's exact test). *GES* nuclear medicine solid gastric emptying scintigraphy, *HRM* high-resolution esophageal manometry

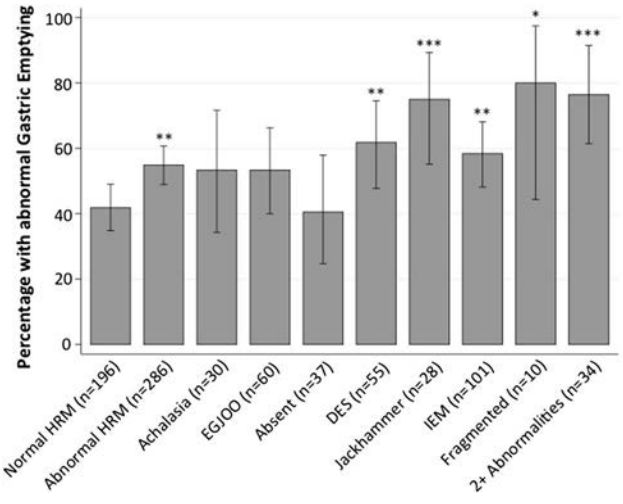

Fig. 2 Prevalence of abnormal gastric emptying stratified by esophageal dysmotility subtypes. Each graphed percentage represents the percentage with an abnormal gastric emptying scintigraphy. Patients with two or more manometric abnormalities appear in that category as well as other categories, depending on the abnormalities. Error bars represent 95% confidence intervals calculated by the binomial distribution. $*p<0.05$; $**p<0.01$; $***p<0.001$ compared to normal HRM (Fisher's exact test). *HRM* high-resolution esophageal manometry, *EGJOO* esophagogastric junction outflow obstruction, *DES* distal esophageal spasm, *IEM* ineffective esophageal motility

Prevalence of Abnormal Gastric Emptying Stratified by Esophageal Dysmotility Subtypes

Overall, more patients had an abnormal GES if they had an abnormal HRM versus if they had a normal HRM (54.9 vs. 41.8%, $p=0.005$) (Fig. 2). The distribution of abnormal gastric emptying was asymmetric among different HRM abnormalities, with distal esophageal spam (DES), jackhammer esophagus, ineffective esophageal motility (IEM), and fragmented peristalsis having higher prevalence in general (Fig. 2). Those patients with 2 or more manometric abnormalities had a particularly high rate of gastric emptying abnormalities (76.5%, $p<0.001$ compared to normal HRM, $p=0.009$ compared to 1 manometric abnormality).

Predictors of an Abnormal HRM Using Logistic Regression

An abnormal GES was a predictor of an abnormal HRM in both adjusted and unadjusted analyses, with an adjusted odds ratio (OR) 2.14, 95% confidence interval (1.41–3.26), $p<0.001$. Other independent positive predictors (after adjustment) of an abnormal HRM were age [OR 1.013 (1.000–1.026) $p=0.05$], scleroderma [OR 6.29 (1.84–22.2) $p=0.004$], and dysphagia [OR 2.63 (1.59–4.33) $p<0.001$]. A single negative predictor was the presence of nausea or vomiting [OR 0.60 (0.39–0.93) $p=0.02$] (Table 2). When evaluating the continuous variable of gastric emptying percentage in separate models, the 2, 3, 4, and final hour time points emerged as independent negative predictors (lower gastric emptying values predicted a higher probability of an abnormal HRM) (Table 2).

Subgroup Analysis

An abnormal GES was an independent predictor of an abnormal HRM in adjusted analyses for patients with a history of diabetes [OR 2.54 (1.18–5.46) $p=0.01$]. There was a trend toward a relationship for patients with a history of transplant but was not significant [OR 2.18 (0.87–5.47) $p=0.10$] (Table 3). The analysis was limited for patients with dysautonomia, scleroderma, and no risk factors for dysmotility given their small sample sizes. Only unadjusted analyses were carried out, and of those all were not statistically significant (Table 3). The results were similar when the analysis was reversed, and abnormal HRM was used as a predictor of abnormal GES (data not shown).

Additional subgroup analyses evaluated questions about time course of events in the same multiple logistic regression model. There were no significant differences when evaluating abnormal GES as a predictor of abnormal HRM if both studies were done within 3 month of each other versus between 3 months and 2 years of each other [OR 2.19 (1.31–3.66); OR 2.82 (1.20–6.61), respectively]. Additionally, there were no significant differences if the GES was done first or HRM was done first [OR 2.30 (1.28–4.15); OR 2.35 (1.23–4.52), respectively] (Table 3). The results were

Table 2 Predictors of an abnormal high-resolution esophageal manometry

Variable	Unadjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value
Abnormal GES	1.69 (1.17–2.44)	**0.005**	2.14 (1.41–3.26)	**< 0.001**
Age	1.015 (1.003–1.027)	**0.01**	1.013 (1.000–1.026)	**0.05**
Male gender	0.74 (0.51–1.09)	0.13	0.87 (0.56–1.35)	0.54
Race (compared to white)				
Black	1.96 (0.61–6.29)	0.26	2.02 (0.59–6.97)	0.26
Hispanic (any race)	0.81 (0.49–1.33)	0.40	0.70 (0.40–1.21)	0.20
Asian	1.03 (0.56–1.91)	0.92	1.04 (0.53–2.07)	0.90
Other	1.75 (0.88–3.48)	0.11	1.71 (0.91–3.80)	0.09
BMI	0.999 (0.968–1.031)	0.96	0.99 (0.96–1.03)	0.59
Diabetes	0.80 (0.55–1.16)	0.24	0.79 (0.48–1.31)	0.36
Heart or lung transplant	0.78 (0.52–1.17)	0.38	0.82 (0.46–1.45)	0.49
Scleroderma	7.54 (2.27–25.1)	**0.001**	6.29 (1.84–22.2)	**0.004**
Autonomic dysfunction	1.28 (0.72–2.25)	0.40	1.31 (0.70–2.45)	0.40
Symptoms				
Nausea or vomiting	0.70 (0.48–1.02)	0.07	0.60 (0.39–0.93)	**0.02**
GERD	1.08 (0.73–1.59)	0.70	0.53 (0.93–2.51)	0.10
Atypical chest pain	1.00 (0.48–2.07)	1.00	1.16 (0.50–2.67)	0.73
Dyspepsia	0.73 (0.51–1.06)	0.10	0.68 (0.45–1.03)	0.07
Dysphagia	2.15 (1.44–3.19)	**< 0.001**	2.63 (1.59–4.33)	**< 0.001**
Other predictor variables				
GES at 1 h	1.003 (0.994–1.012)	0.49	1.002 (0.992–1.012)	0.71
GES at 2 h	0.995 (0.988–1.002)	0.19	0.992 (0.984–1.000)	**0.05**
GES at 3 h	0.991 (0.984–0.998)	**0.014**	0.987 (0.979–0.996)	**0.003**
GES at 4 h	0.987 (0.978–0.995)	**0.003**	0.982 (0.972–0.992)	**0.001**
GES final time point	0.987 (0.979–0.995)	**0.002**	0.982 (0.973–0.992)	**< 0.001**

Bold values indicate p values < 0.05

Logistic regression was used to assess predictors of an abnormal high-resolution esophageal manometry, with the main predictor variable of interesting being an abnormal gastric emptying study

OR odds ratio, *CI* confidence interval, *GES* gastric emptying scintigraphy, *BMI* body mass index, *GERD* gastroesophageal reflux disease

Table 3 Predictors of an abnormal high-resolution esophageal manometry among specific subgroups

Abnormal GES as a predictor of abnormal HRM	Unadjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value
Heart or lung transplant ($n = 131$)	1.66 (0.79–3.49)	0.182	2.18 (0.87–5.47)	0.10
Diabetes ($n = 172$)	1.76 (0.94–3.33)	0.08	2.54 (1.18–5.46)	**0.02**
Autonomic dysfunction ($n = 59$)	0.77 (0.24–2.45)	0.66	Sample too small	
Scleroderma ($n = 33$)	0.29 (0.02–3.57)	0.33	Sample too small	
No risk factors ($n = 34$)	0.94 (0.24–3.77)	0.93	Sample too small	
GES and HRM (within 3 months of each other) ($n = 330$)	1.69 (1.09–2.63)	**0.02**	2.19 (1.31–3.66)	**0.003**
GES and HRM (3 months–2 years apart) ($n = 152$)	1.76 (0.90–3.45)	0.10	2.82 (1.20–6.61)	**0.02**
GES done first ($n = 259$)	1.77 (1.08–2.92)	**0.02**	2.30 (1.28–4.15)	**0.005**
HRM done first ($n = 223$)	1.59 (0.92–2.74)	0.10	2.35 (1.23–4.52)	**0.01**

Bold values indicate p values < 0.05

Logistic regression was used to assess predictors of an abnormal solid gastric emptying scintigraphy specifically in patients with prior heart or lung transplantation, diabetes, autonomic dysfunction, scleroderma, no risk factors for dysmotility, patients in whom GES was done first (and vice versa), and patients in whom GES and HRM were done within 3 months of each other. Both adjusted and unadjusted analyses were done where sample size was sufficient

OR odds ratio, *CI* confidence interval, *HRM* high-resolution esophageal manometry

similar when the analysis was reversed, and abnormal HRM was used as a predictor of abnormal GES in the same logistic regression model (data not shown).

Predictors of an Abnormal GES Using Logistic Regression

An abnormal HRM was a predictor of an abnormal GES in both adjusted and unadjusted analyses, with an adjusted odds ratio of 2.11 (1.39–3.23) $p < 0.001$. Other independent positive predictors (after adjustment) of an abnormal GES were diabetes [OR 1.85 (1.14–3.02) $p = 0.01$], prior heart or lung transplantation [OR 2.61 (1.50–4.55) $p = 0.001$], and the presence of autonomic dysfunction [OR 2.37 (1.26–4.46) $p = 0.007$] (Table 4).

Discussion

Our study was the first large cross-sectional analysis evaluating the relationship between gastric and esophageal dysmotility in a diverse group of patients, with the ability to correct for potential interacting variables and symptoms. Our adjusted logistic regression model suggested a roughly 2.1-fold increase in the odds of having esophageal dysmotility in patients with an abnormal GES and vice versa ($p < 0.001$) (Tables 2, 4). Our data argue for shared pathogenic mechanisms for many gastric and esophageal dysmotility syndromes and suggest a clinically significant overlap that should be considered in the evaluation and subsequent treatment of these patients. While it is conventional to consider the esophagus and stomach as separate compartments and education often focuses on esophageal and gastric dysmotility as separate entities, the anatomy and physiology of the two regions are contiguous and it seems illogical that the effects of common disease processes would affect only one portion.

Indeed, it is not surprising that such overlap exists given that many syndromes known to cause gastric emptying abnormalities are also thought to cause esophageal dysmotility [3–10]. In our study, only patients with diabetes were found to have a positive association between altered gastric emptying and esophageal dysmotility, though there was a trend toward an association in heart/lung transplant patients. Other groups evaluated did not show an association, including those with dysautonomia, scleroderma, and no known risk factors for dysmotility. However, numbers were small in these groups limiting the statistical evaluation. Unlike our study, prior studies in diabetic patients did not show a clear association between gastric and esophageal dysmotility, but

Table 4 Predictors of an abnormal gastric emptying scintigraphy

Variable	Unadjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value
Abnormal HRM	1.69 (1.17–2.44)	**0.005**	2.11 (1.39–3.23)	**< 0.001**
Age	0.994 (0.983–1.006)	0.33	0.992 (0.979–1.005)	0.23
Male gender	1.19 (0.81–1.75)	0.37	1.04 (0.67–1.61)	0.86
Race (compared to white)				
Black	1.41 (0.49–4.06)	0.53	1.16 (0.36–3.76)	0.80
Hispanic (any race)	1.01 (0.62–1.67)	0.96	1.07 (0.62–1.85)	0.80
Asian	0.50 (0.27–0.94)	**0.031**	0.55 (0.27–1.11)	0.09
Other	0.83 (0.44–1.54)	0.54	0.89 (0.46–1.73)	0.73
BMI	1.010 (0.98–1.04)	0.70	1.01 (0.97–1.04)	0.61
Diabetes	2.69 (1.83–3.96)	**< 0.001**	1.85 (1.14–3.02)	**0.01**
Heart or lung transplant	2.98 (1.95–4.55)	**< 0.001**	2.61 (1.50–4.55)	**0.001**
Scleroderma	0.641 (0.31–1.32)	0.29	0.83 (0.37–1.85)	0.65
Autonomic dysfunction	2.37 (1.32–4.23)	**0.003**	2.37 (1.26–4.46)	**0.007**
Symptoms				
Nausea or vomiting	1.22 (0.85–1.78)	0.28	1.38 (0.90–2.11)	0.18
GERD	0.79 (0.54–1.16)	0.23	0.67 (0.41–1.09)	0.32
Atypical chest pain	1.16 (0.57–2.39)	0.68	1.55 (0.71–3.41)	0.28
Dyspepsia	0.62 (0.43–0.88)	**0.009**	0.76 (0.51–1.14)	0.19
Dysphagia	0.96 (0.66–1.39)	0.81	0.89 (0.55–1.44)	0.64

Bold values indicate *p* values < 0.05

Logistic regression was used to assess predictors of an abnormal solid gastric emptying scintigraphy, with the main predictor variable of interest being an abnormal high-resolution esophageal manometry. Both adjusted and unadjusted analyses were done

OR odds ratio, *CI* confidence interval, *HRM* high-resolution esophageal manometry, *BMI* body mass index, *GERD* gastroesophageal reflux disease

these studies may have been limited by smaller sample size and inability to correct for other variables [6, 11, 12]. Associations may exist among other gastrointestinal dysmotility syndromes, though more studies are needed. One such study found that having an abnormal esophageal manometry predicted lower survival and a higher need for TPN in patients with chronic intestinal pseudo-obstruction [13].

The precise underlying pathophysiology of gastrointestinal motility disorders remains unclear, but it is reasonable to assume that similar mechanisms may be at play. In achalasia, histological specimens from both early and late disease exhibit increased inflammatory cell infiltration as compared to controls [16]. Outside of achalasia, inflammatory infiltrates are also observed in other esophageal dysmotility syndromes, including nutcracker esophagus, DES, and IEM at rates higher than controls [17]. Similarly, higher inflammatory cell infiltrates are also seen in gastroparesis [18]. It remains unclear if histological inflammation causes gastrointestinal dysmotility or is a consequence of local factors, such as stasis, that result from dysmotility.

An evaluation of the time course of events showed that gastric dysmotility is not more likely to occur prior to esophageal dysmotility or vice versa based on our subgroup analysis seen in Table 3. Additionally, studies done in closer temporal proximity are not more likely to show concordance than studies done further apart (Table 3). These results suggest that similar underlying pathogenic mechanisms are at play in the GI tract that do not show preference for one organ over the other.

Our study also argues for the importance of gastrointestinal motor over activity in dysmotility syndromes. Patients with rapid gastric emptying had an especially high rate of HRM abnormalities (Fig. 1). Additionally, patients with spastic and hypercontractile disorders of the esophagus had a higher than average rate of altered gastric emptying (Fig. 2). In fact many motility disorders likely have components of hypermotility and hypomotility. For example, rapid gastric emptying is thought to be a result of impaired fundic relaxation/accommodation, greater antral contraction, and/or lower pyloric resistance [19]. In contrast, gastroparesis may have a component of significant pyloric spasm in certain cases along with gastric body hypocontractility. Prior work using functional luminal imaging probe (FLIP) demonstrated a lower pyloric distensibility in gastroparesis as compared to controls, possibly indicating spasm. Such patients showed a good symptomatic response to pyloric dilation [20]. Similarly, a recent non-randomized trial using gastric per oral endoscopic myotomy in patients with gastroparesis showed a response rate of 86% [21]. However, the role of pyloric spasm or hyperactivity in gastroparesis remains unclear, since two small randomized control trials evaluating pyloric botox injection did not show benefit over placebo [22, 23]. Likewise, achalasia often represents a mix of esophageal body motor over activity and under activity, and treatment is targeted mostly at a spastic, non-relaxing lower esophageal sphincter [24].

Our study has several strengths and limitations. First, the number and diversity of our cohort were significant enough to allow meaningful statistical analyses for most variables. Additionally, our cohort was large enough to allow analysis of rapid gastric emptying, a less common phenomenon on which limited study has been performed. Finally, all manometries were re-analyzed using Chicago Classification 3.0. Thus, we were able to analyze each Chicago Classification 3.0 abnormality separately, and determine differential patterns of GES abnormalities. Our study is limited in its evaluation of dysmotility in patients with scleroderma, autonomic dysfunction, Parkinson's disease, and amyloidosis specifically because of smaller sample sizes. Bias might also have skewed our results since our sample was not random and patients were only included in most cases if they had symptoms to warrant both a GES and HRM. Finally, the HRM and GES were not necessarily obtained at the same visit, and it is possible that medical changes occurred in that interim not captured in our review.

In conclusion, our cross-sectional analysis shows patients with gastric dysmotility are more likely to have esophageal dysmotility and vice versa, arguing for a common causative and pathogenic etiology of dysmotility in many cases. Clinicians should consider the presence of an undiagnosed disorder of esophageal dysmotility in patients with abnormal gastric emptying and vice versa.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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NEUROGASTROENTEROLOGY AND MOTILITY DISORDERS OF THE GASTROINTESTINAL TRACT (S RAO, SECTION EDITOR)

New Approaches to Diagnosis and Treatment of Functional Dyspepsia

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Abstract

Purpose of Review The purpose of this article is to review the recent literature and discuss the new approaches to the diagnosis and treatment of functional dyspepsia (FD).

Recent Findings According to the recent American College of Gastroenterology (ACG) and Canadian Association of Gastroenterology (CAG) guideline for dyspepsia, *Helicobacter pylori* (*H. pylori*) eradication is recommended as a first treatment option, and proton pump inhibitors (PPIs), tricyclic antidepressants, and prokinetics are listed as second-line therapy. On the other hand, in the Japanese guideline for FD, PPIs and prokinetics are recommended as the first-line treatment. In Japan, acotiamide, a recently launched prokinetic, showed significant efficacy in several clinical trials performed either in Japan or Europe. Regarding non-pharmacological treatment, recent topics include acupuncture, electrical stimulation, gastric peroral endoscopic myotomy, and meal and lifestyle modification. These treatments have provided significant efficacy, which provides some insights into the main pathophysiology of this disease.

Summary Although FD is common among functional gastrointestinal disorders, it is not easy to relieve the dyspeptic symptoms of FD patients. Combinations of pharmacological and non-pharmacological treatment options are expected.

Keywords Functional dyspepsia (FD) · Diagnosis · Treatment · Proton pump inhibitor (PPI) · Acotiamide · Tricyclic antidepressant (TCA)

Introduction

Functional dyspepsia (FD), one of the major functional gastrointestinal disorders, refers to epigastric symptoms that occur without apparent organic disease, based on the premise that gastric dysfunction may be the cause [1••, 2]. FD is a common condition that affects up to 20% of the population [3] and is known to significantly impair patients' quality of life (QOL). Therefore, FD patients should be appropriately diagnosed and managed. However, the management of FD is challenging. Pharmacological treatment is often used in the clinical setting, yet its effectiveness has been reported to be very low. In this article, the recent literature is reviewed, and the new approaches to diagnosis and treatment of FD are discussed.

This article is part of the Topical Collection on *Neurogastroenterology and Motility Disorders of the Gastrointestinal Tract*

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Diagnosis of FD

Diagnostic Criteria of FD

FD is diagnosed based on symptoms and defined by the Rome criteria, where typical symptoms of FD are described as bothersome postprandial distention, early satiety, epigastric pain, and epigastric burning.

The Rome criteria for FD were revised in 2016 (Rome IV), and the updated guideline of the American College of Gastroenterology (ACG) and Canadian Association of Gastroenterology (CAG) was published in 2017 [1••, 4••]. In Rome IV, FD is classified as postprandial distress syndrome (PDS) or epigastric pain syndrome (EPS), based on the presence or absence of an association with meals, as well as symptom persistence for 3 of the prior 6 months; these descriptors

are similar to those in the Rome III criteria. "Bothersome" symptoms are highlighted in the revised Rome IV criteria more than in the Rome III criteria [5]. As there was an overlap of PDS and EPS in Rome III, in Rome IV, epigastric pain and burning after meal ingestion were included among PDS symptoms to increase specificity and reduce overlap between these categories. In the ACG/CAG guideline, FD is defined by symptoms of dyspepsia (predominantly epigastric pain) that last at least 1 month and are potentially associated with any other upper gastrointestinal symptom, such as epigastric fullness [4••].

In Japan, *Helicobacter pylori* gastritis has been covered by medical insurance since 2013; all regimens for eradication of *H. pylori* infection are covered by national health insurance. The clinical practice guidelines for FD in Japanese and English versions were issued by the Japanese Society of Gastroenterology in 2014 and 2015, respectively [2] (Fig. 1). These guidelines are based on the grading of recommendations assessment, development, and evaluation (GRADE) system, and FD is defined as "a condition chronically presenting symptoms centered in the upper abdomen, such as epigastric pain or discomfort, in the absence of any organic, systemic, or metabolic disease that is likely to explain the symptoms". In this definition, the symptoms and duration of the disease are not specified, unlike in the Rome IV criteria. These statements are written to be widely distributed and inform primary care physicians of this condition. In fact, it is up to each physician to determine whether the patients' symptoms are those of FD or not. In another statement in the Japanese guidelines, it is also stated that "FD is not identical to chronic gastritis" [6]. In Japan, FD is a diagnosis recognized by the insurance plan, and the medical fee for this condition is covered by medical insurance. Further recognition of this condition is needed to provide appropriate management for dyspepsia patients without organic causes.

New Motility Tests for the Diagnosis of FD

In the pathophysiology of FD, gastric motility disorders and visceral hypersensitivity are important factors that are directly associated with symptom deterioration [7–10]. According to previous reports, adaptive relaxation disorders and delayed gastric emptying are particularly associated with upper gastrointestinal symptoms. Barostat testing is generally regarded as the gold standard for measurement of gastric adaptive relaxation, but it has been used only for research purposes at a limited number of institutions because of its invasiveness as a test. A new measurement of the adaptive relaxation response

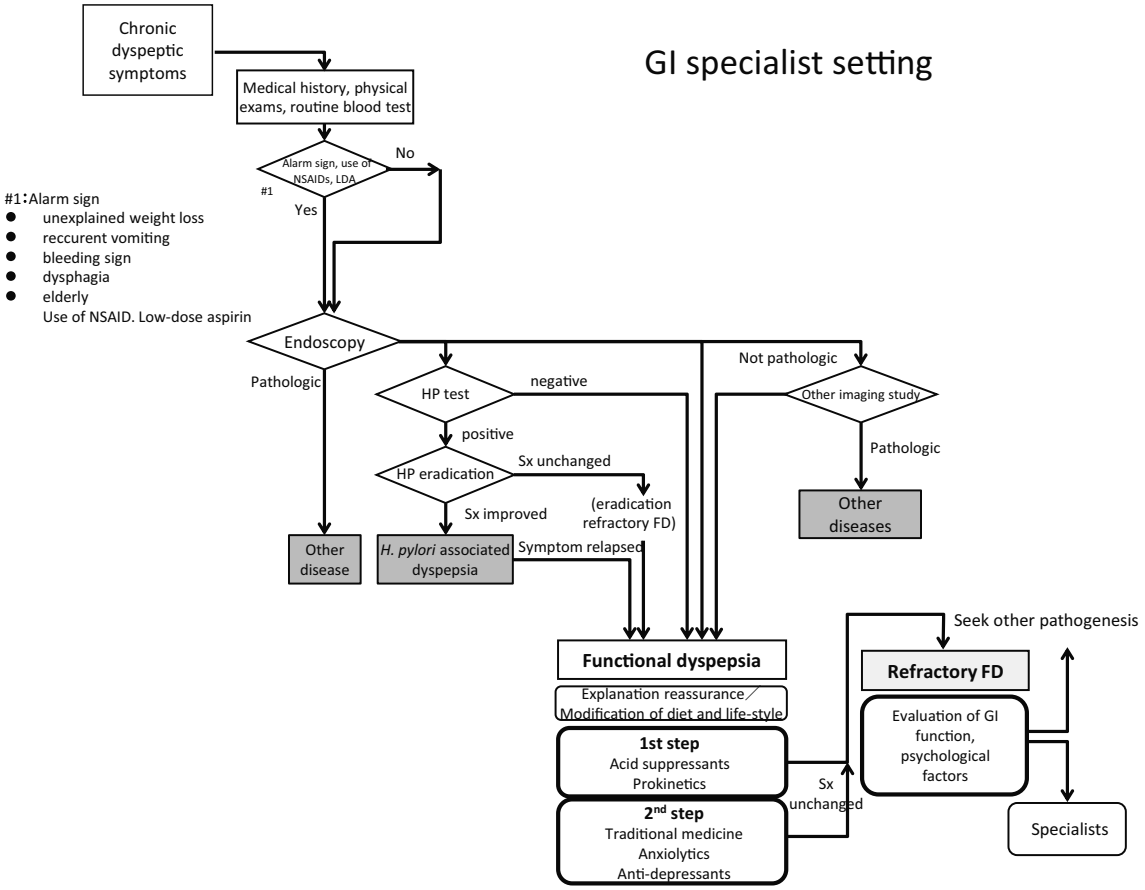

Fig. 1 Algorithm for diagnosis and treatment of FD in Japan (ref. 2). FD, functional dyspepsia

of the stomach using high-resolution manometry has attracted attention as a new noninvasive technique that can even be used in children [11•, 12]. Regarding the evaluation of gastric emptying, the 13-C urea breath test (UBT) is currently often used. There is overlap in the dyspeptic symptoms of idiopathic gastroparesis and FD, but only a minority of FD patients have delayed gastric emptying, suggesting that UBT is of particular value in distinguishing between idiopathic gastroparesis and functional dyspepsia. However, these tests are only regarded as auxiliary methods for making the diagnosis.

Biomarkers of FD

No biomarker of FD has been identified. Although differences in ghrelin, cholecystokinin, serotonin, leptin, gastrin, calcium, and various genetic polymorphisms have been reported, no definite diagnostic indicator has been used in the diagnosis of FD. Nuclear magnetic resonance (NMR)-based analytical approaches to metabolomics have been used to identify changes in levels of glutamine, alanine, proline, high-density lipoprotein, β-glucose, α-glucose, low-density lipoprotein, and very low-density lipoprotein in patients with FD [13].

Treatment of FD

Generally, treatment of FD should be based on the etiology of the condition. However, as is well known, FD is a multifactorial and complex disease, and treatment based on its pathogenesis is difficult. Therefore, the current strategy for pharmacological treatments is empirical. In many guidelines or treatment recommendations, *Helicobacter pylori* (*H. pylori*) eradication is used as the treatment for infection-positive patients. Among many drugs besides *H. pylori* eradication treatment, acid inhibitory drugs, such as proton pump inhibitors (PPIs), are considered as the first treatment option, following the use of psychotropic agents and prokinetics. In the recently published ACG/CAG guideline, *H. pylori* eradication is also recommended as a first-line treatment option, and PPIs, tricyclic antidepressants (TCAs), and prokinetics are listed as second-line therapy (Figs. 2 and 3). Among the prokinetic agents, acotiamide, which has shown significant efficacy in several clinical trials performed either in Japan or Europe, has been introduced in Japan.

Recent non-pharmacological treatments include acupuncture, electrical stimulation, gastric peroral endoscopic myotomy (G-POEM), and meal and lifestyle modification. These treatments have provided significant efficacy, and they provide insight into the main pathophysiology of this disease. However, more evidence is necessary to establish them as treatments for FD (Fig. 3).

H. pylori Eradication Therapy

Eradication of *H. pylori* has been reported as both effective and ineffective for dyspepsia. However, based on the meta-analyses, *H. pylori* infection is thought to be associated with the onset of dyspepsia [14, 15]. *H. pylori* is considered to be a trigger of dyspepsia, but not in all cases of FD. In addition, eradication therapy is at least likely to reduce the risk of peptic ulcer or gastric cancer. The Kyoto global consensus meeting concluded the following: the disappearance or improvement of dyspepsia at 6–12 months after eradication of *H. pylori* defines *H. pylori*-associated dyspepsia and should be regarded as different from FD [16]. This consensus is also in the Rome IV criteria [1••]. Given the above, it is thought that eradication therapy should be performed because it is clearly effective for symptoms, although it is not significantly effective for FD.

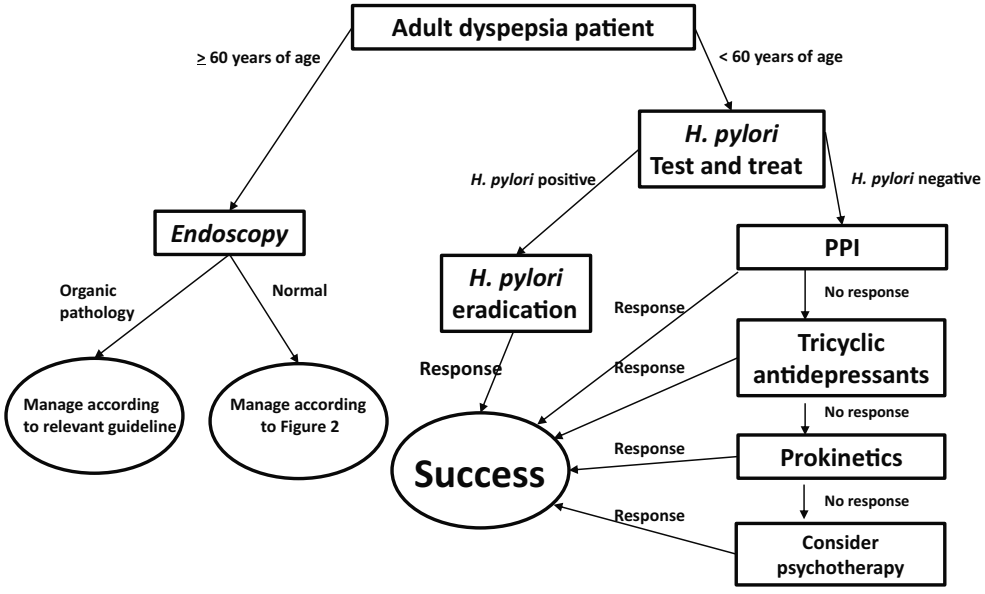

Fig. 2 Algorithm for the management of undiagnosed dyspepsia according to the ACG/CAG clinical guideline (ref. 4). ACG/CAG, American College of Gastroenterology and Canadian Association of Gastroenterology

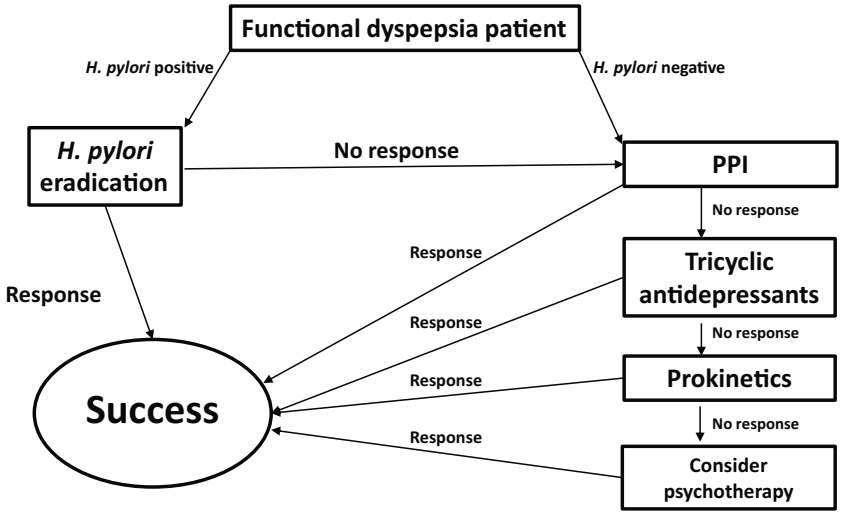

Fig. 3 Algorithm for the management of dyspepsia according to the ACG/CAG clinical guideline (ref. 4). ACG/CAG, American College of Gastroenterology and Canadian Association of Gastroenterology

The recent ACG/CAG guideline also recommends eradication of *H. pylori* as first-line treatment for patients with FD aged 60 years and older who are diagnosed as *H. pylori*-positive after organic diseases are ruled out by esophagogastroduodenoscopy [4••] (Fig. 1). In addition, for patients under the age of 60 years, noninvasive testing is recommended, and patients should be treated for *H. pylori* infection if positive.

Proton Pump Inhibitors

PPIs are considered first-line treatment for patients who are diagnosed with FD. Although many studies and meta-analyses have reported the effectiveness of PPIs, only around 14% of patients with dyspepsia show improvement [17•, 18]. Generally, there is no difference in acid-secretion function between FD and controls. However, it has been reported that postprandial acid exposure in the duodenum is greater in some patients with FD [19]. Patients with FD who have strong acid exposure in the duodenum may experience more severe symptoms than patients with FD who have normal acid exposure. It has been reported that motility and clearance in the duodenum are reduced by instillation of acid into the duodenum, and various upper abdominal symptoms may be more severe in patients with FD [20, 21]. Recent data suggest that low-grade inflammation is present in the duodenum of FD patients. The focus of research in the field has moved to gastric motility, low-grade inflammation, and mucosal permeability of the duodenum [22–24]. A recent study reported that PPIs suppress the duodenal eosinophilia of FD patients [25].

Prokinetic Agents (Acotiamide)

Although meta-analyses have reported the relatively high effectiveness of prokinetic agents, reports are inconsistent. It is likely that publication bias occurred. Moreover, previous studies used cisapride, which has been withdrawn due to cardiovascular side effects. Therefore, many specialists have questioned the efficacy of prokinetic agents. Acotiamide was released in 2013 in Japan, and it improves enterokinesis by acting directly on peripheral synaptic clefts to increase acetylcholine levels, while other prokinetic agents such as mosapride and trimebutine act on the nerve plexus in the gastrointestinal tract. Large-scale, double-blind, placebo-controlled trials have proven that acotiamide is effective for patients who are classified as having PDS-type FD [26, 27]. A recent study using gastric scintigraphy reported that acotiamide significantly improved gastric accommodation and emptying, thereby relieving abdominal fullness in patients with PDS-type FD [28] (Figs. 4 and 5). In addition, chronic administration of acotiamide appears to be safe and effective and improves patients' QOL [29••]. In recent reports, a PPI combined with acotiamide reduced upper abdominal postprandial distention and early satiety more significantly in patients with FD and heartburn. Therefore, greater therapeutic efficacy may result from proper treatment based on a correct understanding of patients' complaints [30].

Tricyclic Antidepressants

The ACG/CAG guideline recommended the use of TCAs for FD following a trial of PPIs, and TCAs should be used prior to prokinetic agents when PPIs are not effective (Fig. 2). The effects of psychoactive agents on FD were examined in 13 randomized controlled trials (RCTs), and their usefulness was reported, with a number needed to treat (NNT) of six [31•, 32, 33]. In addition, three trials reported the usefulness of TCAs [34]. RCTs showed no apparent advantage of selective serotonin reuptake inhibitors (SSRIs) for symptoms of

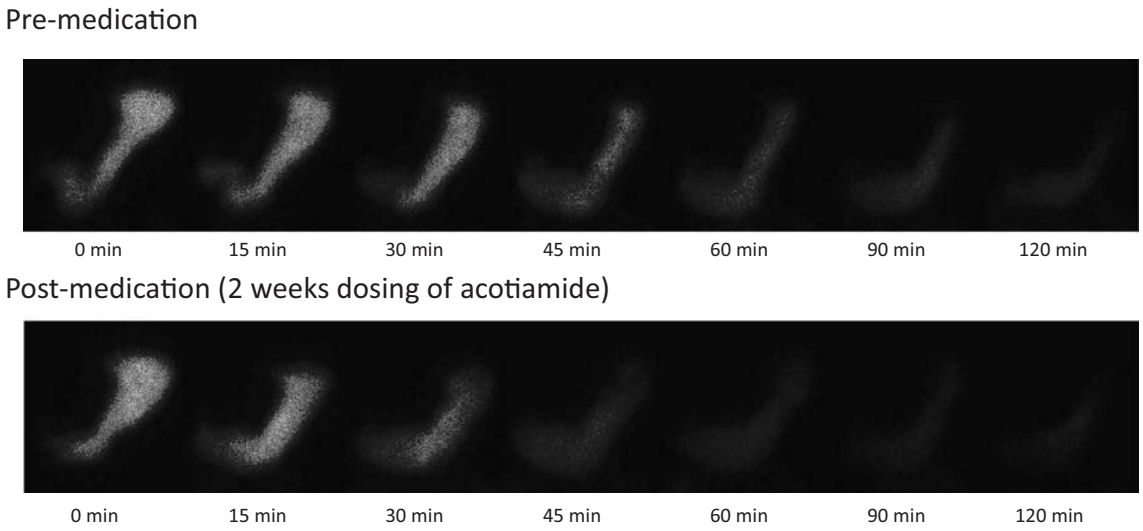

Fig. 4 Effect of acotiamide on gastric motility using gastric scintigraphy. Representative images of the gastric scintigraphy of Japanese FD patients of pre and post medication of acotiamide. The gastric radioactivity was apparently increased after 2 weeks of dosing of acotiamide, suggesting that gastric accommodation was clearly improved. This is a representative image of the patients who participated in our clinical study (ref. 28). FD, functional dyspepsia

FD. In the ACG/CAG guideline, TCA is recommended as second-line therapy following use of PPIs.

Kampo Medicines

In the Japanese FD guidelines, some Kampo medicines are recommended for patients who do not respond to PPIs and prokinetic agents. Although the effectiveness of some Kampo medicines, such as hangekobokuto and rikkunshito, has been reported, no high-quality or well-organized reports have been published. A recent RCT using placebo and rikkunshito showed significant improvement of dyspeptic symptoms in patients with PDS-type FD [35•, 36] (Fig. 5). Recent advances in making placebo with similar smell and taste to rikkunshito could make the evidence along these lines more reliable; it is hoped that further high-quality evidence will become available (Fig. 6).

Acupuncture

Many articles have been published on the use of acupuncture for dyspepsia, mainly in China. However, the sample size was small in all articles, and few reports provided high-level evidence for an active or placebo effect. RCTs comparing acupuncture and sham treatment reported that acupuncture significantly improved dyspeptic symptoms. However, a meta-analysis comparing acupuncture and prokinetic agents found no significant differences in the improvement of dyspepsia [37, 38]. A recent RCT comparing percutaneous electrical and normal acupuncture found that the former was effective for dyspepsia in patients with refractory FD [39]; however, further detailed examination is required because of a faulty study design.

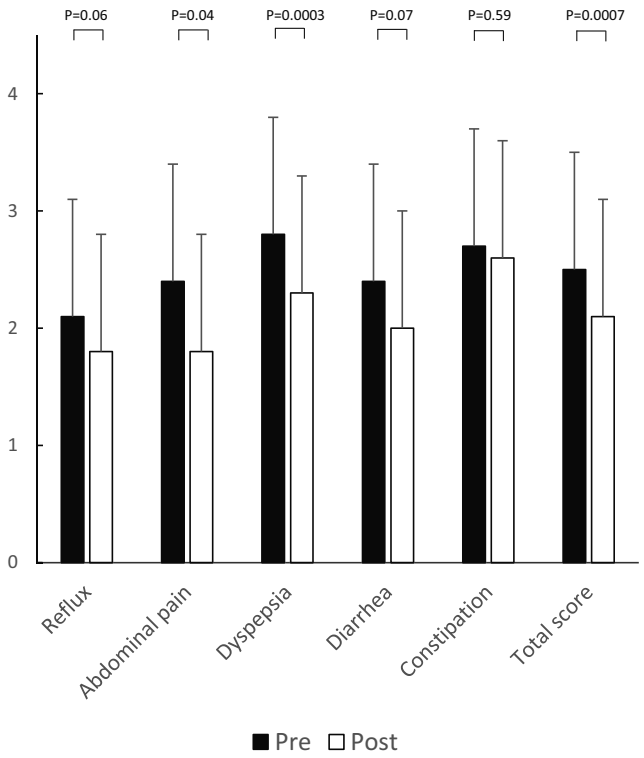

Fig. 5 Effect of acotiamide on GSRS in Japanese FD patients. The abdominal pain and dyspepsia score was significantly improved after study drug administration of acotiamide (abdominal pain: pre, 2.4 ± 1.3; post, 1.8 ± 0.6; $p = 0.04$, dyspepsia: pre: 2.8 ± 0.8, post: 2.3 ± 0.8, $p = 0.0003$). The total GSRS score was also significantly improved in the acotiamide group (pre, 2.5 ± 0.6; post, 2.1 ± 0.6, $p = 0.0007$) (ref. 28). GSRS, Gastrointestinal Symptom Rating Scale; FD, functional dyspepsia

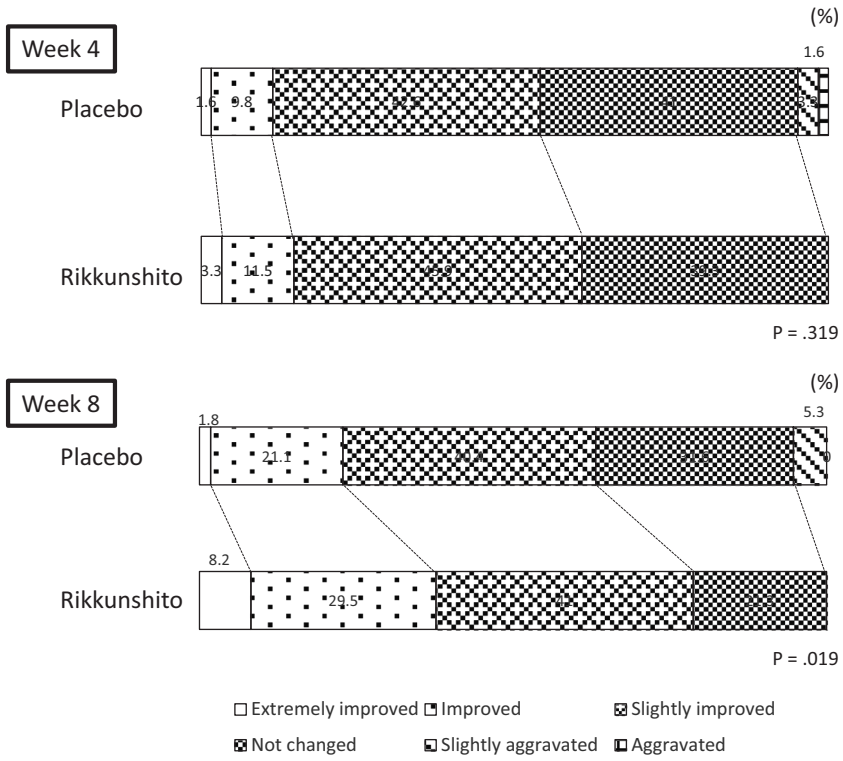

Fig. 6 Effect of rikkunshito on overtreatment efficacy after for 4- and 8-week treatment. The incidence of extremely improved and improved after 8-week treatment of rikkunshito was 8.2% and 29.5% that was significantly higher than those of placebo group (1.8% and 21%) ($p = 0.019$) (ref. 35)

Electrical Stimulation

Gastric electrical stimulation using a laparoscopically implanted device in the abdomen reportedly improves gastric emptying and dyspepsia in patients with gastroparesis [40, 41]. Continuous electrical stimulation may act directly on the gastric myenteric plexus and the vagus nerve (nausea and vomiting are induced by vagus stimulation). This may become a new therapy for refractory gastroparesis.

Gastric Peroral Endoscopic Myotomy

Peroral endoscopic myotomy (POEM) is a novel therapy for esophageal achalasia. G-POEM has reportedly been found to be useful for gastroparesis [42–44]. G-POEM improves gastric emptying by forming a tunnel in the mucosa, similar to that in POEM, and an incision through the muscular layers of the pyloric region enables passage through the pyloric ring. Gastric emptying was significantly improved, and clinical improvement was achieved in 70–80% of patients. G-POEM also improved symptoms of dyspepsia (e.g., nausea, early satiety, and distention) and QOL in the early period after surgery. Although the indications, safety, and efficacy of G-POEM remain controversial, this treatment may prove useful for intractable dyspepsia.

Meal and Lifestyle Modification

RCTs have reported that a high-fat FODMAP (fermentable oligosaccharide, disaccharide, monosaccharide, and polyols) diet and gluten-containing food contribute to the onset of dyspepsia. Wheat protein, milk protein, fruit juice, peppers, chilies, coffee, and alcohol affect sensation and motility in the gastrointestinal tract and induce dyspeptic symptoms [45]. However, some RCTs and meta-analyses showed that ingestion of peppermint, caraway seeds, and STW 5 herbal extracts is effective [46]. FD symptoms may be affected by individual lifestyles, and an improved lifestyle may relieve dyspeptic symptoms. Therefore, patients whose symptoms persist after treatment should attempt lifestyle modification.

Conclusion

This article provided an update of the diagnosis and treatment of FD based on current topics and knowledge. FD is now covered by national health insurance in Japan, and basic concepts of diagnosis and treatment of FD are being established. However, medical doctors and staff have not widely used this disease name, FD. It is expected that dyspeptic symptoms will be appropriately managed based on the concept for the diagnosis and treatment of FD.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the author.

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CLINICAL REVIEW

The evidence for fungus in Crohn's disease pathogenesis

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Abstract
Current evidence suggests the etiology of inflammatory bowel diseases (IBD) involves the confluence of host genetic, environmental, and microbial factors that lead to chronic, and often refractory, disease in susceptible individuals. The involvement of microbial triggers in IBD, including Crohn's disease (CD), is increasingly evident with supporting data provided with advancements in metagenomic sequencing that have identified perturbations in microbial structure and function—broadly termed dysbiosis—in CD patients compared with healthy subjects. This concept is supported by the finding germ-free animals with CD genetic susceptibility fail to develop disease; demonstrating microorganisms are necessary but not sufficient for CD. The vast majority of CD microbiome research has focused on the complex bacterial communities and microbiome dysbiosis in the gut with 16S metagenomic sequencing. However, emerging data capturing eukaryotes suggest fungal opportunistic pathogens are also associated with IBD pathogenesis and chronicity. This hypothesis is further supported by historical observations that CD patient populations display elevated antibodies against fungal targets, even evident before disease diagnosis. This review discusses the current findings in the field, followed by historical and metagenomic evidence for fungal pathogens in the development and recurrence of CD in adult and pediatric populations.

Keywords Inflammatory bowel diseases · Microbiome · Mycobiome · Fungal pathogens · Crohn's disease

Introduction

The prevalence and incidence of inflammatory bowel diseases (IBD) has rapidly increased over the past century and has become a global disease with an alarming increase in the incidence ulcerative colitis (UC) and Crohn's disease (CD) in recently industrialized countries [1]. UC is characterized by superficial, diffuse inflammation and ulceration limited to the colonic mucosa. CD is characterized by chronic transmural inflammation with granulomas and associated with intestinal strictures and fistulae in the gastrointestinal tract. CD most commonly occurs in the terminal ileum or colon. The identification of over 200 genetic mutations associated with IBD in humans now suggests these two disease classes, UC and CD, are likely many closely related conditions that result in similar disease phenotypes, explaining the heterogeneity of disease progression and treatment responsiveness [2]. In particular, many IBD associated gene mutations are in genes regulating immune signaling, Paneth cell function, and autophagy, including *ATG16L1, NOD2, IL-23R, CARD9, IRGM*, implying IBD is related to aberrant immune responses to microorganism sensing and subsequent pro-inflammatory responses.

Despite a clear genetic component, IBD incidence has been increasing in westernized societies for several decades, suggesting environmental triggers [3]. In addition to the numerous genetic mutations associated with disease risk, IBD pathogenesis likely involves lifestyle factors, antibiotic exposure, and diet, which are inexorably linked with perturbations in the intestinal microbiome that colonizes the gut in staggering numbers. While increased antibiotic exposure is often hypothesized to lead to bacterial dysbiosis, the gut complex ecology of interkingdom organisms is interwoven, and imbalances in bacterial taxa likely provide opportunistic fungus and Archaea with new environmental niches. The human gut contains upwards of 10 trillion individual bacteria, representing an estimated 3000–5000 bacterial species

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that potentially outnumbering human cells in the body 1–3:1. Together, the genetic diversity of the microbiome is approximately 150-fold greater its host and these communities provide essential digestive functions, synthesis of de novo vitamins and fermentative products, and stimulate immune function. The gut also harbors numerous species of eukaryotes, including yeast and fungi, termed the mycobiome [3]. The mycobiome is an important component of the intestinal microbiome. A variety of fungal–bacterial interactions, fungal–fungal interactions and fungal–host interactions have been reported and are increasingly recognized for immune and metabolic interactions [4].

While important for normal homeostasis, animal models also demonstrate that the microbiome is necessary, but not sufficient, for IBD development, as animals raised under germ-free conditions do not exhibit IBD [5, 6]. Therefore, the role of the intestinal microbiome and its functions has gained considerable interest for improving our understanding of complex diseases, such as IBD.

Advancements in next-generation sequencing over the last decade have made analysis of the microbial populations more affordable, and research using this technology has exponentially expanded over the same time period. Most microbiome research available up to this point has focused only on bacterial 16S rRNA gene, which excludes the diverse yeast and fungi that also populate the intestine. Nevertheless, emerging data are beginning to understand yeast and fungal species, integrating these organisms into the understanding of IBD pathophysiology. This review will discuss the inexorable relationship between the microbiome and mycobiome, and association between fungal organisms with IBD, specifically ileal Crohn's disease (iCD).

Next-generation sequencing considerations in microbiome studies

Many microbial organisms of the gastrointestinal tract remain uncultured; therefore, culture-independent techniques enable investigation of these organisms in the past 2 decades. Since the successful sequencing of the human genome in 2003, the cost of high-throughput sequencing, called next-generation sequencing (NGS), has decreased by 50,000 fold [7]. Rapid advances occurred in both 'sequence by ligation' and 'sequence by synthesis' short-read platforms over this time. In addition to sequencing the highly conserved 16S rRNA gene found in bacteria and archaea, targeting the highly conserved 18S and Internal Transcribed Spacer (ITS1 or ITS2) in eukaryotic species, such as yeast and fungi, allows for analysis of these organisms. Illumina 'seq' platforms utilize cyclic reversible termination methods on flow cell surfaces that are detected with optics, yielding low levels of homopolymer errors. When longer sequencing reads are desired than Illumina, another sequencing by synthesis optical detection system, 454 pyrosequencing, allows 1000 bp reads. The Sequencing by Oligonucleotide Ligation and Detection (SOLiD) method allows massively parallel sequencing. In contrast to optical detection methods, ion torrent sequencing technology detects changes in pH as incorporated bases release H^+. Illumina may yield > 99.5% accuracy, while some sequencing by ligation techniques, such as SOLiD, may yield > 99.95% [7]. Varied sequencing methods are recommended when an investigator wishes to avoid methodological bias inherent to each platform.

Sequencing fidelity is important when considering downstream data analysis with software programs, such as QIIME [8] and MOTHUR [9], which differentiate operational taxonomic units (OTU) at 97–99% sequence similarity, or 1–3% sequence dissimilarity [10]. Importantly, advanced software approaches are being developed to improve species resolution when OTUs differ by less than 1%, such as minimum entropy decomposition (MED), which detects true differences between closely related organisms from sequencing error [11]. Classical ecology concepts are used to compare and visualize microbial communities. For instance, the Shannon and Simpson Indexes are alpha diversity measure the diversity of species within samples, while beta diversity measurements allow comparison of similarity between samples. The future integration of 16S and ITS sequencing strategies with advanced data analysis approaches of complex mixed communities of bacteria and eukaryotic species will be vital for elucidating the role of the mycobiome in health and disease.

Microbial dysbiosis in inflammatory bowel diseases

Utilizing NGS platforms, many studies demonstrate that dysbiosis is associated with diseases of the intestine and extra-intestinal manifestations. Considering the vital function of the gut microbiota in metabolic and immunomodulatory functions of the gastrointestinal tract, it is not surprising that dysbiosis is now well documented in IBD [12–14]. Most studies of dysbiosis in IBD have examined bacterial compositions. The nexus of microbiota metabolism is inexorably tied to the collective composition of bacterial, fungal, protist, archaea, and viral populations, so disturbances in the microbiome are useful for understanding parallel changes that may occur in the intestinal mycobiome.

While considerable variability is observed in the bacterial microbiota between individuals at the 16S taxa genera level, more than 90% of bacterial species in the human intestine belong to one of four major phyla, and are dominated by the phyla Firmicutes and Bacteroidetes. In contrast, at least 50 phyla are identified in the environment, with total phyla

estimates several folds higher, demonstrating a selective capability for certain microbes by the mammalian intestine. Despite the variability observed at the species level, metagenomic approaches demonstrate there exist core sets of microbial genes that many individuals share. Approximately 40% of the microbial genes found in any individual are also present in approximately half the general population [15]. This observation supports more advanced functional metagenomic approaches to investigating microbial population metabolic capacity compared in contrast to taxonomic membership alone.

Numerous studies in IBD show that microbial diversity is reduced and characterized by reduced diversity in Firmicutes as well as increased relative levels of Proteobacteria [16]. In iCD, the loss of diversity is observed regardless of active or quiescent disease states, while in UC more differences are observed between disease states [17, 18]. Despite consistency in reduced bacterial diversity and altered composition across studies, individual studies have been inconsistent in identifying specific bacterial pathogens present in IBD [19, 20]. This finding casts doubt that a single or handful or pathogenic bacteria are drivers of IBD. Hoffman et al. demonstrated that changes in bacterial composition, driven by diet, are strongly correlated with reproducible shifts in the composition of fungal and archaea communities, where metabolic byproducts of some species or bacteria or fungus serve as substrates for other organisms [21]. While investigation of pro-inflammatory bacterial pathobionts leading to a loss of immunological tolerance continues, bacterial dysbiosis observed in IBD strongly implies compositional shifts also occur in fungal populations. Accordingly, accumulating evidence supports the notion that CD is associated with perturbed fungal diversity compared to healthy controls [22–25].

Mycobiome and the host immune system

Fungi and yeast colonize the mammalian intestinal tract immediately following birth through vertical and horizontal transmission [26]. Fungal communities make up less than 0.1–1% of total intestinal microbial DNA, but since fungi are on average 100× larger than bacterial organisms, the intestinal fungal biomass may be considerably underappreciated in its contribution to intestinal ecology. Accordingly, at least 260 distinct fungal species are found in the human intestine [27]. While these species are likely influenced by genetics and factors shaping colonization niches within the host, including sanitation practices, lifestyle, and administration of antimicrobial drugs, recent work suggests the intestinal fungal community composition is also impacted by dietary sources. David et al. reported differences in the human mycobiome communities based upon whether individuals were eating an animal-based diet or a plant-based diet. Furthermore, certain species of fungi that were detected in fecal samples of healthy subjects were also found in cheese the subjects consumed [28]. Similar findings are likely for *Saccharomyces cerevisiae*, which is commonly used in food and alcohol manufacturing.

The colonized fungal species in the intestine interact with the host immune system via the innate immune receptor, Dectin-1, which recognizes β-1,3-glucans in the fungal cell wall. Detectin-1 and its isoforms activate intracellular signals through CARD9 leading to inflammatory cytokine production and Th1 and Th17 responses [29–33]. This interaction between commensal mycobiome and host immunity appears crucial to maintain host intestinal health. CARD9 variants are reported to be associated with IBD [34]. In addition, Iliev et al. [35] showed that a polymorphism in the gene for Dectin-1 (*CLEC7A*) is strongly linked to a severe form of human UC. Furthermore, they demonstrated that Dectin-1-deficient mice are more susceptible to dextran sodium sulfate (DSS)-induced colitis. Interestingly, Tang et al. [36] reported that inhibition of Dectin-1 signaling attenuates murine DSS-induced colitis associated with an increase of lactobacilli and regulatory T cell expansion. The difference between these findings may underscore the importance to consider base-line microbiota in each research facility [37]. Qiu et al. demonstrated that fungal loads and compositions are different between animals with or without intestinal inflammation using murine DSS-induced colitis model. That work also showed that fungal depletion aggravates acute DSS-induced colitis [38]. Finally, Wheeler et al. [39] showed disruption of commensal fungi by fluconazole exacerbates not only DSS-induced colitis, but also $CD4^{+}CD45RB^{high}$ T cell transfer-mediated colitis in mice. This work collectively suggests a causative effect of fluconazole-induced fungal dysbiosis on peripheral immune responses, however, the direct evidence of a causal link between mycobiome dysbiosis and colitis has not yet been established.

Fungal involvement in pathogenesis of Crohn's disease

Recent studies using advanced sequencing approaches directly measure fungal composition in the intestine during CD. Consistent with the observation that bacterial and fungal community composition and diversity are related [21], and that dysbiosis is often present in IBD, Ott et al. [40] reported that fungal diversity was increased in colonic biopsies collected from CD patients compared with healthy controls. Li et al. analyzed ileal mucosal specimens and fecal samples in patients with CD and demonstrated that fungal diversity, particularly among *C. albicans, Aspergillus clavatus*, and *C. neoformans*, is markedly increased

[23]. Examination of familial CD patients demonstrates greater abundance of the fungus *C. tropicalis* compared with non-diseased first-degree relatives. The levels of *C. tropicalis* also positively correlated with levels of anti-*Saccharomyces cerevisiae* antibody (ASCA), however, overall fungal diversity was reduced [41]. Further supporting a role for *Candida* species, Sokol et al. analyzed fecal samples from IBD patients and showed an increased Basidiomycota/Ascomycota ratio, a decreased proportion of *S. cerevisiae* and an increased relative proportion of *C. albicans* [25]. Interestingly, in that work, the investigators suggested tolerogenic changes to Ascomycota led to Basidiomycota expansion through immunological changes. Liguori et al. [22] recently reported that Cystofilobasidiaceae family and *C. glabrata* species were overrepresented in CD and global fungus load was significantly increased in CD flare compared with healthy subjects. Furthermore, *Saccharomyces cerevisiae* and *Filobasidium* species were found near non-inflamed tissue, while Xylariales was associated with inflamed tissue. A summary of study findings in adult populations is shown in Table 1. The question of causality or association at the mucosal surface remains unanswered, but these findings globally support the concept that fungal organisms are overabundant in many adult CD patients,

Interestingly, pediatric patient populations may have a different host–mycobiome relationship. Chehoud et al. [42] analyzed stool samples in pediatric CD and UC patients and reported lower diversity of fungal microbiota, although two species of *Candida* were found in greater abundance. El Mouzan et al. examined mucosal and fecal samples from treatment-naïve pediatric CD patients and reported that *Psathyrella* and *Gymnopilus* significantly increased in the mucosa while *Gymnopilus* and *Hymenochaete* significantly increased in stool samples. That study also showed that *Monilinia* was significantly decreased in stools. Despite species abundance differences, there were no significant differences in fungal diversity between CD and control patients [43]. However, Lewis et al. [44] examined fecal samples from pediatric CD patients and found five fungal taxa detected in samples (*S. cerevisiae, Clavispora lusitaniae, Cyberlindnera jadinii, C.albicans* and *Kluyveromyces marxianus*) were positively associated with CD diagnosis. A summary of study findings in pediatric populations is shown in Table 2.

There are differences in the clinical presentations, disease phenotypes, and disease course among CD patients according to the age of onset [45] In pediatric-onset CD, ileocolonic disease is dominant and disease extension occurs more frequently than in adult and elderly age groups. Pediatric-onset CD tends to take more aggressive disease courses

Table 1 Changes observed in the mycobiome of adult Crohn's disease patients vs controls or healthy relatives

References	Fungal diversity	Species change	Total fungal load	Sample type
Ott et al. [40]	↑			Colonic biopsy tissue
Li et al. [23]	↑	↑ *Candida* spp., *Gibberella moniliformis, Alternaria brassicicola*, and *Cryptococcus neoformans*		Ileal mucosa surgical specimen
Li et al. [23]	↑	↑ *C. albicans, Aspergillus clavatus*, and *C. neoformans*		Feces
Hoarau et al. [41]	↓	↑ *C. tropicalis*		Feces
Sokol et al. [25]	→	↑ *C. albicans* ↓ *Saccharomyces cerevisiae*		Feces
Liguori et al. [22]	→	↑ Cystofilobasidiaceae family and *C. glabrata* species	↑	Colonic mucosa surgical specimen

Fungal diversity was either increased (↑), decreased (↓), or unchanged (→). Identified species and sample type are displayed

Table 2 Changes observed in the mycobiome of pediatric Crohn's disease patients vs controls or healthy relatives

References	Fungal diversity	Species change	Total fungal load	Sample type
Chehoud et al. [42]	↓	↑ *Candida utilis* and *C. parapsilosis* ↓ *Cladosporium cladosporioides*		Feces
El Mouzan et al. [43]	→	↑ *Psathyrella* spp. and *Gymnopilus* spp		Mucosal biopsy tissue
El Mouzan et al. [43]	→	↑ *Gymnopolius* spp. and *Hymenochaete* spp ↓ *Monilinia* spp		Feces
Lewis et al. [44]		↑ *Saccharomyces cerevisiae, Clavispora lusitaniae (C. lusitaniae), Cyberlindnera jadinii (C. utilis), C.albicans* and *Kluyveromyces marxianus*		Feces

Fungal diversity was either increased (↑), decreased (↓), or unchanged (→). Identified species and sample type are displayed

and perianal disease is also more prevalent in pediatric CD compared to late-onset CD. Given these findings, the differences in results of mycobiome analyses between adult and pediatric patients may reflect the potential differences in the involvement of mycobiome in the pathogenesis of adult and pediatric CD.

Compared to CD, fewer studies focusing on UC and mycobiome dysbiosis have been reported. Sokol et al. [25] reported that fungal biodiversity was decreased only in UC, but not in CD. Recently, Qiu et al. [46] analyzed mucosal samples from UC patients and examined 15 major genera. That work demonstrated *Wickerhamomyces*, unidentified genus of Saccharomycetales, *Aspergillus, Sterigmatomyces*, and *Candida* showed increasing trends, while *Exophiala, Alternaria, Emericella, Epicoccum, Acremonium, Trametes*, and *Penicillium* presented decreasing trends in UC patients compared to the healthy controls.

These studies demonstrate clear disturbances in the intestinal fungal ecology across IBD populations. Strong evidence suggests fungal dysbiosis exists in adults with iCD [25]. Since the immune development is strongly influenced by the microbiome in early life [47, 48], compositional shifts in fungal populations in childhood may be important for understanding disease risk in later life in susceptible individuals. In addition, further basic and translational studies are expected to confirm or rule out causal links between mycobiome dysbiosis and the development of IBD.

Correlation of anti-saccharomyces cerevisiae antibody as a diagnosis marker and predictive factor in Crohn's disease

In response to conserved hyphal cell wall mannans found in Saccharomycetaceae, including *Saccharomyces cerevisiae* and *Candida albicans*, the 'anti-*Saccharomyces cerevisiae* antibody' (ASCA) is an IgG or IgA immunoglobulin associated with CD. Both *S. cerevisiae* and *C. albicans* are potent targets of ASCA and may be the origin of some or most of the immune response [49]. Hoarau et al. recently reported *C. tropicalis* associates with ASCA serum levels when comparing across CD patients, their non-diseased first-degree relatives, and healthy controls [41]. Standaert-Vitse et al. [50] analyzed fecal samples from CD patients and healthy family members and demonstrated that *C. albicans* was more abundant in CD patients and their healthy relatives compared to individuals not related to CD patients.

ASCA is readily detected in 50–60% of CD patients and was historically used as a serological diagnostic marker of ileal CD [51–53]. While endoscopic procedures are the standard of care and direct diagnostic method for CD diagnosis today, elevated ASCA remains a weak secondary indicator of CD compared with UC. In addition to the presence of ASCA in CD, there is also evidence that ASCA levels correlate with disease severity. Forcione et al. [54] demonstrated that ASCA IgA and IgG levels are positively associated with stenosing disease of the ileum in CD and that patients with the greatest levels required surgery at an increased rate. In addition to adult patients, Dubinsky et al. [55] reported that elevated levels of ASCA, along with other immune responses, are associated with more aggressive disease in pediatric CD patients. Tang et al. recently observed median ASCA levels in stool were significantly elevated in patients 19 and younger with active CD than controls and diagnosed disease showed elevated serum and fecal ACSA compared with older diagnoses [56]. Perhaps the strongest indicator of fungal involvement in iCD comes from findings observed by analyzing the banked blood samples from military soldiers prior to disease development. ASCA levels rise in 30% of individuals up to 3 years before clinical diagnosis, representing the strongest metadata predictor of disease [57]. Together these data indicate that CD is associated with elevated intestinal colonization by fungal pathogens, specifically of the family Saccharomycetaceae, and that these organisms stimulate immune responses in the host that are correlated with onset and severity of disease progression.

Potential risk of CD therapy from the perspective of mycobiome

Consistent with aberrant immune responses to microorganisms, current treatment for CD relies on the early introduction of immune targeted therapies to reduce the chronic inflammation that can lead to complications. Therapeutic antibodies are manufactured to target specific molecules and are the most effective medical treatments currently available. The currently available therapeutic antibodies for CD target tumor necrosis factor alpha (TNF-α), IL-12/23, and leukocyte adhesion molecules, mechanisms responsible for inflammatory response to intestinal microorganisms.

Infliximab [58–60], adalimumab [61–64], and certolizumab pegol [65, 66] are therapeutic antibodies targeting TNF-α that are currently approved and widely used for treatment of CD in the United States. Ustekinumab [67, 68] is the first therapeutic antibody to target the p40 subunit of IL-12/23 to be approved for CD. Notably, immune responses to fungal pathogens like *C. albicans* involve TNF-α, IL-17, and IL-23 [69, 70]. Accordingly, there is now strong evidence that some therapeutic strategies are associated with increased incidence of fungal infection in IBD, including histoplasmosis, blastomycosis, and coccidioidomycosis [71] as well as other opportunistic pathogens [72]. Specifically, treatment with the anti-IL-17A antibody (secukinumab) was associated with worsened outcomes compared with placebos, including elevated fecal inflammatory mediators and

increased risk of mucocutaneous candidiasis in clinical trials [73].

Mycobiome and future therapeutic strategies for CD

Thus far, antifungal therapy has not been tested in IBD. Conceptually, targeting of IL-17, IL-23, IL-22, and TNF-α cytokine pathways would result in decreased pro-inflammatory responses mitigating acute mucosal damage, but could subsequently stifle clearance of fungal organisms, providing a cogent explanation for the clinical data observed with fungal pathogenesis. Specifically, the Th17 cytokines, such as IL-22, directly regulates epithelial antimicrobial gene expression, including Reg3γ by enterocytes, and Paneth cell antimicrobial products. Attenuation of these mucosal secretions could potentiate the growth and colonization of fungal species at the mucosal surface. While this has not been empirically tested, data indicating increased fungal load following some immune targeting therapies begs the intriguing hypothesis that these immune responses are in part driven by the fungal pathogens themselves and, accordingly, certain treatment options may mitigate the immunological capacity to contain fungal pathogens within the gut.

Notably, although there has been significant progress on understanding the microbiome in CD, there are no current targeted strategies that effectively manipulate the microbiome or mycobiome for therapy. Fecal microbiota transplantation has been the most extensively investigated to date, but the results in inflammatory bowel disease have been mixed [74]. The largest existing studies show significant clinical remission rates, with a meta-analysis estimating clinical remission in 60.5% of patients, but the studies included only 39 patients total and there was a wide confidence interval (95% CI 28.4%–85.6%) with moderate heterogeneity [75–77]. Studies that evaluate the microbial profiles of the donor and recipient stool focus on bacterial composition, but microbial environment includes fungal elements that participate in complex interactions with the mucosal immune system. Fecal microbiota transplantation is better understood and has been studied as a therapy for ulcerative colitis. A recent randomized placebo-controlled trial in ulcerative colitis showed that a regimen of multidonor fecal microbiota transplantation can induce steroid-free clinical remission with endoscopic remission or response [78]. Microbial 16S rRNA analysis showed that microbial diversity increased and was maintained by fecal microbiota transplant. These findings raise intriguing questions about whether the mechanism for therapeutic effect may apply in Crohn's disease as well; especially in regard to fungal contributions to the interkingdom microbial diversity. Rigorous, adequately powered placebo-controlled trials are needed to help identify the role of fecal microbiota transplant in Crohn's disease. These clinical studies must integrate microbial analysis of both bacterial and fungal organisms to help enhance our understanding when fecal microbiota transplants are associated with positive and negative outcomes. As methods for mycobiome characterization improve, fungal analysis will be important for characterizing donor and recipient microbiome and mycobiome.

Conclusions

The current evidence for the etiology of IBD strongly suggests that environmental triggers influence intestinal microorganism ecology, which conveys the risk for IBD development and subsequent disease course in genetically susceptible hosts. While much attention has been placed on the specific role of bacterial dysbiosis demonstrated in many studies, emerging evidence suggests that changes in fungal community composition occur in parallel, suggesting more complex interkingdom community ecological interactions. The observed changes in fungal diversity and relative abundance of certain fungal taxa within the human gut may have relevant roles in IBD etiology. In light of these collective data and the heterogeneity in treatment responsiveness observed across patients, future research on IBD therapy should carefully consider the potential role of fungal involvement in IBD disease pathogenesis.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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Stomach (P Malfertheiner, Section Editor)

Helicobacter pylori Infection: New Facts in Clinical Management

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Abstract

Purpose The global prevalence of *Helicobacter pylori* remains high in spite of its significant downwards trajectory in many regions. The clinical management of *H. pylori* infection merits guidance to meet ongoing challenges on whom and how to test, prevent, and cure related diseases.
Recent findings Several international guidelines and consensus reports have updated the management strategies for cure of the *H. pylori* infection. The definition of *H. pylori* gastritis as an infectious disease independent of whether or not presenting with clinical manifestations and symptoms has broadened the use of the *test and treat* strategy. Patients on selected long-term medications, such as aspirin, other anti-platelet agents, NSAIDs, and PPIs should be considered for *H. pylori* test and treat. Important progress is made with initiatives in primary and secondary gastric cancer prevention. Uncertainties persist in the interpretation of the role of *H. pylori* in association with extragastric diseases. Selection of therapies needs to address individual antibiotic resistance and regional surveillance of resistance for the adoption of an effective treatment algorithm.
Conclusion Clinical aspects of *H. pylori* infection have evolved over time and the therapeutic management requires continuous adaptation. A vaccine is still a non-fulfilled promise. The future will tell us more about the role of *H. pylori* in interactions with the gut microbiome.

Introduction

The prevalence of *Helicobacter pylori* (*H. pylori*) infection remains high with an estimate of 4.4 billion people infected on a global scale but differs significantly among populations in various geographical regions [1]. There is a definite trend of decreasing *H. pylori* prevalence in the western world already at the turn of the third millennium, in Europe (from 48.8% before 2000 to 39.8% after 2000), Northern America (42.7% before to 26.6% after 2000), opposed to Asia (53.6% before vs. 54.3% after 2000), and Latin America and the Caribbean (62.8% before vs. 60.2% after 2000). A further decrease in the prevalence of *H. pylori* infection is reported from more recent studies in several western countries [2, 3], and this trend has been extended also to some countries in Asia [4]. Over time, this will lead to a further decline in gastroduodenal pathologies, including peptic ulcer with or without complications and gastric cancer. For now, locally adapted management strategies for cure and prevention of clinical consequences are required to obtain a meaningful reduction in the *H. pylori*-related disease burden. In the clinical setting, we continue to face ongoing challenges every day and everywhere with the key questions, whom and how to test and treat, and how to treat in times of rising antibiotic resistance.

Several international guidelines and consensus conferences have revised and updated their advice and recommendations to optimize the clinical management of the *H. pylori* infection and will be given consideration in this update [5•, 6•, 7•, 8•].

Whom to test and treat

Test and treat in the symptomatic patient

In all patients referred for clinical symptoms related to the upper gastrointestinal tract, proper, diagnosis should always include the assessment of *H. pylori* infection, which depending on the clinical scenario will be based on the analysis of biopsies taken during the upper endoscopy or on non-invasive testing. The 13C urea breath test and the fecal monoclonal antigen test have both a high sensitivity and specificity in the detection of *H. pylori* infection. These tests are superior to serum antibody testing which requires local validation for optimal accuracy and has the limitation that antibodies may remain detectable even up to years after *H. pylori* has disappeared from the stomach. This is the reason why serological testing is inappropriate to control the effect of *H. pylori* eradication therapy which remains the domain of 13C-UBT (alternatively the fecal monoclonal antigen test).

Besides the conventional indications for *H. pylori* therapy, there is general agreement that all those infected should receive treatment (Fig. 1). In patients with dyspepsia, the initial options are a) prompt endoscopy with therapy tailored to findings with either PPI or *H. pylori* eradication, b) empiric therapy based on acid suppression with PPI, or c) *H. pylori* test and treat. The test and treat strategy is superior to PPI on long term, and superior to the endoscopy-based strategy from a cost-benefit perspective [5•, 7•]. Test and treat is advised in patients with dyspeptic symptoms, in the absence of alarm symptoms, by respecting a regionally defined age cut-off and by use of non-invasive tests, the 13C urea breath test (13C-UBT), alternatively the fecal monoclonal antigen test (FMA). The age cut-off considered in the USA for patients suitable for the test and treat strategy is up to 60 years. This is different in Europe for patients with dyspepsia above the age of 50 for whom the endoscopy-based strategy is recommended [5•, 7•]. The rationale behind considering different age cut-offs for the test and treat strategy is to avoid missing preneoplastic gastric lesions

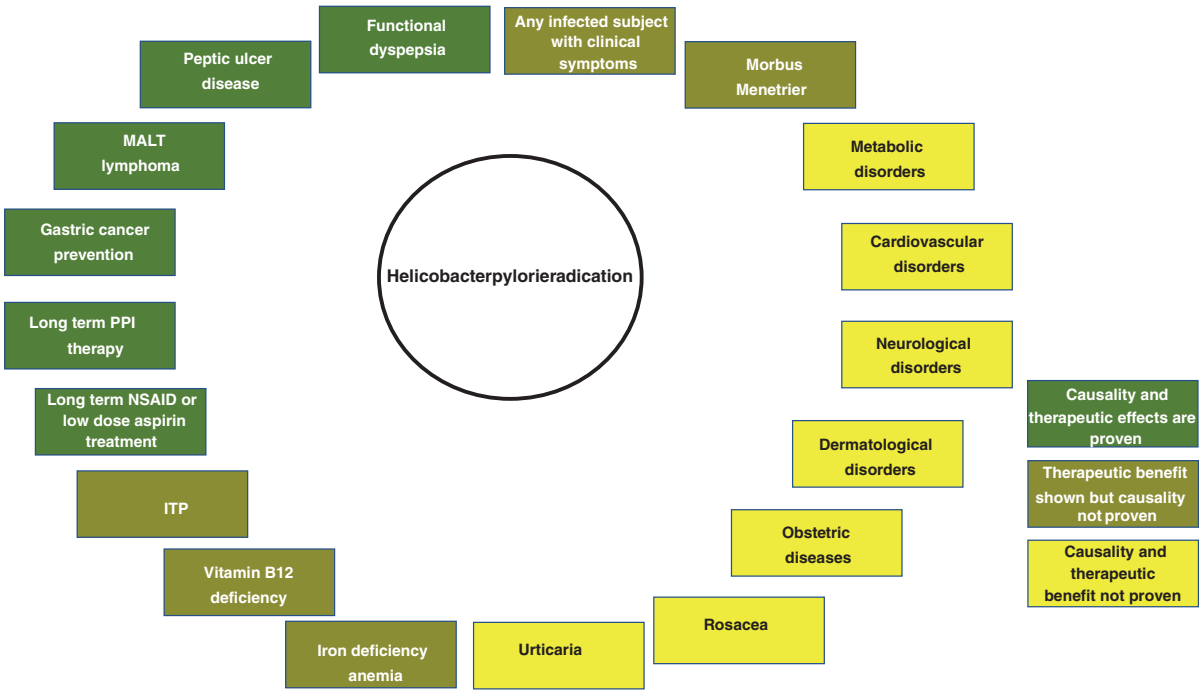

Fig. 1. Indications for *H. pylori* eradication therapy. Inspite of the fact that some associations are not confirmed to be causally linked once *H. pylori* gastritis (=infectious disease) is detected eradication therapy should be offered to all and is mandatory for those with causal relationship.

or delaying the diagnosis of gastric cancer, which presents with variable age-related incidence in various geographical regions.

H. pylori in extragastric diseases

H. pylori testing in patients without symptoms related to the upper gastrointestinal tract remains an issue of uncertainty. The list of extragastric diseases associated with *H. pylori* is growing with new positive associations for cardiovascular, neurological, dermatological, obstetric, and metabolic diseases [9]. However, causality is not proven in most of these conditions and a therapeutic benefit as of today for *H. pylori* eradication is documented only for few hematological disorders including, iron deficiency anemia, immune-thrombocytopenic purpura, and vitamin B 12 deficiency after excluding other potential causative factors than *H. pylori* (Fig. 1). This should not prevent consideration of a test and treat strategy in individual patients with suspected infection triggered rosacea, urticaria, or some distinct autoimmune disease [10, 11].

There are still many uncertainties in the area of *H. pylori* associations with extragastric conditions such in recent reports concerning the relationship of *H. pylori* with eosinophilic esophagitis [12, 13].

Claims for "preserving" *H. pylori* in the stomach as part of the indigenous good microbiome are extrapolated from several studies showing a negative association of *H. pylori* with atopic and allergic diseases, nearly all of them reported in children and in experimental settings [14, 15, 16]. None of these

findings is strong enough to oppose and argue against the enormous benefit of *H. pylori* eradication in infected adults [7•].

Also, the assumption that use of antibiotics is associated with an increased risk of developing both new-onset Crohn's disease and ulcerative colitis [17] cannot be attributed to a specific effect of previous *H. pylori* eradication therapy as claimed from few case reports [18].

H. pylori eradication in patients on medications

According to current *H. pylori* guidelines, there are two important considerations for *H. pylori* eradication in patients on medications: a) in patients on medications with harmful effects on the stomach to prevent peptic ulcer complications and b) in patients on long-term PPI [5•, 7•].

In a recent international guideline update on the management of non-variceal upper gastrointestinal bleeding, no consideration is given on how to integrate the management of *H. pylori* infection in the context of drug-induced peptic ulcer bleeding [19]. Studies providing a high grade of evidence are indeed missing in this area but it has previously been shown that peptic ulcer rebleeding in *H. pylori* positive patients on low-dose aspirin could significantly be prevented by *H. pylori* eradication [20].

In a large monocentric retrospective experience in over 1700 patients, the *H. pylori*-associated risk for peptic ulcer bleeding is confirmed to be increased in patients on low-dose aspirin and NSAIDs as previously shown [21] and has been extended to other non-aspirin antiplatelet drugs with a fourfold increased risk [22]. The risk for peptic ulcer bleeding in *H. pylori* carriers to be independent of PPI intake. *H. pylori* positive patients on combined antiplatelet therapy carry the highest risk for peptic ulcer bleeding. This observation may have important implications in clinical practice and advise to reduce the additional *H. pylori* conferred risk by eradication.

An increased risk for peptic ulcer bleeding was not observed in *H. pylori* positive patients on anticoagulants, selective serotonin reuptake inhibitors or corticosteroid therapy [22].

H. pylori gastritis and long-term PPI

PPIs provide the highest gastroprotective and healing effect in the broad clinical range of peptic ulcer disease [23] and are standard of care in treatment of gastroesophageal reflux disease [24].

H. pylori eradication is recommended in patients requiring long-term PPI because of the aggravation of the fundus-corpus gastritis, which is accompanied by an accelerated loss of oxyntic glands with development of atrophic gastritis [7•, 25]. The resulting gastritis phenotype carries a significantly increased risk for gastric cancer development. Profound changes in gastric physiology with induction of a hypoacidic gastric milieu and colonization by nitrosating bacteria are mechanisms suggested to be involved in the pro-carcinogenetic process [25]. Clinical concern had first been raised in a population-based cohort study conducted in Denmark in which the incidence of gastric cancer was increased among PPI users and was most striking in those on PPIs that had received *Helicobacter pylori* eradication [26]. This, certainly, contrary to what one would expect, could be explained by the missing information on whether *H. pylori* eradication had been successful in these patients or whether patients had

already preneoplastic changes (point of no return in gastric carcinogenesis) at the time when they received eradication therapy. *H. pylori* as the major confounding factor was not taken into account in other observational studies, and, therefore, the estimated risk of PPI-related gastric cancer in a recent meta-analysis was not dramatic (relative risk (RR) 1.43, 95% CI 1.23–1.66) [27]. The relationship of *H. pylori* and PPI has recently become more intriguing with the report from a large population-based cohort in Hong Kong, on 63,397 eligible subjects in which 153 (0.24%) developed gastric cancer during a median follow-up of 7.6 years following *H. pylori* eradication. PPI use was associated with an increased gastric cancer risk (HR 2.44; 95% CI 1.42–4.20**)**. The risk increased with duration of PPIs use (HR 5.04 [95% CI: 1.23–20.61], 6.65 [95% CI: 1.62–27.26] and 8.34 [95% CI: 2.02–34.41] for ≥ 1 year, ≥ 2 years, and ≥ 3 years [28].

The study has raised great concern about long-term use of PPI use in general but more so because of the increased gastric cancer risk in subjects after the intended elimination of the major cancer risk through *H. pylori* eradication. Significant flaws in the study are the absence of reporting the eradication success, the gastric mucosal status at the time of eradication, the indication for the PPI use after eradication but on the other side the study generates the hypothesis for the carcinogenetic effects of other microbes that get established in the hypoacidic gastric environment [29].

Screen and treat in asymptomatic individuals

The rationale to extend testing for *H. pylori* is because infection invariably leads to chronic gastritis with changes in the gastric mucosal structure and perturbations of gastric physiology. Although in most infected subjects H.pylori gastriris causes no symptoms, over the long term, it predisposes the infected person to a variety of clinical manifestations including dyspepsia and peptic ulcer. In the worst-case scenario, *H. pylori* gastritis progresses to gastric cancer. Based on these aspects, the Kyoto consensus defines *H. pylori* gastritis as an infectious disease with the implied consequence that cure of the infection should be offered to all infected individuals even in the absence of symptoms to prevent progression of gastritis and eventful complications [30]. Here, we enter the field of preventive medicine with the primary aim to prevent gastric cancer. The effectiveness of primary gastric cancer prevention by *H. pylori* eradication is well documented and has been demonstrated in 7 randomized clinical trials and 8 cohort studies recently revisited and analyzed in two meta-analyses [31, 32].

Successful gastric cancer prevention by *H. pylori* eradication is now confirmed also in a Western population conducted in Sweden. In a retrospective population-based cohort study of 95,176, patients who received *H. pylori* eradication therapy, followed-up after 5 to 7.5 years, showed a decrease of gastric cancer [33].

An uncertainty has remained as to whether the time of intervention by *H. pylori* eradication to prevent gastric cancer had surpassed the critical point of no return once preneoplastic changes (atrophy, intestinal metaplasia) are established in the gastric mucosa. To this purpose, an important contribution has been made in a prospective randomized placebo-controlled trial conducted in Korea. Authors reported a 50% reduction in incidence of secondary (metachronous) gastric cancer in a 5-year follow-up by *H. pylori* eradication

compared to placebo after the endoscopic removal of early gastric cancer [34••]. The decreased metachronous gastric cancer rate was accompanied by a significant reduction of gastric atrophy after successful *H. pylori* eradication. This important finding does not obviate the necessity to perform surveillance in patients with advanced atrophic gastritis after successful *H. pylori* eradication.

In conclusion, new data confirm and strengthen the important role of *H. pylori* eradication in gastric cancer prevention and support the recommendation to adopt screen and treat strategies in individuals at increased risk (i.e., first-degree relative with gastric cancer) and in regions with high gastric cancer risk but encourage such strategies also in regions with intermediate and low risk as well [7•, 35]. This has not been included in other recent guidelines [5•]. A prevention strategy we think of is the inclusion of non-invasive screening for premalignant gastric lesions in national colorectal gastric cancer screening programmes [36].

Non- invasive screening may either make use of the 13C-UBT in the younger population or of serology (based on pepsinogen) which allows identification of asymptomatic subjects with already advanced gastritis and include them in further diagnostic assessment and treatment [37, 38].

Recent studies from Japan and Korea, reporting on gastric cancer screening programs, based on serology (pepsinogen I) and/or endoscopy found an increased detection rate of early gastric cancer and thus in a curable stage with significant reduction in mortality from the disease [39, 40]. Initiatives in the Western world are lagging behind. In a recent study on a Finnish population of 329 gastric cancer patients, pre-diagnostic low serum pepsinogen I and anti-*H. pylori* antibodies were significantly associated with increased gastric cancer risk [41, 42].

Therapy

Efficacy of anti-*H. pylori* therapy depends on formulation, dose, duration of treatment, and patient adherence, but antibiotic resistance remains the most critical factor. The selection of therapies for cure of *H. pylori* infection should thus be guided ideally by knowing the *H. pylori* antibiotic susceptibility of the individual patient which is not what happens in usual clinical practice. Selection of the antibiotic regimen can be influenced by asking patients about previous antibiotic exposure but in fact, many patients do not recall the names of previous antibiotics taken. For management in clinical practice, therefore, a regional monitoring of *H. pylori*-resistance rates to conventional antibiotics included in eradication regimen is advised. In patients with migration background, if available, national registries or published studies on antibiotic resistance from their country of origin provide useful information for the selection of the optimal first-line therapy.

Current *H. pylori* therapies are still based on a restricted range of individual antibiotics that include, clarithromycin, metronidazole, amoxicillin, levofloxacin (sitafloxacin in Japan), tetracycline, rifabutin, furazolidone, and on the exclusively luminally active antibacterial bismuth. These antibiotics are combined in various regimen which always require the addition of a potent acid suppressant (PPI, vonoprazan a new P-cab available in Asia only) [43, 44].

The previously successful use of clarithromycin in triple combinations has now been abandoned in many parts of the world due to high rates of treatment

failures in areas with clarithromycin $R > 15\%$ [6•, 7•]. Clarithromycin continues to be part of recommended concomitant quadruple therapies even in circumstances of high resistance. This appears illogical since frequent failure of clarithromycin including quadruple regimen has to be anticipated because of the frequent simultaneous metronidazole resistance [45]. Where available, bismuth-based quadruple without clarithromycin is now recommended as first-line treatment. The preference for this regimen is the absence of *H. pylori* resistance to bismuth or tetracycline and the irrelevance of metronidazole resistance, which is overcome by these other two components [46, 47]. The challenge with this regimen is to keep the patient compliant given the large number of pills required. The duration of therapy is 10 to 14 days with bismuth quadruple and 14 days for all other regimens [6•, 7•].

If first-line treatment fails, there are not many options for second- and third-line therapies, which need to consider the additional resistance depending on the initial therapy used (Fig. 2 algorithm).

The challenge of antibiotic resistance

In February 2017, the WHO categorized *H. pylori*, in terms of antibiotic resistance, a high-priority issue [48]. We are facing, if any, minimal resistance problems with amoxicillin, tetracycline, and rifabutin and, therefore,

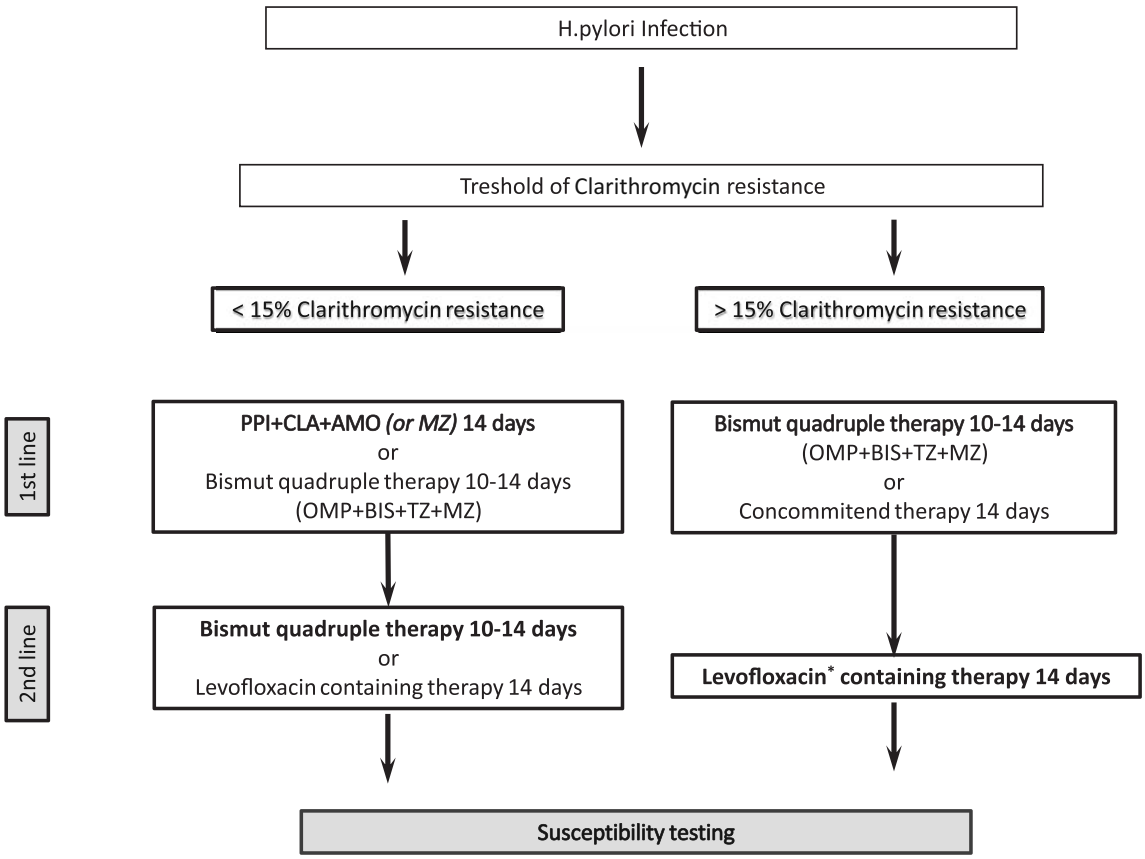

Fig. 2. *H.pylori* eradication algoritm according to primary clarithromycin resistance and managment in case of treatment failure.

these substances continue to be choices for rescue therapies, although if they are included in previous regimen their efficacy in second line therapies may be problematic. The high metronidazole resistance reported from most countries is of little relevance as some of the other antibiotic components of quadruple therapies can compensate for it.

However, the more recently reported dramatic increase of levofloxacin resistance [49, 50] is of great concern as the use of levofloxacin is recommended as second-line regimen in all current international guidelines [5•, 6•, 7•, 8•]. *H. pylori* antibiotic susceptibility testing becomes a clear need in a second-line approach to avoid unnecessary courses of antibiotics utilizing antibiotics with high probability of resistance such as levofloxacin. Already now, *H. pylori* eradication testing for antibiotic susceptibility is recommended prior to proceeding with third-line therapies. In the future, selection of individual therapy based on susceptibility testing might become the standard of care. Tailored, culture-based treatment is highly effective [51]. For overcoming practical shortcomings such as availability, delay and costs with culture-based methods, molecular-based antibiotic resistance tests are on the horizon which will provide accurate results more rapidly [52, 53].

Probiotic as add-on

H. pylori eradication is associated with antibiotic-related side effects, primarily dysgeusia, transient abdominal symptoms, antibiotic-associated diarrhea (in less than 10%), and rarely severe events (*C. difficile* infection). To minimize side effects, add-on therapy with probiotics has been advocated. Only for certain strains including specific *Lactobacillus* strains, *Bifidobacterium* strains [54, 55], and *S. boulardii* have their efficacy been documented [56, 57]. For other strains as in a most recent RCT the probiotic supplementation containing *Lactobacillus Plantarum* and *Pediococcus acidilactici* to *H. pylori* treatment neither decreased side effects nor improved compliance with therapy or eradication rates [58]. In some studies, probiotics are reported to increase *H. pylori* eradication rate by reducing side effects related to eradication therapy. Guidelines are currently restrictive [7•] or nihilistic (5) in advising for the use of probiotics with *H. pylori* eradication therapies. At present, the advice should be on an individual basis with particular consideration in patients who had previously experienced side effects during treatment course with antibiotics.

Compliance with Ethical Standards

Conflict of Interest

Peter Malfertheiner is involved in speakers bureau/or consulting: Biocodex, Biohit, Danone, Mayoly- Spindler. Marino Venerito declares that he has no conflict of interest. Christian Schulz declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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RESEARCH ARTICLE

Circular stripes were more common in Barrett's esophagus after acetic acid staining

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Abstract

Background: The diagnosis of Barrett's esophagus (BE) is disturbed by numerous factors, including correct gastroesophageal junction judgment, the initial location of the Z-line and the biopsy result above it. The acetic acid (AA) could help to diagnose BE better than high resolution imaging technology or magnifying endoscopy, by providing enhanced contrast of different epithelium. We have noticed AA could produce multiple white circular lines, forming circular stripes (CS), at lower esophagus, which hasn't been reported by others. This study aimed to investigate whether the CS is a special marker in BE patients.

Methods: A total of 47 BE patients and 63 healthy people were enrolled from March 2016 to October 2016, and 2% AA staining had been operated routinely at lower esophagus under high resolution gastroscopy. We observed whether there were CS after AA staining and the images were compared between the two groups.

Results: CS were confirmed in 42 patients (89.36%) in the BE group and 5 (7.94) in the control group (($\chi^2 = 72.931$, $P < 0.001$)). The average width of CS was 0.76 ± 0.25 cm in BE group, which was similar to that in the control group (0.88 ± 0.11 cm). Villous or punctate or reticular pattern usually existed above or below the CS.

Conclusions: CS could be found at lower esophagus in most BE patients with AA staining, and this special feature might be valuable in diagnosing, evaluating and following up of BE patients.

Keywords: Barrett's esophagus, Chromoendoscopy, Esophagogastric junction, Intestinal metaplasia

Background

Barrett's esophagus (BE) is defined as the replacement of squamous epithelium of the lower esophagus by single layer columnar epithelium [1–4], with or without the intestinal metaplasia (IM), which may be accompanied by risk of progression to carcinoma [4–6]. In recent years, the morbidity of esophageal squamous cell carcinoma and gastric carcinoma has been decreasing, while the incidence of esophageal adenocarcinoma is gradually increasing [5]. Therefore BE has attracted more attention as the most important precancerous lesion of esophageal adenocarcinoma.

The diagnosis of BE is disturbed by numerous factors clinically. Firstly, judgment for esophagogastric junction (EGJ) is subjective to some extent [7]. EGJ is usually defined by the top of the gastric folds, or the location of esophagus palisade blood vessel [8–13], both of which will be influenced by respiration, the volume of gas injected, the pressure of esophagus, even the relative position of diaphragmatic hiatus [9]. Secondly, the shape of the Z-line is another disturbing factor, and the biopsy result of columnar epithelium is meaningless if the initial or correct location of Z-line is wrong. Therefore, the accurate diagnosis of BE is based on correct EGJ judgment, the initial location of the Z-line, and the biopsy result above the Z-Line.

High resolution imaging technology and magnifying endoscopy have greatly improved the observation of mucosal micro-pattern. However, chromoendoscopy is irreplaceable. The acetic acid (AA) could provide better contrast of different epithelium. It is a kind of dye that can react specifically and reversibly with the columnar

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cells, however the exact mechanism is unclear yet. It is speculated that reversible degeneration of cellular proteins causes aceto-whitening reaction [14]. There are studies confirming that the AA used for BE epithelial staining could identify the mucosal microstructure especially highlight dysplasia and early cancer [15–18]. Meanwhile AA can increase the contrast between the squamous and columnar epithelium, producing white line at the junction which is coincident with Z-line in the healthy people. We have noticed that AA could produce multiple white circular lines, forming circular stripes (CS) at lower esophagus, and this feature was more common in BE patients. So a retrospective study was conducted to investigate whether the CS is a special marker for BE patients.

Methods

Patients

Both BE patients and control group participants were selected from Data of Endoscopy Center of The 2nd Affiliated Hospital of Xi'an Jiaotong University from March 2016 to October 2016.

Inclusion criteria: The BE patients should be diagnosed as full range or tongue type at least once before the research with pathological evidenc according to biopsy standard of ACG Clinical Guideline**,** 18 to 85 years old, male or female, outpatient or hospitalization. In this research, there are also some typical endoscope features can be seen to support BE diagnose, including columnar epithelium above esophagogastric junction (EGJ) where should be squamous epithelium normally, repositioned Z-line (upward the normal position > 0.5 cm) and the orange esophageal epithelium below the Z-line which is carnation in healthy people, and paliform vessel below the Z-line can be observed. If without the imaging evidence above, the patient will be removed out from the study.

In the control group, healthy physical examination participants with similar age and sex were selected. Esophageal AA staining was performed in both groups during the routine gastroscopy procedure and the images were fully integrated to identify the epithelial structure of lower esophagus near the Z-line.

Exclusion criteria: Patients with esophageal epithelial erosion which is always lead by gastroesphageal reflux disease (GERD) and will impact the mucosa observation after AA staining (Additional file 1), patients with esophageal or gastric cancer, patients with acute gastrointestinal bleeding, and patients with upper gastrointestinal surgery were excluded. Inappropriate patients, such as normal Z-line location in BE group, or BE in control group, were excluded either (Fig. 1).

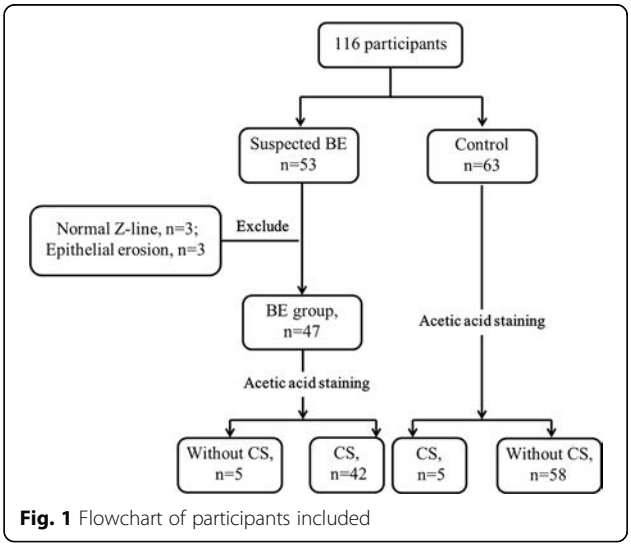

Fig. 1 Flowchart of participants included

Materials and equipments

AA was prepared as a 2% solution by diluting 5 mL of 6% acetic acid in (with) 10 mL of distilled water used for dyeing. High resolution gastroscopes (EG29-i10; PENTAX, Japan) were used for recording pictures and videos.

Protocol

The endoscope would be placed at the lower esophagus, or proximal end of the lesion if there was obvious metaplasia. The mucosa should be cleaned by injecting water before taking photos. Then 10 – 15 ml AA would be sprayed by spraying pipe onto cleaned mucosa, and further observation began after 30 s [19]. The biopsy were taken in and out of CS region with 1~ 2 pieces separately. Patients were given spasmolytic (Anisodamine 10 mg im.) and sedation (Midazolam 5 mg im.) before the examination to reduce the discomfort.

Diagnostic criteria for CS

Made the Z-line and the submucosa of the esophageal folds evenly with moderate air under endoscope. The imaging features before AA staining were recorded. Thirty seconds after AA staining, the mucosa of lower esophagus and cardia turned white at the same time, which meaned the albino acetate reaction. Then, white linear stripes, so-called circular stripes, generally with a length of more than 0.5 cm, could be observed below the Z-line. These stripes had clear boundaries and distributed in circular or a certain quadrant. The images after AA staining were recorded. About 4–6 min later, the whitening area gradually returned to normal color and shape. This phenomenon is defined as CS positive in this study, which is approved and reviewed by two experienced endoscopic physicians.

All patients signed the informed consent of gastroscopy examination and AA staining. All aspects of the study were conducted using de-identified photographs and videos. Because all the photographs and videos existed before initiation of the study, this study was granted exempt status by the Xi'an Jiaotong University Human Research Committee.

Outcomes

The primary end point of the study was whether there were CS. The secondary outcome included the distribution and width of the CS, the length from the Z-line to the EGJ (presumed by CS) which was recorded using the Prague C&M criteria [20], the fine structure of the mucosa below the Z-line according to Guelrud M's study [21],and the clinical symptoms of patients in BE group.

Statistical methods

All analyses were performed with SPSS 18.0 software. χ^2 test or Fisher's exact test were used to compare the categorical variables. As the age for each group was normally distributed and had equal variance, *t*-test was conducted to test their mean difference. Statistical difference was considered to be significant at the level of 0.05.

Results

The general characteristics of the subject

A total of 110 people were enrolled in the study and there were 47 patients in the BE group. Consistent with our patient population, the majority of the patients were over-aged with a roughly equal distribution between males and females (Table 1). In the BE group, the main clinical symptoms were not the same. 19 patients (40.42%) had acid reflux and heartburn, 12 (25.53%) had upper abdominal pain, 7 (14.89%) had abdominal distention, 5 (10.64%) had abdominal discomfort, and 4 (8.51%) had no typical symptom. In control group, there was no patient having symptom, consistent with the healthy screening population. There were not significant differences between two group of their taste preference (Table 1).

Outcomes

In the BE group, the average M length of BE epithelium was 1.35 ± 0.48 cm (Prague criteria), and the C length was 0.50 ± 0.32 cm (Table 2). There was no long segment BE patient, and there were 38 (80.85%) patients with 1 cm ≤ M < 3 cm and 9 (19.14%) patients with M < 1 cm respectively. After acetic acid staining, CS was showed in a total of 42 patients (89.36%) in the BE group, which was significantly higher than that in the control group (5/63, 7.94%). There was a significant difference between the two groups ($\chi^2 = 72.931$, $P < 0.001$). CS could be found in the control group, which indicating movement of Z-line in 5 cases. The average width of CS was 0.73 ± 0.25 cm in the BE group, which was similar with that in the control group (0.88 ± 0.11 cm, $t = -1.270$, $P = 0.211$).

In BE group, mucosa patterns were always abnormal above the CS, including 33.33% villous (Fig. 2a), 30.95% reticular and 33.33% punctate pattern. Blow the CS, the reticular (50.00%) and punctate patterns (45.24%) were observed after staining (Fig. 2b, c). There were some

Table 1 Baseline characteristics of the two groups

	BE group $n = 47$	Control group $n = 63$	χ^2	*P* value
Age, mean ± SD	53.68 ± 14.39	49.41 ± 11.51	1.728[a]	0.087
Female, n (%)	16 (34.0)	28 (44.4)	1.214	0.271
Fissure hernia, n (%)	6 (12.8)	4 (6.3)	1.341	0.320*
Taste preference				
Peppery	14 (29.79)	21 (33.33)	5.283	0.152
Sweet	11 (23.40)	18 (28.57)		
Sour	7 (14.89)	15 (22.22)		
Plain food	15 (31.91)	9 (14.29)		
Sympotoms (%)				
Acid reflux or heartburn	19 (40.42)	0		
Upper abdominal pain	12 (25.53)	0		
Abdominal distention	7 (14.89)	0		
Abdominal discomfort	5 (10.64)	0		
Asymptomatic	4 (8.51)	63 (100.00)		

*Fisher's exact test
[a]t value from t-test

Table 2 Results of gastroscopy and pathology in BE group vs control group

	BE group $n = 47$	Control group $n = 63$	χ^2 or t value	*P* value
M value(cm), mean ± SD	1.35 ± 0.48	–		
C value(cm), mean ± SD	0.50 ± 0.32	–		
CS below the Z-line, n(%)	42 (89.36)	5 (7.94)	72.931	< 0.001
Width of CS (cm), mean ± SD	0.73 ± 0.25	0.88 ± 0.11	-1.270[a]	0.211
Above the CS				
Punctate pattern, n (%)	14 (33.33)	1 (20.00)	–	*
Reticular pattern, n (%)	13 (30.95)	3 (60.00)	–	*
Villous pattern, n (%)	14 (33.33)	0 (0.00)	–	*
Below the CS				
Punctate pattern, n (%)	19 (45.24)	2 (40.00)	–	*
Reticular pattern, n (%)	21 (50.00)	2 (40.00)	–	*
Villous pattern, n (%)	0	0	–	*
Without the CS	5 (10.6)	58 (92.1)	72.931	< 0.001
Punctate pattern, n	2 (40.00)	1 (1.72)	–	*
Reticular pattern, n	2 (40.00)	0 (0.00)	–	*
Villous pattern, n	1 (20.00)	0 (0.00)	–	*
Pathology confirmed intestinal metaplasia (IM), n (%)	23 (48.94)	3 (4.76)	29.101	< 0.001
The region of CS, n (%)	8 (34.78)	2 (66.67)	–	*
Above the CS, n (%)	13 (56.52)	1 (33.33)	–	*
Below the CS, n (%)	2 (8.70)	0 (0.00)	–	*

*The sample size of the variable is too small to do the hypothesis testing
[a]t value from t-test

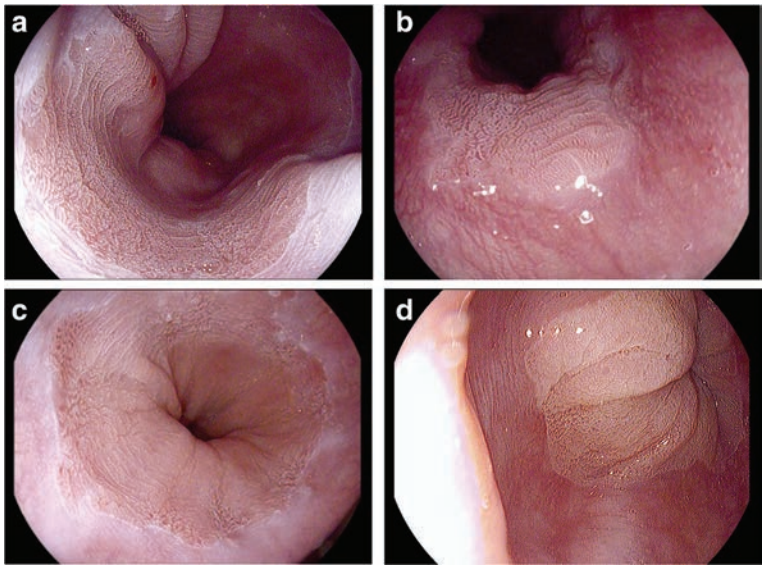

Fig. 2 Aceto-whitening reaction for the diagnosis of BE after instillation of 2% acetic acid. **a**, **b**, **c** After spraying acetic acid, the mucosal surface shows multiple CS near EGJ, with the surface pattern could be identified by either reticular or punctate or villous. **d** normal mucosa with punctate pattern without CS

abnormal mucosa patterns observed in control group (Table 2).

Without CS, the mucosa patterns were always normal, just 1 participant in control group without CS (1/58) was observed to have punctate pattern (Fig. 2d). But in BE group, although 5 patients didn't have CS, their mucosa patterns were all abnormal.

Pathological examination showed that 23 (48.94%) patients had intestinal metaplasia (IM), which was significant more than control group (3/63, 4.76%). The patients with IM all had CS meanwhile. In BE group, 34.78% IMs were detected in the region of CS, 56.52% were above the CS and just 8.70% were below the CS.

Discussion

Because the AA could give a good enhancement on the mucosa pattern at lower esophagus, we were using AA staining as a routine procedure for gastroscopy and the CS were unexpected discovery. In this study, CS were mostly observed in the BE group. There might be three potential mechanisms underneath this phenomenon.

Firstly, the CS might be caused by columnar epithelial metaplasia following squamous epithelium retraction. The AA might emphasize the gap between columnar epithelium in different periods. It could be found that the squamous epithelium was in circular pattern near the cardia in healthy people and the CS partially crossed from columnar epithelium to squamous epithelium (Fig. 3a, b), both supporting this explanation. However, the CS were only confined at the cardia within 2 cm. The emergence of new columnar epithelium in the higher position no longer formed CS anymore and could be found in punctate, reticular or villous pattern while the previous two mainly. On the other hand, there could be absence of CS in 5 BE patients and villous or punctuate mucosa pattern were observed (Fig. 3c). Therefore, the generation of the CS cannot be fully explained only by the regression and metaplasia theory.

The second possible explanation was that the CS might be specifically performed at the EGJ region and its scope exactly represented the range of EGJ. The hypothesis, that EGJ was not a simple line, but a small portion of the lower esophagus to the cardia, was suggested by previous studies [22–24]. The dense squamous epithelium covering the EGJ might be the reason why we could less likely observe CS in the control group. When the squamous epithelium gradually became thinner or replaced by columnar epithelium, the CS would be revealed. However, more evidence needs to be found.

Thirdly, the CS might be the result of repeated hyperplasia and substitution of the cardiac epithelium. The columnar epithelium of the cardia in BE patients might be affected by inflammation [25] or mechanical motion, which led to the edema or protein change of epithelium cell. For example, some healthy people were observed with circular appearance of the cardia in the inferior position occasionally. However, there was difference between non-metaplastic CS and metaplastic CS, that stripes in the former were flatter, while the latter were often stacked (Fig. 3d).

Based on the above assumptions, CS might help to identify the EGJ, sometimes obscure in white light

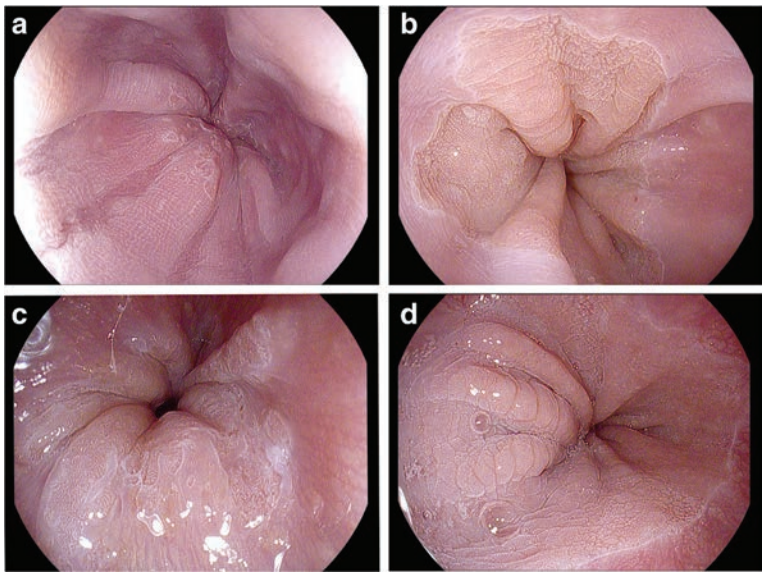

Fig. 3 Special features after instillation of the 2% acetic acid. **a** Squamous epithelium was in circular pattern near the cardia in healthy people, **b** CS partially crossed from columnar epithelium to squamous epithelium, **c** Absence of CS in 2 BE patients and only villous or punctuate mucosa pattern were observed, **d** Flat circular appearance was observed in healthy people

images, even in magnified or NBI images. EGJ is an important marker for endoscopists to get biopsy, which is necessary for the diagnosis of BE in some guidelines, such as British Society of Gastroenterology guidelines and American Gastroenterological Association on the diagnosis of BE [1, 9]. Most studies suggested that EGJ was a marker for pointing the initial position of squamous epithelium, and further evaluating distance between the EGJ and the ascending squamo-columnar junction (SCJ) precisely [1, 9–11, 26]. Paris Workshop showed that the EGJ was located in the abdomen, just below the diaphragmatic pinch with the upper margin of the longitudinal gastric folds coinciding with the SCJ in the normal situation. The length of the metaplastic columnar segment is the distance between the neo-formed SCJ and the anatomical EGJ [26], and the reliability of the evaluation depends on the precision of the determination of the EGJ under endoscopy [26]. Mistake in EGJ judgement has little influence on diagnosis of long-segment BE, however it would mislead the diagnosis of short or ultra-short segment BE, while the majority of the Asian BE patients are in short segment [11, 26–31]. Ishimura showed that the prevalence of long segment BE was extremely low in East Asia, while the prevalence of short segment BE was very high only in Japan [11], which was similar to Okita K and Amano Y [30, 31]. Chang CY showed that short segment BE (75.6%, $n = 31$) was more prevalent than long segment BE (24.4%, $n = 10$) in Chinese population [28, 29]. Therefore, more effective method is needed to determine EGJ. If the CS after AA staining are related to the newly hyperplastic columnar epithelium, the length of the BE epithelium can be evaluated from distal end of CS; if CS are limited to the EGJ region, then the proximal end can be borderline for hyperplastic epithelium. Multiple biopsies may prolong the time of procedure and increase patients' suffering, and cause too much bleeding to get the high-risk lesions [18, 32]. The CS helps to outline the target area and make emphasis on the microstructure of the surface. Meanwhile the fading effect after the AA staining can help to identify the abnormal mucosal lesions [14].

The research of the CS could also help to understand the origin and development of BE. Pathologists generally believe that the BE epithelium consists of three tissue types: [1] proximal end is intestinal epithelial cells including goblet cells, [2] in the middle, it is connection type epithelium that is cardiac mucosa without goblet cells, [3] the distal end is the basal epithelium contains both parietal cells and primary cells [33–36]. Our study indicates that CS may be a useful marker representing the connected epithelium perfectly and furthermore pathological evidences are required to support this theory.

It is generally believed that BE is closely associated with gastroesophageal reflux disease [1]. This study showed that BE patients mainly had gastroesophageal reflux symptoms, including acid reflux or heartburn, but 59.58% of patients had no symptoms of gastroesophageal reflux, which was similar to the literature reports [37–39]. These findings suggested that there may be other etiological factors, such as race, environment, diets, use of alcohol or smoking. Therefore, we should pay attention to the people without gastroesophageal reflux symptoms. Combined with magnifying endoscopy, Toyoda improved the mucosal microstructure classification through the study of patients with BE, including 3 types: normal pits, slit-reticular pattern, and gyrus-villous pattern. The sensitivity and specificity of gyrus-villous pattern for IM were 88.5% and 90.2%, and the overall accuracy was 90.0% [35]. In this study, we observed there was no IM in the punctate and the reticular area in the BE group, while the accuracy rate of IM was 100% in the villous area, which was consistent with Toyoda. This result suggested that AA staining combined with high resolution endoscopy could also improve the yielding of BE diagnosis without magnifying endoscopy, NBI or BLI, especially in screening.

The deficiency of this research was that the sample size was limited as a pilot study. In addition, we did not classify the different types of BE because of the small sample size. So further research is needed to explore the differences and the occurrence mechanism of different types of BE, and to explore the effectiveness of different endoscopic techniques in the diagnosis of BE epithelial range and nature.

Conclusion

This is the pilot study that mentions and describes CS as a special feature under high resolution endoscopy with AA staining, and CS may become an important reference in the diagnosis and treatment of BE.

Additional file

Additional file 1: The features after 2% acetic acid in patients with esophageal epithelial erosion. The esophageal epithelial erosion is always lead by gastroesphageal reflux disease and will impact the mucosa observation after acetic acid staining. (JPEG 666 kb)

Abbreviations

AA: Acetic acid; BE: Barrett's esophagus; CS: Circular stripes; EGJ: Esophagogastric junction; IM: Intestinal metaplasia; SCJ: Squamo-columnar junction

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Availability of data and materials
The relevant raw data from this study can be readily available on request for non-commercial purpose per request from the corresponding author.

Authors' contributions
YS participated in the collection and analysis of data and writing of the manuscript. SM and LD participated in conception and oversight of the study, supervision, data analysis and manuscript editing. LF and JW participated in the data collection and analysis. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate
The study protocols were approved by the Ethical Committee at the Second Affiliated Hospital of Xi'an Jiaotong University (No. 2017023). The written informed consent was obtained from each participant.

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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RESEARCH ARTICLE

Length of Barrett's segment predicts failure of eradication in radiofrequency ablation for Barrett's esophagus: a retrospective cohort study

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Abstract

Background: We aim to investigate factors that may contribute to failure of eradication of dysplastic Barrett's Esophagus among patients undergoing radiofrequency ablation treatment.

Methods: A retrospective review of patients undergoing radiofrequency ablation for treatment of Barrett's Esophagus was performed. Data analyzed included patient demographics, medical history, length of Barrett's Esophagus, number of radiofrequency ablation sessions, and histopathology. Subsets of patients achieving complete eradication were compared with those not achieving complete eradication.

Results: A total of 107 patients underwent radiofrequency ablation for Barrett's Esophagus, the majority white, overweight, and male. Before treatment, 63 patients had low-grade dysplasia, and 44 patients had high-grade dysplasia or carcinoma. Complete eradication was achieved in a majority of patients (57% for metaplasia, and 76.6% for dysplasia). Failure of eradication occurred in 15.7% of patients. The median number of radiofrequency ablation treatments in patients achieving complete eradication was 3 sessions, compared to 4 sessions for failure of eradication ($p = 0.06$). Barrett's esophagus length of more than 5 cm was predictive of failure of eradication ($p < 0.001$).

Conclusions: Radiofrequency ablation for dysplastic Barrett's Esophagus is a proven and effective treatment modality, associated with a high rate of complete eradication. Our rates of eradication from a center starting an ablation program are comparable to previously published studies. Length of Barrett's segment > 5 cm was found to be predictive of failure of eradication in patients undergoing radiofrequency ablation.

Keywords: Barrett's esophagus, Radiofrequency ablation, Endoscopy, Esophagus

Background

Barrett's Esophagus (BE) is a condition in which the stratified squamous epithelium that lines the distal esophagus is replaced by metaplastic columnar epithelium that predisposes to the development of dysplasia and adenocarcinoma [1]. Esophageal adenocarcinoma (EAC) incidence has been on the rise, most drastically in the Caucasian segment of the American population [2, 3]. Therefore, it is important to adequately address dysplastic precursor lesions to EAC. A relatively recent addition to gastrointestinal endoscopy is radiofrequency ablation (RFA) using the HALO system (BARRX Medical, Inc., Sunnyvale, CA, USA), which has been shown to be safe and effective for the treatment of BE, including low-grade dysplasia (LGD) and high-grade dysplasia (HGD) [4–6]. Not only is RFA associated with decreased neoplastic progression compared to surveillance endoscopy [7, 8], a recent meta-analysis of the literature showed a pooled complete eradication of intestinal metaplasia (CE-IM) rate of 78% (95% CI 70–86%) and complete eradication of dysplasia (CE-D) rate of 91% (95% CI 87–95%) [9].

Despite high rates of eradication, as many as one-third of patients experience recurrence after complete eradication [10]. Some cited predictors of recurrence are older

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age, non-Caucasian race and longer length of pretreatment BE [11, 12]. Additionally, some patients do not respond to RFA or require multiple sessions to obtain complete eradication. While some have not been able to determine any significant predictors of response to therapy [13], others have found that active reflux disease, longer history of dysplasia, increased hiatal hernia size as well as increased length of BE are all predictors of RFA failure [14–16].

The success of RFA is such that it has become integrated at many large institutions in combination with resection techniques, such as endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD), which are needed to remove macroscopically visible lesions [17]. Given this increased use, it is vital to determine which patients may be at high risk for not responding to RFA and thus neoplastic progression. The current literature is conflicting, as studies that have found predictors of RFA failure differ in their results. For instance, Lyday et al. found CE-IM to be inversely related to BE length [15], while van Vilsteren et al. did not find BE length to be statistically significant [14], leading to the conclusion that further investigation is warranted.

The goals of this study were as follows: (1) to determine factors that may predict failure of CE-IM and CE-D in patients treated with RFA, and (2) to report the rates of CE-IM and CE-D at a large institution that recently began offering RFA and compare them to those previously published in the literature.

Methods

Study design

After the study was reviewed and approved by the University of Alabama at Birmingham Institutional Review Board (IRB), a retrospective review of consecutive patients undergoing RFA between December 2009–February 2015, for treatment of Barrett's Esophagus at the University of Alabama at Birmingham (UAB) was performed. All study participants provided informed written consent prior to study enrollment. Data was entered and stored in a de-identified spreadsheet. Data abstracted for analysis included patient demographic characteristics, medical history, pathological findings, endoscopic findings, endoscopic procedures, adverse events, treatment, and biopsies with histopathology findings on surveillance. A standard four quadrant biopsy protocol based on the Seattle protocol was used for sampling [18]. As part of this protocol, targeted biopsy using narrow band imaging was performed. All biopsies were examined by the same experienced GI pathologist, and were reviewed again by a separate pathologist for documentation of consensus. Histopathology was graded and classified as high grade dysplasia, low grade dysplasia, or intestinal metaplasia. Both endoscopic inspection and biopsy results were used to determine which patients needed additional rounds of RFA to try to achieve CE. The biopsy protocol at our institution was as follows: We would continue RFA sessions until BE appeared endoscopically cleared, and then biopsies would be obtained at that time. Patients in our study required between two to ten RFA sessions to achieve complete eradication (mean = 3 sessions), and after any number of RFA sessions, if the patient appeared to have endoscopic clearance of BE, then biopsies would be obtained at that time to document complete eradication. Similarly, if a patient had an endoscopically visible lesion that needed targeted biopsy, then biopsies would be obtained at that time. Based on the results of mucosal biopsies after endoscopic treatment, patients were then divided based on histopathology into complete eradication (CE) of dysplasia (CE – D) or intestinal metaplasia (CE – IM). Subsets of patients achieving CE were compared with those not achieving CE. Those patients with mucosal biopsies demonstrating persistent dysplasia or intestinal metaplasia after treatment with RFA were considered failure of CE. Thus, failure was based upon histology, not endoscopy. Patients were considered lost to follow-up if post-treatment biopsies were not obtained.

Procedure description

Patients were placed in the decubitus supine position. All procedures were performed with patients under monitored anesthesia care (MAC). Measurements of BE were done using the Prague Classification [19]. Patients underwent ablation using the circumferential device (HALO^{360} system) or a focal device (HALO^{90} both from Covidien GI Solutions) according to the extent of disease and investigator preference, as previously described. Subsequent ablation sessions were performed every 2 months, until complete endoscopic and histological eradication of Barrett's Esophagus. At each ablation session, if any visible nodular lesions were identified, these were first treated with Endoscopic Mucosal Resection (EMR) using the Band ligation with the Duette Multi-Band Mucosectomy Device (Wilson-Cook, Winston-Salem, NC, USA) as previously described [20]. Then, the gastro-esophageal junction was ablated circumferentially, irrespective of its endoscopic appearance. Our protocol for ablation therapy has been previously described [21]. In more detail, the protocol for circumferential ablation and focal ablation included endoscopy with visual inspection, reading landmarks, sizing balloon, selection of ablation type, first pass ablation, clearing the face, and then second pass ablation. Focal ablation RFA was performed for treating shorter segments or islands of tongues of BE. Energy was delivered at settings of 12 J/cm2. A similar second pulse of energy was given after cleaning the electrode. Each target area received

a total of 4 energy ablations for focal ablation and 2 for circumferential ablation respectively. The average length of each RFA treatment was 15.6 min.

CE-IM and CE-D were defined as complete eradication of IM and dysplasia, respectively, as documented by histopathology from mucosal biopsy obtained by white-light endoscopy (GIF-HQ190 Olympus, Tokyo, Japan). Time to CE-IM or CE-D was measured from the date of first RFA to the first follow-up EGD with normal histopathology reported for biopsy specimens. Recurrence was defined as the presence of IM or dysplasia in standard surveillance biopsies. The neosquamocolumnar junction was assessed in every case by white-light endoscopy with biopsies. For surveillance, 4-quadrant biopsies were performed at 1 cm intervals of the original extent of the Barrett's Esophagus, starting at 1 cm proximal to the top of the gastric folds. In addition, any suspicious visible lesions were targeted, biopsied, and placed in separate jars. Remission of intestinal metaplasia/ dysplasia was confirmed with endoscopic findings and the four quadrant biopsy protocol.

Statistical analysis

Unadjusted univariate and bivariate comparisons were made, utilizing chi-square or Fisher exact test for categorical variables and two tailed t-tests or Wilcoxon Rank Sums for continuous variables, where appropriate. Negative binomial logistic regression was used to model predictors of failure for CE-IM and CE-D utilizing stepwise selection. Significance was determined by a $p < 0.05$. All statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

A total of 107 patients underwent RFA for BE. Overall, 96.3% ($n = 103$) of the patients were white, and 86.9% ($n = 93$) were male. The median age was 64 years (range 58–72 years), and the mean length of Barrett's esophagus was 6.7 cm (range 2-8 cm, median 5 cm). Most patients were overweight, with mean BMI 29.1 (range 25.5–32.6). On average, each patient underwent 3 (range 2–10, median 3) RFA procedures. The median time until CE-IM was 238 days (119–474) and the median time until CE-D was 251 days (133–525). There were 20 patients (15.7%) who did not obtain post-treatment biopsies, and were considered lost to follow up. Of the patients included in the study, 41.1% had HGD, and 58.9% had LGD. After RFA treatment, 57.0% of patients achieved CE-IM, and 76.6% achieved CE-D. 4.7% of patients progressed to esophageal adenocarcinoma. The average time to progression from dysplasia to adenocarcinoma was 170 days. The initial pathology for all patients that progressed to esophageal adenocarcinoma was HGD. Focal ablation was performed only for shorter segments of BE, as this tends to be more effective than circumferential ablation for these lesions. Comparing eradication rates between focal and circumferential ablation was not the main study objective, so our study did not directly compare differences in circumferential ablation versus focal ablation. Also, many longer segment BE lesions initially treated with circumferential ablation were later followed up with focal ablation, making it difficult to directly compare circumferential and focal ablation. There were no statistically significant differences in rates of CE-IM or CE-D for patients with HGD versus those with LGD. There were no statistically significant differences in BE segment length in patients with HGD (mean 6.2 ± 4.2 cm) versus LGD (mean 5.3 ± 3.8 cm).

Independent predictors of failure to achieve CE-IM [see Table 1] included age > 64 years, (OR: 2.6, (1.20–5.79); $p < 0.02$), and having a BE segment length greater than 5 cm (OR: 4.03(1.78–9.09); $p < 0.001$). On adjustment, both age (OR: 4.508, (1.72–11.84), $p < 0.0022$) and length of segment (OR: 7.064, (2.62–19.06), $p < 0.001$) remained significant predictors of failure to achieve CE-IM.

Independent predictors of failure to achieve CE-D [see Table 2] included having hypertension (OR: 3.33(1.21–9.17); $p = 0.02$), and having a BE segmental length greater than 5 cm (OR: 2.60 (1.04–6.51); $p = 0.04$). On adjustment, both hypertension (OR 3.86; 1.32–11.31, $p < 0.01$) and length of segment (OR 3.08; 1.12–8.46, $p < 0.03$) remained significant predictors of failure to achieve CE-D (see Table 3). The number of patients who developed adenocarcinoma was very small, so no independent predictors were identified.

There were no major complications from RFA therapy in our study population. Specifically, there was no stricturing or bleeding noted on follow-up EGD. Retrospective review from the medical records did not reveal any documented complications related to anesthesia/ sedation.

Discussion

Many studies have analyzed the durability and recurrence of BE associated with RFA, but few studies to date have examined factors which affect the rate of eradication of CE-IM or CE-D with RFA. We analyzed multiple factors including patient characteristics such as age, comorbidities such as GERD, hypertension, or diabetes mellitus, risk factors such as tobacco use or duration of reflux, and endoscopic characteristics such as length of Barrett's segment and number of treatments with RFA. The number of patients in our study population who drank alcohol was low, so this risk factor was not analyzed. In our study, the overall rate of CE-IM and CE-D was 57 and 76.6%, respectively. The rates of CE-IM and CE-D in our study are similar to other published studies, which demonstrate rates of CE-IM ranging from 41 to

Table 1 Factors Affecting Complete Eradication of Intestinal Metaplasia

	All Patients[a]		Complete Eradication of Intestinal Metaplasia		Incomplete Eradication of Intestinal Metaplasia		
	107	100.0%	61	57.0%	46	43.0%	*p*-value[b]
Patient Characteristic							
Race							
White	103	96.3%	59	96.7%	44	95.7%	0.773
Other	4	3.7%	2	3.3%	2	4.3%	
Sex							
Male	93	86.9%	51	83.6%	40	86.9%	0.242
Female	14	13.1%	10	16.4%	6	13.1%	
Age	[c]64	(58-72)	[c] 63	(57–72)	[c] 67	(59–76)	0.117
BMI	[c] 29.1	(25.5–32.6)	[c] 30.9	(26.5–33.1)	[c] 28.3	(24.5–30)	0.077
Dysplasia							
HGD	44	41.1%	24	39.3%	20	43.5%	0.667
LGD	63	58.9%	37	60.7%	26	56.5%	
Comorbidities							
GERD	75	70.1%	30	49.2%	32	69.6%	0.339
Hyperlipidemia	26	24.3%	17	27.9%	11	23.9%	0.322
Diabetes	25	23.4%	15	24.6%	10	21.7%	0.730
Hypertension	59	55.1%	30	49.2%	31	67.4%	0.154
History of Tobacco Usage							
Yes	60	56.1%	30	49.2%	29	63.0%	0.225
No	33	30.8%	21	34.4%	13	28.3%	
Unknown	14	13.1%	10	16.4%	4	8.7%	
Endoscopic Treatments Received							
EMR	24	22.4%	16	26.2%	8	17.4%	0.326
Length							
Median, IQR	5	(2–7)	3	(2–7)	7	(2–8)	< 0.001
</= 5 cm	64	59.8%	44	72.1%	20	43.4%	< 0.001
> 5 cm	43	40.2%	17	27.9%	26	56.6%	
Number of RFA's							
Median, IQR	3	(2–5)	3	(2–5)	4	(2–5)	0.023
≤ 3	62	57.9%	41	67.2%	21	45.7%	0.008
> 3	45	42.1%	20	32.8%	25	54.3%	

[a]Data presented as N (%) or median (IQR)
[b]*p*-value ≤0.05 is significant
[c]average; not total number of patients

67% [9]. Some other studies with higher rates of CE-IM and CE-D typically treated shorter lengths of BE than our current study [21, 22] which had 42.1% of patients with BE segment greater than 5 cm. We found that length of Barrett's segment length greater than 5 cm was independently predictive of a higher rate for failure of complete eradication in patients undergoing RFA. Of those patients in our study with failure of CE-IM or CE-D, 56.6 and 60.0% of patients, respectively, had a pretreatment BE length of > 5 cm. Longer segments of BE have been associated with potentially more aggressive behavior and with a resultant higher risk of progression, which may explain the lower rates of CE in our study [11, 12]. These studies demonstrate similar findings with longer segments of BE associated with higher rates of eradication failure and recurrence. In addition, their finding that BE of length 4.8 vs. 3.8 cm had significantly higher recurrence after treatment correlates closely with our data showing that BE length > 5 cm predicts failure with RFA. Although the reasons for the association are unclear, a longer pretreatment segment may be a marker for more severe acid exposure and injury [12]. Recent

Table 2 Factors Affecting Complete Eradication of Dysplasia

	All Patients[a]		Complete Eradication of Dysplasia		Incomplete Eradication of Dysplasia		
	107	100.0%	82	76.6%	25	23.4%	*p*-value[b]
Patient Characteristics							
Race							
White	103	96.3%	80	97.6%	23	92.0%	0.232
Other	4	3.7%	2	2.4%	2	8.0%	
Sex							
Male	93	86.9%	69	84.1%	24	96.0%	0.180
Female	14	13.1%	13	15.9%	1	4.0%	
Age	[c]64	(58-72)	[c] 64	(57–72)	[c] 66	(59–76)	0.336
BMI	[c] 29.1	(25.5–32.6)	[c] 30.5	(26.5–33.1)	[c] 27.45	(24.5–30)	0.045
Dysplasia							
HGD	44	41.1%	30	36.6%	14	56.0%	0.084
LGD	63	58.9%	52	63.4%	11	44.0%	
Comorbidities				0.0%			
GERD	75	70.1%	60	73.2%	15	60.0%	0.208
Hyperlipidemia	26	24.3%	20	24.4%	6	24.0%	0.968
Diabetes	25	23.4%	18	22.0%	7	28.0%	0.532
Hypertension	59	55.1%	40	48.8%	19	76.0%	0.017
History of Tobacco Usage							
Yes	60	56.1%	43	52.4%	17	68.0%	0.350
No	33	30.8%	28	34.1%	5	20.0%	
Unknown	14	13.1%	11	13.4%	3	12.0%	
Endoscopic Treatments Received							
EMR	24	22.4%	18	22.0%	6	24.0%	0.732
Length							
Median, IQR	5	(2–7)	4	(2–7)	6	(2–8)	0.150
</= 5 cm	64	59.8%	52	63.4%	12	48.0%	0.038
> 5 cm	43	40.2%	30	36.6%	13	52.0%	
Number of RFA's							
Median, IQR	3	(2–5)	3	(2–5)	4	(2–5)	0.066
≤ 3	62	57.9%	52	63.4%	10	40.0%	0.064
> 3	45	42.1%	30	36.6%	15	60.0%	

[a]Data presented as N (%) or median (IQR)
[b]*p*-value ≤0.05 is significant
[c]average; not total number of patients

studies also show a spatial preference for dysplasia being more common in proximal areas of the Barret's segment [23]. In addition, recent literature studying cryotherapy as a modality for BE refractory to RFA has also revealed that RFA failure groups have longer Barrett's segments. [24, 25]. We believe the length of Barrett's segment ablated was the main reason for the large range of RFA sessions required for eradication of BE, as longer segments tended to require more frequent RFA sessions to achieve eradication. The choice of focal or circumferential ablation was standardized based on the protocol as discussed above, and we feel that the choice of ablation technique was not a contributing cause to the range of RFA sessions required for eradication. A greater BMI seems to be associated with longer segment of BE, however we did not find the BMI to be an independent predictor for failure in our study [26].

Another finding of our study was that having a greater number of RFA treatments was predictive of failure of CE-IM. Those patients who required more than 3 RFA treatments were significantly more likely to have failure of CE-IM. Of the 46 patients who failed to achieve

Table 3 Predictors of Failure

Covariate	Incomplete Eradication of Intestinal Metaplasia						Incomplete Eradication of Dysplasia					
	Univariate Analysis			Multivariate Analysis			Univariate Analysis			Multivariate Analysis		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Not White	1.34	0.18–9.89	0.77				3.48	0.46–26.07	0.23			
Female	2.06	0.60–7.04	0.25	4.157	0.94–18.40	0.060						
65 years or older	2.63	1.20–5.79	0.02	4.508	1.72–11.84	0.0022	2.16	0.857–5.45	0.10	Ref.	Ref.	0.10
Obese, BMI > 30	2.60	1.11–6.14	0.01				2.29	0.85–6.20	0.08			
HGD	1.19	0.55–2.58	0.67				2.21	0.89–5.47	0.09	2.151	0.80–5.80	0.13
GERD	1.50	0.65–3.45	0.34				Ref.	Ref.	0.21			
Hyperlipidemia	1.59	0.63–3.98	0.32				Ref.	Ref.	0.97			
Diabetes	1.17	0.47–2.92	0.73				1.38	0.50–3.83	0.53			
Hypertension	1.76	0.81–3.85	0.16				3.33	1.21–9.17	0.02	3.86	1.32–11.31	0.01
Tobacco Usage	2.50	0.71–8.85	0.23				1.45	0.36–5.85	0.36			
Length > 5 cm	4.03	1.78–9.09	< 0.01	7.064	2.62–19.06	< 0.01	2.60	1.04–6.51	0.04	3.08	1.12–8.46	0.03
> 3 RFA's	2.16	0.96–4.89	0.06				1.15	0.45–2.93	0.78			
				c statistic:	0.766					c statistic:	0.761	
				r squared	0.285					r squared	0.2215	

CE-IM, 54.3% had greater than 3 RFA treatments. This is similar to findings from other studies such as Agoston et al., which also suggest increased number of treatments predicts failure of eradication [11] [27]. These results may actually be explained by a more aggressive neoplastic phenotype as opposed to a result of treatment, and could explain differences in measured rates of achieving CE-IM. Other notable statistically significant predictors of failure in our study included age greater than 64 years old for CE-IM. While the significance of this finding is unclear, it has been suggested that elderly people may have more prolonged exposure to carcinogens and are therefore more likely to accumulate somatic mutations [12]. Hypertension was also found to be a statistically significant predictor of failure of CE-D. We suspect that this is likely just a statistical finding related to small study size. Further evaluation is necessary to support these factors as predictors of eradication failure.

These findings are important because RFA is used commonly for the treatment of BE. Identifying factors which place patients at higher risk of not responding to RFA may also help identify individuals with a greater risk of progressing to neoplasia. Those patients with longer pretreatment BE are at greater risk of failure of complete eradication with RFA, and may benefit from a more invasive treatment approach such as EMR. Patient's with persistent BE after greater than 3 treatments with RFA are at greater risk of failure of complete eradication, and this could be helpful for directing further therapy as continued RFA sessions may be less beneficial. At this point, other treatment modalities such as APC, cryotherapy, or EMR could be considered. Other contributing factors such as medication noncompliance or lack of appropriate follow-up should also be considered. It should also be noted that studies have shown that RFA is a cost-effective strategy for treatment of dysplastic Barrett's esophagus [28].

Our study had limitations which should be considered when interpreting the data. The study was retrospective in nature, and data was collected entirely from our own single institution: a large, academic, tertiary-care hospital which is relatively new to the technique of radiofrequency ablation for eradication of Barrett's Esophagus. Another limitation is that there was a moderate percentage that were lost to follow up, thus limiting the results. However, in general, our data is similar to that of other large, academic hospitals with high rates of complete eradication of both intestinal metaplasia and dysplasia. The retrospective nature of our study also makes misclassification possible. Also, some patients were lost to follow up for unknown reasons, which could affect the reported rates of eradication.

The lower eradication rate is another limitation of our study. This lower rate is likely related to the number of patients lost to follow-up, as well as the fact that our patient sample is entirely from a tertiary care referral center, likely dealing with the most complex cases.

Conclusion

In conclusion, we have identified pathologic factors as well as endoscopic factors which are associated with a higher risk of failure to achieve CE-IM or CE-D with

RFA treatment of BE. Knowledge of these predictors can help identify patients at higher risk for treatment failure and subsequent increased risk for neoplastic progression. This knowledge may be beneficial to prompt a more aggressive initial therapy to prevent unnecessary procedures or neoplastic progression.

Abbreviations

BE: Barrett's Esophagus; BMI: body mass index; CE: Complete eradication; CE-D: Complete eradication of dysplasia; CE-IM: Complete eradication of intestinal metaplasia; EAC: Esophageal adenocarcinoma; EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection; HGD: High grade dysplasia; LGD: Low grade dysplasia; MAC: Monitored anesthesia care; RFA: Radiofrequency ablation

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Availability of data and materials

The data used and analyzed during the current study are included in this published article, and are also available from the corresponding author on reasonable request. Technical appendix, statistical code, and dataset are also available from the corresponding author on reasonable request.

Authors' contributions

TL, JE, KC, and SP made substantial contributions to the conception and project design. TL and KC made substantial contributions to the acquisition and analysis of data. AG and TL made substantial contributions to the statistical analysis of the data. TL, KC, CA, and SP made substantial contributions to the drafting of the manuscript, as well as critical revisions. All parties have given final approval of the version to be published, and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

The study was reviewed and approved by the University of Alabama at Birmingham (UAB) Institutional Review Board. All study participants provided informed written consent prior to study enrollment.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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RESEARCH ARTICLE

Ultrasonic assessment of liver stiffness and carotid artery elasticity in patients with chronic viral hepatitis

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Abstract

Background: This study investigated the relationship between liver stiffness and carotid artery elasticity in patients with chronic viral hepatitis. We used an acoustic radiation force impulse (ARFI) technique to measure stiffness, and a radio frequency (RF) vascular quantitative ultrasound technique to measure changes in common carotid artery elasticity and vascular function.

Methods: Two-hundred seventeen patients with chronic viral hepatitis caused by either hepatitis B virus (HBV) or hepatitis C virus (HCV) were enrolled. We divided the patients into two groups, one comprising 147 patients with chronic hepatitis B (CHB) (98 men and 49 women, average age 46.5 ± 12.2 years) and another comprising 70 patients with chronic hepatitis C (CHC) (47 men and 23 women, average age 47.6 ± 12.1 years). Additionally, 64 healthy age- and sex-matched participants (43 men and 21 women, average age 47.8 ± 5.1 years) were selected as the control group. The ARFI technique was used to measure liver stiffness and the RF ultrasound technique was used to measure carotid artery elasticity parameters including intima-media thickness (IMT), pulse wave velocity (PWV), arterial wall dilation coefficient (DC), compliance coefficient (CC), sclerosis indices α and β, and augmentation index (Aix). Clinical indicators, liver stiffness, and carotid artery elasticity parameters were observed and compared between the different age groups to investigate the correlation between carotid artery elasticity parameters and liver stiffness.

Results: The ARFI values for the CHB and CHC groups were significantly higher than those for the control group (1.84 ± 0.52 vs. 1.04 ± 0.11 m/s; 1.86 ± 0.37 vs. 1.04 ± 0.11 m/s, respectively; $P < 0.001$). When compared to the control group, both CHB and CHC groups showed an IMT of the same order, but had significantly higher elasticity parameters, such as α and β, as well as lower DC and CC values ($P < 0.001$). The PWV of the CHC group was significantly higher than that of the control group (7.98 ± 1.42 vs. 6.09 ± 0.90 m/s, $P < 0.001$). In the CHB group, all parameters including ARFI, IMT, PWV, DC, CC, α and β, were significantly different between the two age groups ($P < 0.05$). Within the CHC group, all parameters including IMT, PWV, DC, α and β, were significantly different between the two age groups ($P < 0.05$), except for ARFI, wherein the difference was not statistically significant. The correlation analysis and stepwise multiple linear regression analysis indicated that for patients with CHB, age was an independent predictor of common carotid artery IMT ($R^2 = 0.468$, F = 54.635, and $P < 0.001$). For patients with CHC, age and blood sugar were independent predictors of common carotid artery IMT ($R^2 = 0.465$, F = 29.118, and $P < 0.001$).

Conclusion: Although based on ARFI and RF ultrasound, the carotid artery IMT in patients with CHB and CHC was not significantly higher than that in the control group, their functional elasticity parameters had already changed. This finding serves as a useful reference for the clinical diagnosis of vascular diseases in patients with viral hepatitis.

Trial registration: ClinicalTrials: ChiCTR1800015859 25/04/2018.

Keywords: Ultrasound, HBV, HCV, Carotid artery elasticity

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Background

Vascular wall elasticity is an important indicator of abnormal lipid metabolism in vascular disease, which is caused by pathogen-mediated chronic liver damage [1, 2]. Signals in the radio frequency (RF) vascular quantitative ultrasound technique quantify the intima-media thickness (IMT) and arterial stiffness within blood vessels and can serve as a sensitive indicator of early changes in vascular wall stiffness. The progression of liver fibrosis changes liver morphology and hepatic haemodynamics and decreases liver function. Liver biopsy has been the gold standard to measure and classify liver fibrosis, but because of its invasiveness, its clinical use is limited. Recently, elastography, a novel non-invasive technique for evaluating the degree of liver fibrosis, has gradually been applied in clinical practice and has been included in several liver disease diagnosis and treatment guidelines [3]. Preliminary experiments found that carotid artery elasticity parameters in patients with coronary artery disease and diabetes differed from those of healthy subjects [4]. However, whether these parameters were correlated with the degree of liver fibrosis in patients with chronic viral hepatitis have not been not studied using the new vascular measurement technologies. As a result, we conducted relevant clinical investigations and experiments to understand the extent of the changes in carotid artery morphology and function in patients with chronic viral hepatitis. These findings would help to determine whether the liver fibrosis is related to macrovascular diseases.

Methods

Research targets and grouping

A total of 217 consecutive patients with chronic viral hepatitis that were treated at our hospital between December 2015 and March 2017 were enrolled. The study population comprised 147 patients with Chronic hepatitis B (CHB) and 70 patients with Chronic hepatitis C (CHC). Patients were admitted into the CHB group according to the standard chronic hepatitis B diagnostic criteria [5], defined as hepatitis B surface antigen-positive without detectable ascites. Decompensated patients with symptoms such as ascites and lower oesophageal varices were excluded from this group. In the CHB group, there were 98 men and 49 women, with an average age of 46.5 ± 12.2 years. Among them, 34 patients had biopsy-proven liver fibrosis. Patients enrolled in the CHC group were hepatitis C virus antibody- (HCVAb) positive and had decompensated liver function. The exclusion criteria for the CHC group were the same as those for the CHB group. The CHC group consisted of 47 men and 23 women, with an average age of 47.6 ± 12.1 years. Among them, a biopsy confirmed liver fibroses in 21 patients. Contemporaneously, 64 healthy subjects comparable in age and sex, which was defined by no history of liver disease, hepatitis B virus surface antigen (HBsAg) and HCVAb negativity, normal haemogram, liver, and kidney laboratory examinations, and no detection of liver diseases via 2-D ultrasound examination. Patients with disorders, such as high blood pressure, hypercholesterolemia, and dyslipidemia were excluded. The control group included 43 men and 21 women, with an average age of 45.8 ± 10.6 years.

Instruments and methods

Liver Elastography ultrasound

The acoustic radiation force impulse (ARFI) technique uses the 4C1 convex probe from the Color Doppler Diagnostic Ultrasound Scanner (Siemens, Siemens Acuson S2000) with a frequency of 3.0 to 4.5 MHz. During ARFI imaging, measurements were performed with the probe placed between the rib bones with the patient lying in either a dorsal decubitus or left recumbent position, taking normal breaths under a resting state or holding their breath, with displays of real-time 2-D images of the right liver lobe. When the image became clear, the operator used the cursor to locate a 5 mm × 10 mm-sized region of interest that targeted the liver parenchyma area and was free of vessels and bile duct. The measurement depth was fixed at 3 cm. When echoing was uniform within the sampling region, the operator pressed the probe button to freeze the image and display the depth and shear wave velocity (in m/s) in the region of interest. This measurement procedure was repeated 10 times for each patient. The median value of the measurements was recorded as the final result [6] (Fig. 1a).

Quantitative measurement of the common carotid artery

Measurements of the common carotid artery were obtained using the LA523 vascular probe from the Esaote Mylab Color Doppler Diagnostic Ultrasound Scanner with a frequency of 4 to 13 MHz. The ultrasound scanner was equipped with the RF-data technique and the Mylab Desk analysis working station. To take quantitative measurements of the common carotid artery, the patient was placed in the dorsal decubitus position, and instructed to breath normally in a resting state. After the patient's systolic and diastolic blood pressures in the right upper limb were measured, the patient's neck was sufficiently exposed. The ultrasonic probe was moved down longitudinally from the beginning of the common carotid artery, skipping the bifurcation area by 1 cm and the plaque sites. The operator then moved the sampling frame to the region for measurement, and the scanner automatically recorded the IMT and the elasticity parameters during 6 cardiac cycles. When the standard deviation of the IMT was less than 30, the indicator would turn green, suggesting that the measurements were stable. At that time, the values of parameters such as IMT, pulse wave velocity (PWV), arterial wall dilation

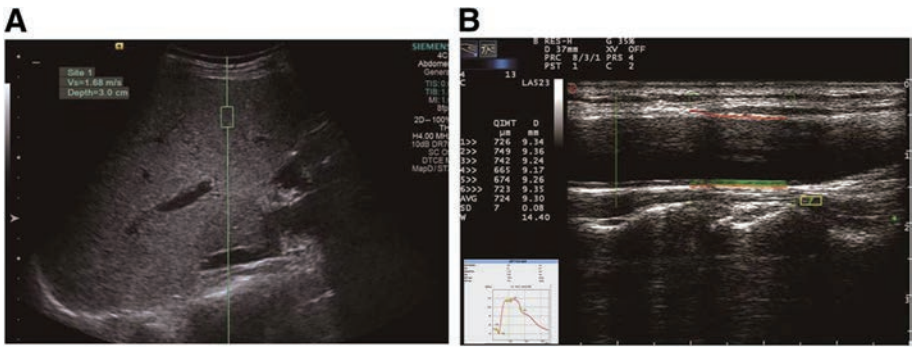

Fig. 1 ARFI liver stiffness images and common carotid artery RF ultrasound images in patients with CHB: **a** Use of the ARFI technique to measure liver stiffness in patients with CHB using a conventional ultrasound interface. **b** Use of QIMT and QAS techniques to quantify the carotid artery intima-media thickness (IMT) and vascular elasticity in patients with CHB

coefficient (DC), arterial wall compliance coefficient (CC), sclerosis indices α and β, and augmentation index (Aix) were exported as the final results [7] (Fig. 1b).

Clinical information

For all subjects, the following information was collected: (1) laboratory indicators including blood sugar, glycated haemoglobin, total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, alanine aminotransferase, aspartate aminotransferase, albumin, globulin, and platelets. (2) blood pressures including systolic and diastolic blood pressures.

Statistical analysis

Statistical analysis was performed using SPSS statistical software (version 19.0, IBM Corp., Armonk, NY, USA). Metrological data were expressed as average ± standard deviation ($\overline{X}$ ± S), while classification data were expressed as percentages (%). Inter-group measurement data were compared using one-way analysis of variance (ANOVA) and Levene's homogeneity of variance test. The average values of the two groups of measurement data were compared using the independent sample t test. A determination of correlation was conducted using Pearson's linear correlation method. Casual relationships of various intra-group parameters were investigated using either linear regression analysis or stepwise multiple linear regression analysis.

Results

General clinical data of the subjects

General clinical data for all study participants are listed in Table 1. We observed that there was no significant difference in age or sex among the three groups. Compared to the control group, the CHB group demonstrated a higher blood sugar level (5.3 ± 1.1 vs. 4.9 ± 0.5 mmol/L; $P = 0.010$) as well as higher levels of other parameters such as platelets, albumin, AST and ALT, whereas the systolic and diastolic blood pressures were similar. The CHC group demonstrated a higher blood sugar level (5.3 ± 1.1 vs. 4.9 ± 0.5 mmol/L, $P < 0.001$), higher glycated haemoglobin level (6.8 ± 1.6 vs. 5.3 ± 0.5%, $P < 0.001$), and higher levels of other parameters such as AST, ALT, and platelets compared to the control group. However, there was no significant difference in the cholesterol, triglycerides, HDL, and LDL levels.

Comparison of liver stiffness and elasticity parameters among groups

Results measured by the ARFI technique showed that both the CHB group (1.84 ± 0.52 m/s) and the CHC group (1.86 ± 0.37 m/s) had significantly higher liver elasticity parameters than the control group, and the inter-group differences were statistically significant (F = 90.806, $P < 0.001$) (Fig. 2a).

Comparison of carotid artery IMT values measured by RF ultrasound showed statistically significant differences ($P = 0.015$) between the control group (534.08 ± 134.25 μm), the CHB group (529.56 ± 131.04 μm), and the CHC group (587.34 ± 162.70 μm). An intergroup comparison showed significant difference between the CHB and the CHC group ($P = 0.032$) (Fig. 2b).

The PWV measurements of the CHC group (7.98 ± 1.42) were evidently higher than those of the CHB and the control groups, which were 6.70 ± 1.32 and 6.09 ± 0.90 m/s respectively. Differences in the intergroup comparisons were statistically significant (F = 40.310, $P < 0.001$) (Fig. 3a). The α values of the CHC and the CHB group were significantly higher than those of the control group, which were 3.03 ± 0.79, 4.13 ± 1.68, and 5.77 ± 2.29, respectively, with statistically significant differences under intergroup comparisons (F = 44.036, $P < 0.001$) (Fig. 3b). Similarly, the β values of the CHC and the CHB group were significantly higher than those of the control group, which were 6.17 ± 1.58, 8.42 ± 3.37, and

Table 1 General clinical data of all study participants

	Control Group $n = 64$	Patients with chronic viral hepatitis		F	*P*
		Patients with CHB $n = 147$	Patients with CHC $n = 70$		
Age, Years	45.8 ± 10.6	46.5 ± 12.2	47.6 ± 12.1	0.400	0.670
Men Percentage, Count (%)	43(67)	98(67)	47(67)	–	–
Systolic pressure, mmHg	115.7 ± 11.6	116.2 ± 13.1	119.7 ± 12.8	2.166	0.177
Diastolic, pressure mmHg	71.2 ± 8.7	73.2 ± 8.6	71.2 ± 8.6	1.872	0.156
Blood Glu, mmol/L	4.9 ± 0.5	5.3 ± 1.1	6.0 ± 1.7	15.933	< 0.001
Glycated haemoglobin, %	5.3 ± 0.5	5.5 ± 1.4	6.8 ± 1.6	30.804	< 0.001
BMI, Kg/m^2	23.2 ± 2.6	23.5 ± 3.0	24.4 ± 3.4	2.936	0.055
Cholesterol, mmol/L	3.8 ± 1.1	3.8 ± 1.3	3.7 ± 1.1	0.373	0.689
Triglycerides, mmol/L	1.2 ± 0.7	1.2 ± 0.5	1.2 ± 0.6	0.185	0.831
LDL, mmol/L	1.9 ± 0.8	1.8 ± 0.8	1.7 ± 0.8	0.695	0.500
HDL, mmol/L	1.0 ± 0.3	1.2 ± 0.4	1.1 ± 0.5	3.115	0.046*
Platelets, 10^9 /L	187.6 ± 70.2	137.4 ± 77.8	138.3 ± 64.3	11.598	< 0.001
AST, U/L	23.8 ± 8.7	45.8 ± 34.2	37.7 ± 24.6	13.927	< 0.001
ALT, U/L	28.5 ± 13.8	46.1 ± 38.5	43.1 ± 40.4	5.765	0.004
Albumin, g/L	43.8 ± 7.4	40.9 ± 8.9	40.8 ± 8.2	3.104	0.046*
Globulin, g/L	24.2 ± 6.3	25.3 ± 7.2	24.9 ± 7.1	0.566	0.569

Note: *CHB* chronic hepatitis B, *CHC* chronic hepatitis C, *BMI* Body mass index, *LDL* Low-density lipoprotein, *HDL* High-density lipoprotein, *AST* Aspartate aminotransferase, *ALT* Alanine aminotransferase. * $P < 0.05$ when compared to the control group

11.67 ± 4.53, respectively, with statistically significant differences under intergroup comparisons (F = 44.493, $P < 0.001$) (Fig. 3c). On the contrary, the DC and CC values of the CHC and CHB group were significantly lower than those of the control group. The DC values of the three groups were 0.32 ± 0.008, 0.030 ± 0.008, and 0.017 ± 0.008 1/kPa, respectively (Fig. 3e), with statistically significant intergroup differences (F = 3.897, $P = 0.021$). The CC values were 1.290 ± 0.248, 1.054 ± 0.385, and 0.815 ± 0.378 mm^2/kPa, respectively (Fig. 3d), with statistically significant intergroup differences (F = 29.717, P < 0.001). The DC and CC values of the CHC group were also significantly lower than those of the CHB group ($P < 0.001$). Aix measurements showed no statistically significant differences among the 3 groups (F = 2.237, $P = 0.0109$) (Fig. 3f).

Comparison of liver stiffness and left common carotid elasticity parameters among different age groups

All subjects were divided into two groups according to their age (Tables 2 and 3). with age 50 years being the dividing line. A comparison of the two groups showed no significant difference in ARFI values. However, in terms of carotid elasticity parameters, except for Aix, all other parameters including IMT, PWV, DC, CC, α, and β were significantly different between the two groups (all *P* values < 0.001). In the CHB group, ARFI values, as well as parameters including IMT, PWV, DC, CC, α, β, and Aix were all significantly different between the two age groups (all *P* values < 0.05). In the CHC group, IMT, PWV, DC, α, β, and Aix were significantly different between the two age groups (all *P* values < 0.05).

Relationship between liver stiffness with carotid artery elasticity in patients with chronic viral hepatitis

Stepwise multiple linear regression analysis indicated that for patients with CHB, age was an independent predictor of common carotid artery IMT ($R^2 = 0.468$, F = 54.635, and $P < 0.001$) (Fig. 4a). For patients with CHC, both age (β = 8.291,$t = 6.847$,P < 0.001) and blood glucose (β = 22.436, $t = 2/573$, $P = 0.012$) were independent predictors of common carotid artery IMT ($R^2 = 0.465$, F = 29.118, and $P < 0.001$) (Fig. 4b, c).

Discussions

Chronic liver disease can cause abnormal lipid metabolism, which, in severe cases, can directly change the peripheral blood vessel walls. Timely and convenient measurements of changes in the peripheral blood vessel walls have a positive effect on preventing detrimental cardiovascular and cerebrovascular events [1, 8]. Previous studies reported that HCV infection can alter in vivo glucose homeostasis and lipid metabolism leading to liver and peripheral insulin resistance [9, 10]. Our study found that patients with CHC not only had thickened IMT compared to the control and the CHB groups, but also had higher PWV, α, and β parameters and lower DC and CC values than the control group. This

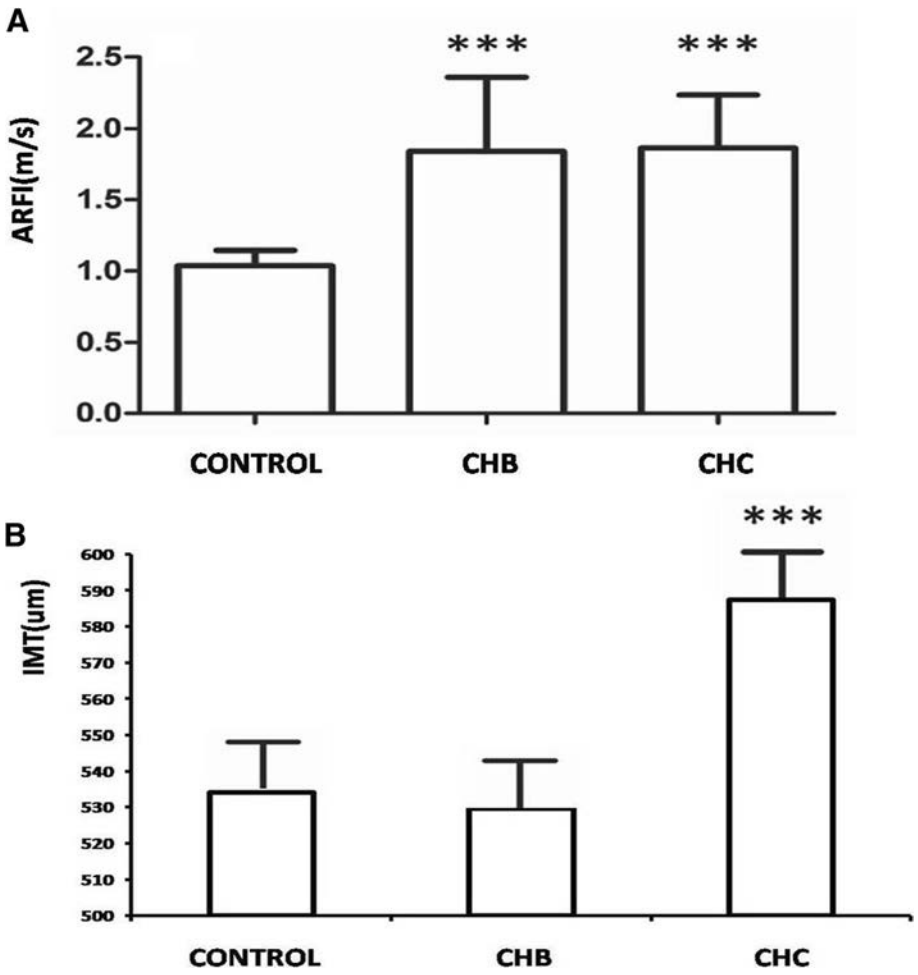

Fig. 2 Comparison of liver stiffness and carotid artery IMT values among the 3 groups: **a** Use of the ARFI technique to measure liver stiffness in patients with CHB, CHC and control group using a conventional ultrasound interface. **b** Use of QIMT and QAS techniques to quantify the carotid artery intima-media thickness (IMT) and vascular elasticity in patients among the 3 groups. Note: ARFI: acoustic radiation force impulse; IMT: intima-media thickness; CHB: chronic hepatitis B; CHC: chronic hepatitis C; ***: when compared to the control group, $P < 0.001$

indicated that both the carotid artery structure and function parameters of the CHC group had changed compared to the control group. This finding was in line with the argument that HCV infection is a risk factor for atherosclerosis [10, 11] . For patients with CHB, although their carotid artery IMT were not thickened, their other parameters such as α and β were higher, and their CC value was lower than those in the control group. These results indicated that, despite normal carotid artery wall structures, their carotid arteries and the elasticity parameters of their carotid artery walls had already changed. A possible explanation is that carotid atherosclerosis could cause both structural and functional changes. One major indicator for structural changes was an increase in the carotid IMT, while functional changes were mainly indicated by changes in carotid artery elasticity [12, 13]. Although structural changes in the carotid artery can cause changes in its elasticity, such elasticity changes may also indicate that IMT thickening is not the only cause of arterial wall composition changes. It is speculated that carotid artery functional changes in patients with CHB may occur before the structural changes. Therefore, this study showed that patients with viral hepatitis maybe suffer a higher risk of cardiovascular events than healthy people, and this finding can provide some reference value for clinical diagnosis and treatment of these patients.

Age is also a critical factor affecting the potential for development of arteriosclerosis [14, 15]. In this study, participants were divided into two groups according to their age, with those aged 50 or above in one group and those aged under 50 in the other. It was found that within the CHB group, the ARFI value ($P = 0.001$) and the carotid artery elasticity parameters (all P values < 0.05) differed significantly between the two age groups, indicating that these parameters might be related to the

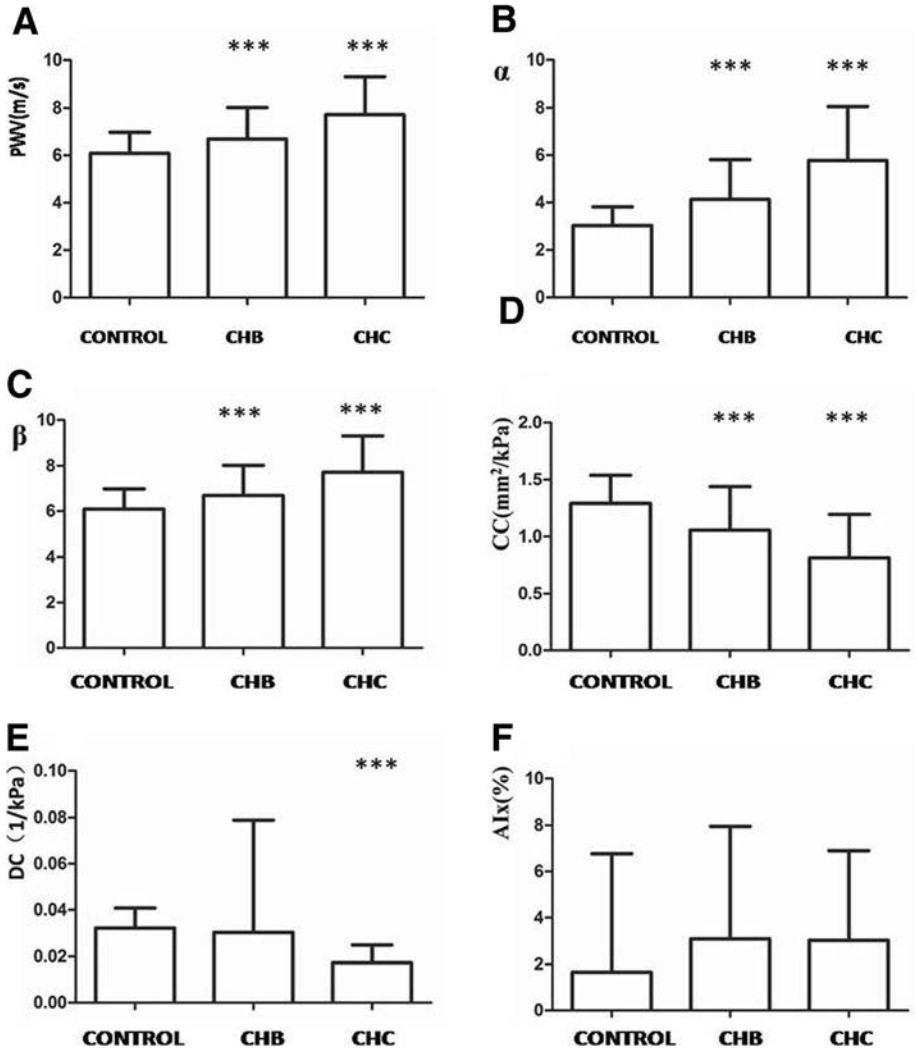

Fig. 3 Carotid elasticity parameters and comparisons among the 3 groups: **a** PWV values and comparisons among the 3 groups; **b** α values and comparisons among the 3 groups; **c** β values and comparisons among the 3 groups; **d** CC values and comparisons among the 3 groups; **e** DC values and comparisons among the 3 groups; **f** Aix values of the 3 groups. CHB: chronic hepatitis B; CHC: chronic hepatitis C; PWV: pulse wave velocity; CC: arterial wall compliance coefficient; DC: arterial wall dilation coefficient; Aix: augmentation index. Note: ***: when compared to the control group, $P < 0.001$

Table 2 Liver stiffness and left common carotid elasticity parameters in the different age groups

	Age, Year	No.	ARFI, m/s	IMT, μm	PWV, m/s	DC, 1/kPa
Control Group	< 50	41	1.02 ± 0.09	478.44 ± 107.86	5.72 ± 0.73	0.035 ± 0.007
	≥50	23	1.06 ± 0.13	633.26 ± 120.02	6.75 ± 0.80	0.026 ± 0.007
	P	–	0.127	< 0.001*	< 0.001*	< 0.001*
CHB Group	< 50	86	1.72 ± 0.50	456.70 ± 87.56	6.26 ± 1.35	0.038 ± 0.062
	≥50	61	2.02 ± 0.50	630.30 ± 112.17	7.32 ± 1.00	0.020 ± 0.007
	P	–	< 0.001*	< 0.001*	< 0.001*	0.009*
CHC Group	< 50	41	1.84 ± 0.38	526.63 ± 130.03	7.70 ± 1.57	0.018 ± 0.009
	≥50	29	1.89 ± 0.36	673.17 ± 167.48	8.36 ± 1.09	0.015 ± 0.006
	P	–	0.569	< 0.001*	0.041*	0.079

ARFI radio frequency, *IMT* intima-media thickness, *PWV* pulse wave velocity, *DC* arterial wall dilation coefficient
* $P < 0.05$ when compared to the control group

Table 3 Liver stiffness and left common carotid elasticity parameters in the different age groups

	Age, Year	No.	CC, mm^2/kPa	α	β	Aix,%
Control Group	< 50	41	1.376 ± 0.259	2.70 ± 0.54	5.54 ± 1.10	0.82 ± 5.18
	≥50	23	1.137 ± 0.129	3.63 ± 0.81	7.30 ± 1.70	3.14 ± 4.76
	P	–	< 0.001*	< 0.001*	< 0.001*	0.082
CHB Group	< 50	86	1.139 ± 0.420	3.71 ± 1.69	7.58 ± 3.41	1.55 ± 4.41
	≥50	61	0.935 ± 0.291	4.72 ± 1.51	9.61 ± 2.96	5.25 ± 4.68
	P	–	0.001*	< 0.001*	< 0.001*	< 0.001*
CHC Group	< 50	41	0.854 ± 0.423	5.31 ± 1.97	10.65 ± 3.79	1.75 ± 3.45
	≥50	29	0.761 ± 0.303	6.42 ± 2.57	13.11 ± 5.13	4.84 ± 3.75
	P	–	0.317	0.044*	0.024*	0.001*

CC arterial wall compliance coefficient, *Aix* augmentation index
* $P < 0.05$ when compared to the control group

time span of HBV infection. Older patients with chronic hepatitis B are likely to carry the virus for a longer period of time and, consequently, experience a higher degree of liver stiffness. This difference was not observed in the control group, which indicated that aging is not related to the natural aging and fibrosis of the liver. In a study of 459 chronic HBV carriers, liver biopsies showed that the liver tissue inflammatory activity level and degree of liver fibrosis gradually increased with age [16], which was in line with our findings.

However, within the CHC group, the ARFI did not differ significantly between the two age groups ($P > 0.05$). A possible explanation was that patients with CHC have a higher risk of increased blood glucose levels. Studies on using the ARFI technique to grade liver stiffness and fibrosis showed that fat in the liver is an important factor that affects the accurate measurement of the ARFI value [17, 18]. Since 40% of the patients with CHC in this study had elevated blood glucose, the abnormal lipid metabolism caused by abnormal blood sugar levels led to fat deposition in their liver, thus affecting the ARFI values. Although the accuracy of ARFI measurements were affected by abnormal blood glucose contents, measurements of carotid artery elasticity parameters were also found to be significantly abnormal. This indicated that HCV not only significantly affected liver stiffness but also changed carotid artery elasticity. Stepwise multiple linear regression analysis demonstrated that both

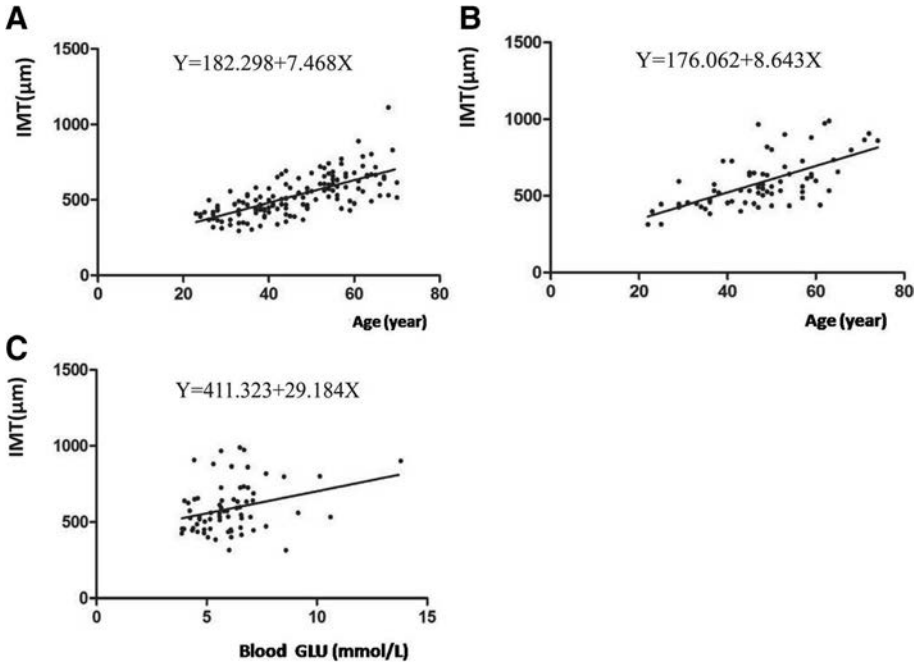

Fig. 4 a There was a relationship between IMT and age of CHB patients. **b** Relationship between IMT and age of CHC patients. **c** Relationship between IMT and blood glucose meter of CHC patients. Note: IMT: intima-media thickness; CHB: chronic hepatitis B; CHC: chronic hepatitis C

age and blood sugar are independent predictors of IMT in patients with CHC. Therefore, we speculated that, in addition to lipid metabolism, patients with chronic viral hepatitis also have metabolic syndromes caused by viral infections. The macrovascular damages caused by blood viscosity and hyperglycaemia also affect the structural changes of the carotid artery.

This study was limited by the fact that patients' diagnoses were made based on clinical diagnosis primarily. Biopsy was used to obtain pathological results in only a limited number of cases. The next step is to obtain results to classify liver fibrosis into different pathological levels, to further exclude any confounding factors.

Conclusions

In summary, using RF ultrasound and ARFI techniques to measure liver stiffness and carotid artery elasticity in patients with chronic viral hepatitis is beneficial for assessing the liver fibrosis and the structural and functional changes of the carotid artery. This serves as a reference for clinicians to monitor any vascular diseases in these patients.

Abbreviations

Aix: Augmentation index; ANOVA: Analysis of variance; ARFI: Acoustic radiation force impulse; CC: Compliance coefficient; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; DC: Arterial wall dilation coefficient; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HCVAb: Hepatitis C virus antibody; IMT: Intima-media thickness; PWV: Pulse wave velocity; RF: Radio frequency

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Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Authors' contributions

YLY conceived the idea. YLY, NZ and JHL was responsible for conception and participation in design, experimental work and collection of data, analysis and interpretation of results, drifting and substantial editing the manuscript. BYM, XZS, YYD were responsible for experimental work and collection of data, analysis and interpretation of results. YLY was responsible for interpretation of results and critically revising the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Tangdu Hospital (TDLL-2013082) and written informed consent was obtained from the patients in our center.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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BMC Gastroenterology

RESEARCH ARTICLE

Macrocytic anemia is associated with the severity of liver impairment in patients with hepatitis B virus-related decompensated cirrhosis: a retrospective cross-sectional study

Jian Yang[1†], Bin Yan[1†], Lihong Yang[1], Huimin Li[2], Yajuan Fan[2], Feng Zhu[3], Jie Zheng[1] and Xiancang Ma[2*]

Abstract

Background: Macrocytic anemia is common in liver disease. However, its role in hepatitis B virus (HBV)-related decompensated cirrhosis remains unknown. The aim of the present study was to determine the association between macrocytic anemia and the severity of liver impairment in patients with HBV-related decompensated cirrhosis according to the Model for End Stage Liver Disease (MELD) score.

Methods: A total of 463 participants who fulfilled our criteria were enrolled in this cross-sectional study. Patients were classified into three groups according to anemia types, diagnosed based on their mean corpuscular volume level. Multivariate linear regression analyses were used to determine the association between macrocytic anemia and the MELD score for patients with HBV-related decompensated cirrhosis.

Results: Patients with macrocytic anemia had evidently higher MELD scores (10.8 ± 6.6) than those with normocytic anemia (8.0 ± 5.5) or microcytic anemia (6.3 ± 5.1). The association remained robust after adjusting for age, gender, smoking, drinking, and total cholesterol (β = 1.94, CI: 0.81–3.07, $P < 0.001$).

Conclusions: Macrocytic anemia was found to be associated with the severity of liver impairment and might be a predictor for short-term mortality in patients with HBV-related decompensated cirrhosis.

Keywords: Macrocytic anemia, HBV-related decompensated cirrhosis, MELD score, Severity of liver impairment

Background

Cirrhosis is an end-stage disease that invariably leads to death. It is the 14th most common cause of death in adults worldwide and results in 1.03 million deaths per year [1]. Chronic infection with hepatitis B virus (HBV) is one of the major causes of cirrhosis and 30% of deaths are attributable to HBV [2, 3]. China is a highly endemic area of HBV, where 78% of patients with cirrhosis are HBsAg positive [4]. In patients with cirrhosis, the 5-year probability of decompensation is 15–20%, while the 5-year survival rate decreases from 84 to 14–35% once clinical decompensating events occur [5–7].

Anemia is a common comorbidity in cirrhosis that is associated with poor prognosis [8]. Erythrocyte abnormalities were clinically important and frequent findings in patients with chronic disease. Mean corpuscular volume (MCV), a measurement of the average volume of red blood cells (RBCs), has been documented to be associated with an increase in many clinical conditions [9–12]. Typically, anemia can be classified into macrocytic anemia (> 100 fL), normocytic anemia (80–100 fL), and microcytic anemia (< 80 fL) based on the patient's MCV level. A recent study has reported that the elevated MCV level was associated with increased liver cancer mortality, especially in men who are hepatitis B surface antigen (HBsAg)

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positive [13]. Therefore, in this study, we hypothesized that a common association might exist between macrocytic anemia and the severity of liver impairment in patients with HBV-related decompensated cirrhosis.

We used the Model for End Stage Liver Disease (MELD) score for evaluating the severity of liver impairment of HBV-related decompensated cirrhosis. The MELD score was developed to predict the short-term mortality of end-stage liver disease because of the shortage of donated livers. It had been validated subsequently as an accurate predictor of survival among different populations of patients with advanced liver disease and was adopted for organ allocation for liver transplantation instead of the older Child-Pugh score in the USA since 2002 [14–16]. Liver transplantation is generally recommended for patients with MELD score of > 15, if possible [17].

The goal of the present study is to investigate whether the MELD score is higher in the macrocytic anemia group in patients with HBV-related decompensated cirrhosis.

Methods

Study population

From May 2013 to July 2016, data of 1445 patients diagnosed as having HBV-related decompensated cirrhosis were extracted from the HIS Database at the First Affiliated Hospital of Xi'an Jiaotong University. For patients to be diagnosed as having HBV-related decompensated cirrhosis, the following conditions must be present: HBsAg carrier for ≥6 months; pathological or clinical evidence of cirrhosis; and occurrence of complications, such as ascites, upper gastrointestinal bleeding, spontaneous bacterial peritonitis, or hepatic encephalopathy [6, 18–20]. Anemia was defined according to WHO's haemoglobin thresholds, which is haemoglobin level of < 130 g/L in male and < 120 g/L in female [8]. After strictly screening according to the inclusion criteria and exclusion criteria, 463 patients were enrolled in this hospital-based cross-sectional study (Fig. 1). The study was approved by the Ethics Committee of the First Affiliated Hospital, Xi'an Jiaotong University. Since this is a retrospective study, a written consent is waived by the Ethics Committee and is deemed unnecessary. All methods were carried out in accordance with appropriate clinical practice guidelines and national legal requirements.

Data collection

Demographic characteristics were obtained from an interview during the patients' admission to our hospital. Venous blood samples were collected from the participants after an overnight fasting for laboratory assessments. Smoking was defined as having ≥1 cigarette per day and drinking was defined as alcohol intaking > 20 g per day for at least a year [21, 22]. Estimated glomerular filtration rate (eGFR) was calculated using a formula adapted from the Modification of Diet in Renal Disease (MDRD) equation [23, 24]. Unfortunately, body mass index (BMI) and HBV DNA data were not included in the analysis due to excessive missing values.

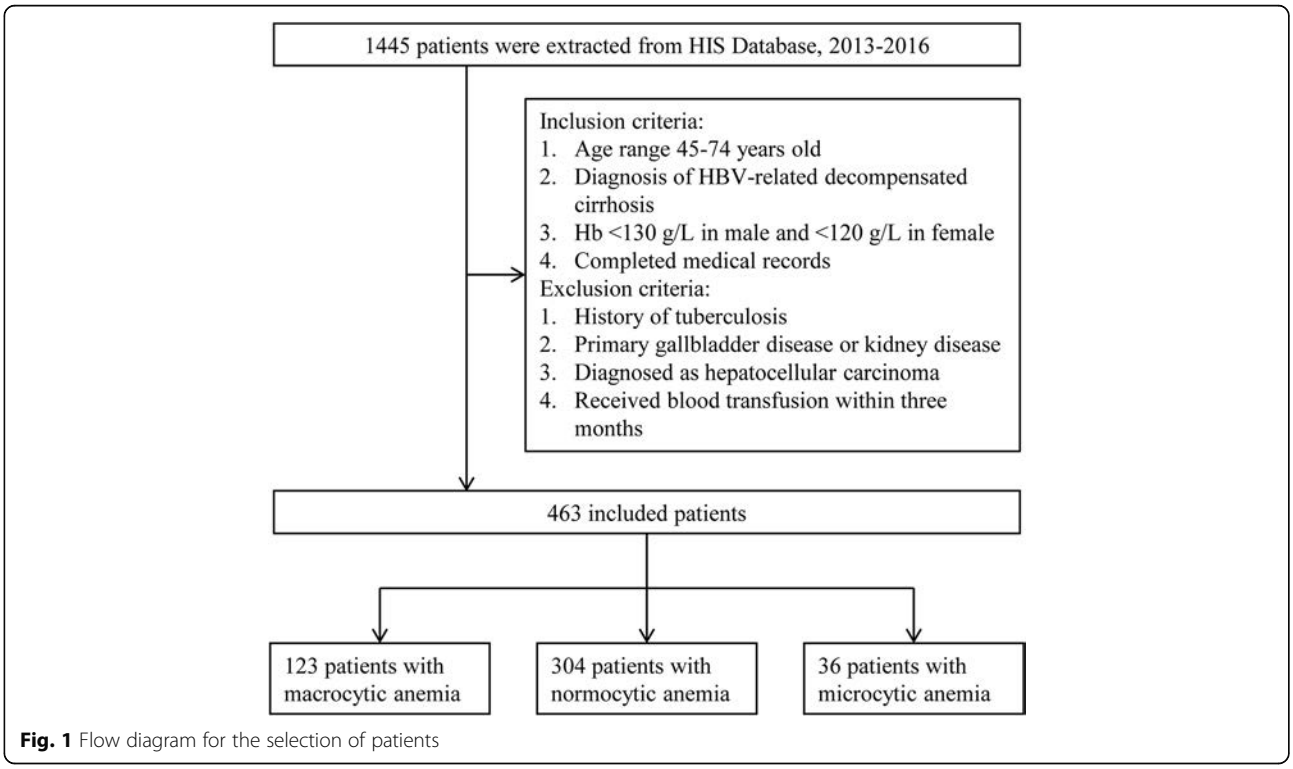

Fig. 1 Flow diagram for the selection of patients

MELD score

The MELD score was calculated using the following formula: $9.57 \times \log_e$ (creatinine mg/dl) + $3.78 \times \log_e$ (bilirubin mg/dl) + $11.2 \times \log_e$ (INR) + 6.43, where INR is the international normalised ratio and 6.43 is the constant for liver disease aetiology [16].

Statistical analysis

Statistical analyses were conducted using R software (version 3.1.3). Continuous data were presented as mean ± SD, and categorical variables were presented as count and percentage. All participants were divided into three groups according to their anemia classification. We used one-way ANOVA to determine the differences among the three groups in terms of the continuous variables, because the variables were all normally distributed and homogeneous in variance. Simultaneously, the chi-square test was used for categorical variables. Univariate and multivariate linear regression analyses were used to examine the associations of the MELD score with macrocytic anemia. Variables with P value < 0.05 in univariate models were then included in the multivariate analyses. A two-tailed test was used to calculate the P value, and the results were considered statistically significant when the P value < 0.05.

Results

Characteristics of participants

Table 1 presents the baseline characteristics of the participants, which were divided into three groups according to anemia types. Among the 463 eligible participants, 304 had normocytic anemia, 123 had macrocytic anemia and 36 had microcytic anemia. The average age of participants was 54.3 (SD = 7.3) years and 63.5% of them were male. Our data showed that patients with macrocytic anemia were older and had higher levels of bilirubin, international normalized ratio (INR) and alkaline phosphatase (ALP) compared to patients with normocytic or microcytic anemia. MELD score was also observed to be higher in the macrocytic group. Oppositely, the total cholesterol and albumin were relatively low. There were no significant differences observed in terms of gender, smoking, drinking, hypertension, systolic blood pressure, diastolic blood pressure, creatinine, eGFR, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The haemoglobin level and prevalence of diabetes in the microcytic group were slightly different from that in the other two groups, but this difference was negligible.

Assessment of the association between MELD score and possible risk factors

We next assessed the correlation between the MELD score and possible risk factors using the univariate linear regression analyses (Table 2). Our results revealed a positive association between the MELD score and male, smoking and drinking. In addition, a negative association between the MELD score and the total cholesterol level was observed.

Association between macrocytic anemia and MELD score

Patients in the macrocytic group had evidently higher MELD scores than patients in the other two groups (Fig. 2). In univariate regression analysis, we found that there was a significant association between macrocytic anemia and the MELD score (estimated coefficient [β] = 2.80, 95% confidence interval [CI]: 1.59–4.01, P value [P] < 0.001), using the normocytic group as the reference. Furthermore, the association remained robust (β = 1.94, CI: 0.81–3.07, P < 0.001) after adjusting for age, gender, smoking, drinking and total cholesterol in multivariate analysis (Table 2).

Discussion

In this retrospective study, we demonstrated that macrocytic anemia, defined as anemia in which the RBCs are larger than their normal volume (100 fL), is associated with the severity of liver impairment in patients with HBV-related decompensated cirrhosis. This finding remains substantial even after adjusting for demographics and laboratory parameters, such as age, gender, smoking, drinking and total cholesterol.

An MCV level greater than 100 fL, which is also known as macrocytosis, may not always be associated with anemia. Moreover, it presents independently from anemia in most cases [10]. Nevertheless, we chose anemia as one of our inclusion criteria because 84.2% of the 1445 pre-screened patients have anemia. This result was consistent with the finding of another study, which reported that about 75% of patients with chronic liver disease have a diverse aetiology of anemia [25]. Furthermore, patients with cirrhosis may have anemia due to a lack of haematopoietic factors, shortened erythrocyte survival, reduced bone marrow function, or gastrointestinal bleeding. All these conditions indicate impaired liver function and a high risk of mortality. Therefore, patients without anemia were excluded from the data analysis to avoid potential bias in our present study.

The importance of macrocytic anemia or macrocytosis seems to be underestimated in the past. Only a few studies focused on its risk of adverse events or death [9–13]. Among these studies, Yoon et al. documented that the elevated MCV level was associated with increased liver cancer mortality in men [13]; this finding was consistent with the result of our study. A small-sample study also found a markedly higher MCV in patients with chronic liver failure than in healthy subjects [26]. These observations, though not directly, provided evidence for our conclusion that patients with HBV-related decompensated cirrhosis

Table 1 Demographic and biochemical characteristics of the study participants ($N = 463$)

Variable	Macrocytic anemia	Normocytic anemia	Microcytic anemia	*P* value
Number of subjects	123	304	36	
Mean corpuscular volume, fL	102.7 ± 2.6	91.2 ± 5.1†	74.2 ± 4.6†¥	< 0.001
Age, years	56.1 ± 7.6	53.9 ± 7.1†	51.8 ± 6.2†	0.002
Male, n(%)	78(63.4)	191(62.8)	25(69.4)	0.738
Drinking, n(%)	24(19.5)	74(24.3)	13(36.1)	0.118
Smoking, n(%)	48(39.0)	107(35.2)	15(41.7)	0.618
Diabetes, n(%)	10(8.1)	39(12.8)	9(25.0) †	0.026
Hypertension, n(%)	14(11.3)	36(11.8)	4(11.1)	0.985
Hemoglobin, g/L				
> 90	97(78.9)	230(75.7)	10(27.8)†¥	< 0.001
60–90	21(17.1)	63(20.7)	20(55.6)†¥	< 0.001
< 60	5(4.1)	11(3.6)	6(16.7)†¥	0.002
Total cholesterol, mmol/L	2.4 ± 0.7	2.7 ± 0.9†	2.7 ± 0.8	0.012
Systolic blood pressure, mmHg	117.9 ± 17.6	117.8 ± 15.2	113.8 ± 13.8	0.350
Diastolic blood pressure, mmHg	72.6 ± 11.7	73.4 ± 10.0	70.9 ± 8.6	0.354
Bilirubin, mg/dL	3.4 ± 3.4	2.6 ± 3.2†	1.8 ± 3.4†	0.011
Creatinine, mg/dL	0.8 ± 0.8	0.7 ± 0.4	0.7 ± 0.4	0.147
INR	1.5 ± 0.3	1.4 ± 0.4†	1.3 ± 0.1†	0.006
eGFR, mL/min/1.73m^2	123.6 ± 54.5	126.9 ± 43.4	130.3 ± 39.1	0.686
Albumin	27.0 ± 4.7	29.1 ± 4.7†	31.7 ± 4.8†¥	< 0.001
AST	78.3 ± 147.3	84.2 ± 196.6	41.7 ± 39.5	0.394
ALT	45.3 ± 42.4	59.4 ± 114.3	29.3 ± 31.8	0.113
ALP	122.9 ± 55.5	106.4 ± 61.1†	85.2 ± 32.7†	0.001
MELD score	10.8 ± 6.6	8.0 ± 5.5†	6.3 ± 5.1†	< 0.001
Complications, n(%)				
UGB	6(4.9)	34(11.2)	5(13.9)	0.093
SBP	36(29.3)	75(24.7)†	3(8.3)†	0.037
HE	14(11.4)	22(7.2)	3(8.3)	0.377

Values are presented as mean ± standard deviation or numbers (percentage)
INR international normalized ratio, *eGFR* estimated glomerular filtration rate, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *MELD* model for end stage liver disease, *UGB* upper gastrointestinal bleeding, *SBP* spontaneous bacterial peritonitis, *HE* hepatic encephalopathy
P indicates the difference among the three groups. †Indicates significance ($P < 0.05$) compared to macrocytic anemia; ¥Indicates significance ($P < 0.05$) compared to normocytic anemia

who have macrocytic anemia were more likely to present worse liver condition.

There are several potential pathological mechanisms that explain why macrocytic anemia is associated with the severity of liver impairment. First, patients with advanced liver damage are more likely to have vitamin B_{12} or folate deficiencies [27], which directly result in macrocytic anemia. Vitamin B_{12} and folate coenzymes are required for thymidylate and purine synthesis, thus, their deficiencies result in retarded DNA synthesis and eventually will develop into macrocytic anemia [28–30]. Second, macrocytic anemia in liver disease may be due to an increased deposition of cholesterol on the membranes of circulating RBCs [31, 32]. This deposition effectively increases the surface area of the erythrocyte. Third, hemolytic anemias are common in advanced liver failure. In this case, excessive destruction of RBCs and increased reticulocyte count can be observed. The immature erythrocytes are approximately 20% larger compared to the mature erythrocytes, which result in macrocytic anemia [25]. Moreover, erythrocyte morphology is affected by various factors in liver disease, such as causes, degree of liver damage, and drugs used. Complicated mechanisms, which allow the synchronized performance of their independent or collaborative functions, determine the shape of RBCs. Nevertheless, we firmly believe that there is a positive correlation between macrocytic anemia and the severity of liver impairment in patients with HBV-related decompensated cirrhosis.

Table 2 Univariate and multivariate linear regression analysis for MELD score

Variable	Univariate		Multivariate	
	β (CI 95%)	*P* value	β (CI 95%)	*P* value
Age	0.05(−0.02,0.13)	0.160	0.07(0.01,0.15)	0.028
Male	2.25(1.15,3.36)	< 0.001	1.49(0.29,2.70)	0.015
Smoking	1.93(0.82,3.03)	< 0.001	0.21(−1.03,1.44)	0.742
Drinking	1.59(0.34,2.85)	0.013	0.73(−0.56,2.01)	0.269
Diabetes	0.53(− 1.11,2.16)	0.527		
Hypertension	0.15(−1.53,1.84)	0.857		
Hemoglobin, g/L				
> 90	Ref	–		
60–90	1.09(−0.21,2.39)	0.100		
< 60	−1.35(−3.90,1.20)	0.298		
Total cholesterol	−2.77(−3.37,-2.16)	< 0.001	−2.53(− 3.14,-1.93)	< 0.001
Systolic blood pressure	−0.01(− 0.04,0.03)	0.863		
Diastolic blood pressure	−0.01(− 0.06,0.05)	0.900		
Anemia classification				
Normocytic anemia	Ref	–	Ref	–
Macrocytic anemia	2.80(1.59,4.01)	< 0.001	1.94(0.81,3.07)	< 0.001
Microcytic anemia	−1.73(−3.72,0.27)	0.089	− 1.77(− 3.59,0.05)	0.057

MELD model for end stage liver disease, *β* estimated coefficient, *95% CI* 95% confidence interval

In addition, we used the MELD score, which is a formula comprising creatinine, bilirubin, and INR values, to evaluate the severity of liver impairment and risk of death. In our study, patients with macrocytic anemia had higher levels of bilirubin and INR, but no significant difference was observed in creatinine levels and eGFR. Thus, macrocytic anemia might be unrelated to kidney damage in patients with HBV-related decompensated cirrhosis.

There were a few limitations in this study. First, we used the MELD score for evaluating the severity of liver impairment in patients with HBV-related decompensated cirrhosis. Although the MELD score could provide an accurate prediction of short-term mortality of patients with cirrhosis, a follow-up data might be better and more credible. Second, the analysis did not include data on serum vitamin B_{12}, folate, reticulocyte count, drugs, and measures of haemolysis, which could

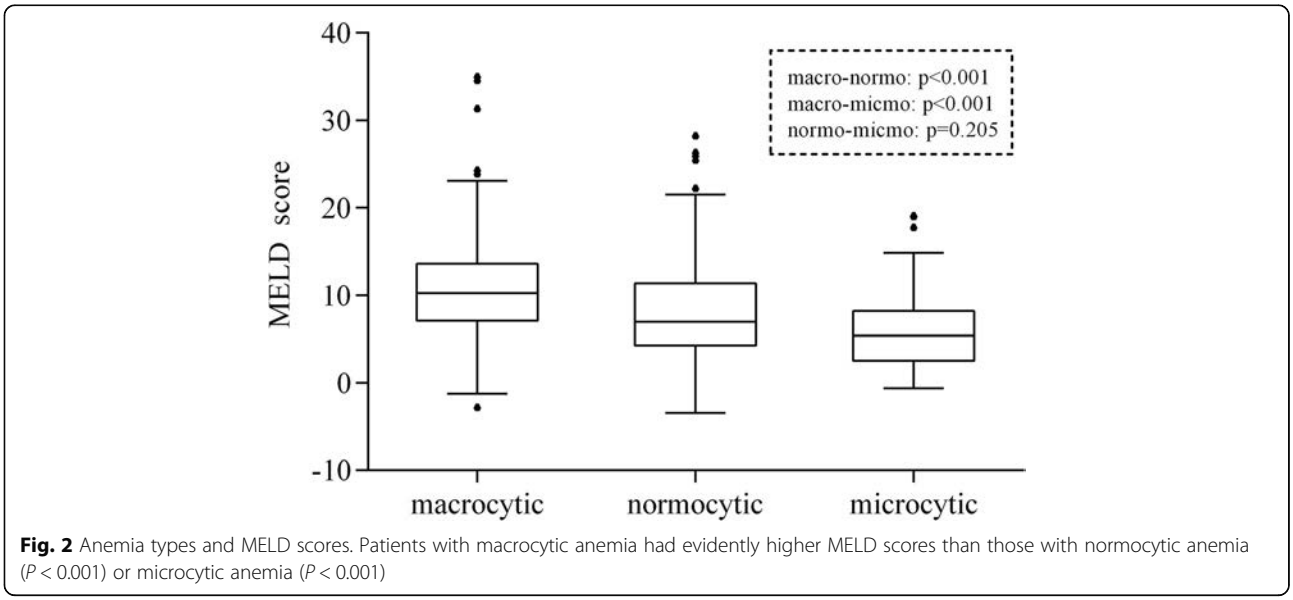

Fig. 2 Anemia types and MELD scores. Patients with macrocytic anemia had evidently higher MELD scores than those with normocytic anemia ($P < 0.001$) or microcytic anemia ($P < 0.001$)

contribute to better understand the mechanisms of macrocytic anemia in patients with cirrhosis.

Conclusions

Macrocytic anemia was found to be associated with the severity of liver impairment and might be a predictor for short-term mortality in patients with HBV-related decompensated cirrhosis. However, a large-scale cohort study is recommended to confirm the present results and to elucidate the mechanisms underlying the observed correlations between macrocytic anemia and the severity of liver impairment in patients with HBV-related decompensated cirrhosis.

Abbreviations

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; eGFR: Estimated glomerular filtration rate; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HE: Hepatic encephalopathy; INR: International normalised ratio; MCV: Mean corpuscular volume; MDRD: Modification of diet in renal disease; MELD: Model for end stage liver disease; RBCs: Red blood cells; SBP: Spontaneous bacterial peritonitis; UGB: Upper gastrointestinal bleeding

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Availability of data and materials

The datasets used and analysed during the current study will be available from the corresponding author on reasonable request.

Authors' contributions

XM, JY and BY designed the study. LY, HL and YF compiled the data and helped with the data interpretation. JY and BY analysed the data and drafted the manuscript. XM, JZ and FZ revised the manuscripts for important intellectual content helped with the data interpretation. All authors reviewed the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the First Affiliated Hospital, Xi'an Jiaotong University. Since this is a retrospective study, a written consent is waived by the Ethics Committee and is deemed unnecessary.

Consent for publication

Not applicable

Competing interests

The authors declare no competing financial interests.

Publisher's Note

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Evaluation of ballooned hepatocytes as a risk factor for future progression of fibrosis in patients with non-alcoholic fatty liver disease

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Abstract

Background The prevalence of non-alcoholic fatty liver disease (NAFLD) has increased. Non-alcoholic steatohepatitis (NASH) shows progression of liver fibrosis in NAFLD. It remains unclear which patients with NAFLD will show progression of liver fibrosis. Therefore, we aimed to investigate the risk factor associated with the progression of liver fibrosis among patients with NAFLD.

Methods This observational study enrolled 157 patients with biopsy-proven NAFLD. Thirty-two patients were excluded because of lack of data. The accuracy of the formulae for estimating liver fibrosis, i.e., the FIB-4 index, APRI, and Forns index, was compared. Using serial changes of the best formula for liver fibrosis, we identified factors associated with the progression of liver fibrosis. Histological liver fibrosis was quantified using the Brunt stage.

Results Sixty-three patients were diagnosed as having NASH. The FIB-4 index provided the best diagnostic accuracy for liver fibrosis [Brunt stage 0 versus 1–4, areas under the curve (AUC) 0.74; 0–1 versus 2–4, AUC 0.77; 0–2 versus 3–4, AUC 0.78; and 1–3 versus 4, AUC 0.87]. The association between body mass index, sex, observation period, and histological findings (liver fat content, bridging fibrosis, and hepatocyte ballooning) with the change in the FIB-4 index was evaluated among patients with NASH, using multivariate analysis. Among these factors, hepatocyte ballooning was associated with an increase in the FIB-4 index.

Conclusion The FIB-4 index was the best formula for estimating liver fibrosis in patients with biopsy-proven NAFLD, and the presence of ballooned hepatocytes was a risk factor for the progression of liver fibrosis.

Keywords NAFLD · Ballooning · FIB-4 index · NASH · NAFL · Fibrosis

Abbreviations

ALT	Alanine transaminase
APRI	Aspartate transaminase to platelet ratio index
AST	Aspartate transaminase
AUROC	Area under the receiver operating characteristic
BF	Bridging fibrosis
BH	Ballooned hepatocyte
BMI	Body mass index
FIB-4	Fibrosis 4
γGT	Gamma-glutamyl transferase
HOMA-R	Homeostatic model assessment for insulin resistance
M2BPGi	Mac-2 binding protein glycan isomer
MR	Magnetic resonance
NAFL	Non-alcoholic fatty liver
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
ROC	Receiver operating characteristic
SHH	Sonic hedgehog
T4C7s	Type 4 collagen 7s
TC	Total cholesterol

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Introduction

Non-alcoholic fatty liver disease (NAFLD), which manifests as the liver form of metabolic syndrome, is a severe health issue because the prevalence of NAFLD has strikingly increased in western countries [1]. Non-alcoholic steatohepatitis (NASH) is a stage of NAFLD that shows progression of fibrosis due to inflammation [2]. Because of the increased prevalence of NAFLD, NASH has been focused on as the cause of liver cirrhosis [1]. Since fibrosis is associated with mortality in patients with NASH [3], anti-fibrosis is considered as a therapeutic target for NASH.

Although NASH is characterized by inflammation and fibrosis in the liver, the histological hallmark of NASH is ballooning of the hepatocytes [4, 5]. Indeed, Matteoni et al. reported that the presence of ballooned hepatocytes is associated with patients' prognosis [5]. However, diagnostic criteria for ballooned hepatocytes vary among pathologists; therefore, these findings might be subjective [6, 7]. Ballooned hepatocytes are significant in the pathophysiology of NASH, although they may be difficult to be objectively used as the diagnostic hallmark of NASH.

Fibrosis of the liver can be objectively evaluated using elastography [8, 9]. Magnetic resonance (MR) elastography and transient elastography are now available for evaluating fibrosis. Although these methods are non-invasive, repeatable, and safe, elastography requires expensive equipment. Since the prevalence of NAFLD is increasing among individuals in western countries, the assessment of fibrosis using several serum laboratory data and general information is also useful for general physicians. Several formulae calculated by laboratory data, such as the Fibrosis 4 (FIB-4) index and Forns index, have been proposed for the evaluation of liver fibrosis [10–12].

Several clinical trials have investigated the pharmacologic treatment of NASH [13]. Because the prevalence of NASH has drastically increased and use of a new therapeutic agent is expensive in general [14], the medical cost for NASH is incalculable. To reduce the medical cost and increase the efficacy of treatment for NASH, patients with NASH who require treatment need to be identified. Specifically, patients with NAFLD at risk for progression of liver fibrosis in the future need to be identified. Therefore, we aimed to investigate the risk factor associated with the progression of liver fibrosis among patients with NAFLD.

Materials and methods

Patients

One hundred fifty-seven consecutive patients who were diagnosed as having NAFLD by liver biopsy between December 2008 and March 2016 were screened for the present study (Fig. 1). Seven of the 157 patients eligible for the study were excluded because of an incomplete dataset. An additional 25 of the 157 patients were excluded because either the observation period was < 12 months or they voluntarily withdrew from the study.

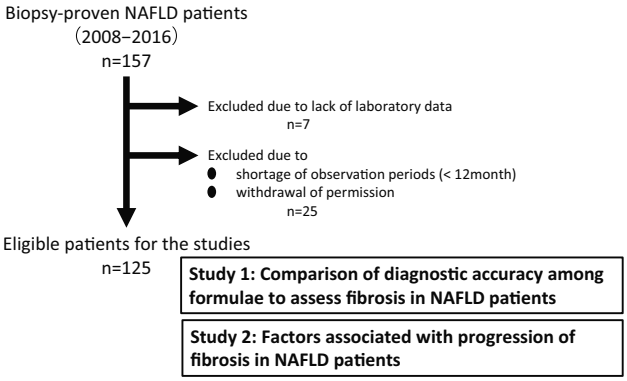


Fig. 1 Flow chart of the study design. *NAFLD* non-alcoholic fatty liver disease

Informed consent was obtained from all patients. All protocols reported in this study were approved by the Institutional Review Board of Iwate Medical University (approval number: H27-56).

To evaluate the accuracy of several formulae, which are described later, histological findings of fibrosis were compared (part 1 of the study). The factor associated with a change in the value, as calculated by the best formula for evaluating liver fibrosis, was evaluated. To evaluate the risk factor associated with the progression of liver fibrosis, the formula that predicted accurate fibrosis stage was used to assess fibrosis during the last visit in our department (part 2 of the study). The difference between the value at the last visit and that at liver biopsy was considered as a change in the fibrotic state.

Measurements and calculations

Body mass index (BMI) was calculated using the following formula: BMI = weight (kg)/(height $\times$ height) (m^2). The aspartate transaminase (AST) to platelet ratio index (APRI) [12], FIB-4 index [11], and Forns index [10] were calculated using the following formulae:

- $\text{APRI} = \left\{\frac{\text{AST(acutualvalue)}}{\text{AST}}\right.$ $\left.(\text{upperlimitofnormal})\right\}/\text{plateletcount}(10^9/\text{L})$.
- FIB-4 index $= \{\text{Age(years)} \times \text{AST}\}/\{\text{plateletcount}$ $(10^9/\text{L}) \times\sqrt{\text{ALT}}\}$.
- Forns index $= 7.811 - 3.131 \times \text{Ln(platelet count}$ $(10^9/\text{L}) + 0.781 \times \text{Ln(gamma - } \textit{glutamyltransferase}[])$ $+3.467 \times \text{Ln(age)} - 0.014 \times$ total cholesterol (TC).

To evaluate insulin resistance, homeostatic model assessment for insulin resistance (HOMA-IR) was used. These values were calculated using the following formula:

- HOMA-IR = insulin × fasting glucose level/405.

Liver biopsies and histological assessments

Percutaneous needle biopsies were performed on liver segment 6 under ultrasonography, using a 16-gauge (G) needle. To diagnose NAFLD or NASH definitively, all liver biopsy specimens were examined for fibrosis, steatosis, ballooning hepatocytes, and portal inflammation. Although these findings were scored using the NAFLD activity score [15], we evaluated whether ballooning was absent or present to avoid subjective grading of ballooning hepatocytes. NASH was defined based on the following findings: (1) more than 5% of fat in the liver and (2) existence of inflammation in any zone of the liver. NAFL was defined as more than 5% of fat in the liver without inflammation. Histological findings were evaluated by a single pathologist who was blinded to the patients' clinical characteristics. The fibrosis stage was classified using the Brunt staging system. Because the patients in this study had NAFL and fibrosis was absent in NAFL, NAFL was classified as Brunt stage 0.

Laboratory data

All blood samples were collected on the day of liver biopsy and at every visit to our unit. The levels of AST, alanine transaminase (ALT), γ-GT, fasting glucose, ferritin, insulin, type IV collagen (T4C7s), and TC were analyzed using an autoanalyzer (JCA-BM2250, JEOL, Tokyo, Japan).

Statistical analysis

Continuous variables are presented as mean ± standard deviation. The Mann–Whitney *U* test was used to compare the laboratory data, BMI, and age between patients with NAFLD who were divided into the NASH and NAFL groups. The diagnostic performance of the formula for detecting the Brunt stage was assessed using the receiver-operating characteristic (ROC) curve method. The cut-off values of the APRI, FIB-4 index, and Forns index in each analysis were estimated using the area under the ROC (AUROC). After evaluating the performance of each formulae used to assess fibrosis in part 1 of the study, serial change of the best formula was calculated for the patients in part 2 of the study. Because the best formula contained laboratory data and age, linear regression analysis of serial change in the value of the formula was analyzed in BMI, sex, age, body weight (BW) change during the observation period, the observation period, and histological findings (fat in the liver, bridging fibrosis, and ballooned hepatocytes). Serial change of the formula was defined as delta: (formula using data at the day of biopsy) − (formula using data at the day of the last visit). All statistical analyses were performed using the SPSS 17.0 software program (SPSS Inc., Chicago, IL, USA). Results were considered significant when the p value was < 0.05.

Results

Patients' characteristics

The relevant characteristics of patients are summarized in Table 1. Based on histological findings, 62 patients were sub-classified in the NAFL group, and 63 were sub-classified in the NASH group. The two groups were comparable with regard to the BMI distribution. Distributions of Brunt stages in the NASH group were as follows: stage 1, 23 patients; stage 2, 14 patients; stage 3, 21 patients; and stage 4, 5 patients. Among patients in the NASH group, bridging fibrosis was identified in 17, and ballooning hepatocytes were identified in 49. Patients were older in the NASH group (mean age, 54.9 years) than in the NAFL group (mean age, 46.6 years). Levels of the following serum markers were higher in the NASH group than in the NAFL group: AST, 62 versus 51 IU/mL; ferritin, 248 versus 224 mg/dL; glycated hemoglobin, 6.2 versus 5.7%; and T4C7s, 6.09 versus 3.91 ng/mL. However, the platelet count was lower in the NASH group than in the NAFL group (213 versus 234 × 10^4). There were no between-group differences with regard to the levels of ALT, TC, insulin, and HOMA-IR. As expected, fibrosis scores were higher in the NASH group than in the NAFL group: FIB-4 index, 1.04 versus 2.03; APRI, 0.56 versus 1.05; and Forns index, 5.24 versus 6.48.

FIB-4 index had the best diagnostic accuracy of liver fibrosis

Although each formula used to estimate liver fibrosis showed a high value among the subjects, it remained unclear which formula accurately evaluated the fibrosis stage. To evaluate serial change of fibrosis during the observation period, we needed to identify the best formula for evaluating liver fibrosis. For this purpose, the diagnostic accuracy of each formula was evaluated among the patients with NAFLD in the NASH and NAFL groups using the ROC curve method. To identify the patients with Brunt stage 0, the FIB-4 index, APRI, and Forns index showed

Table 1 Patients' characteristics in this study

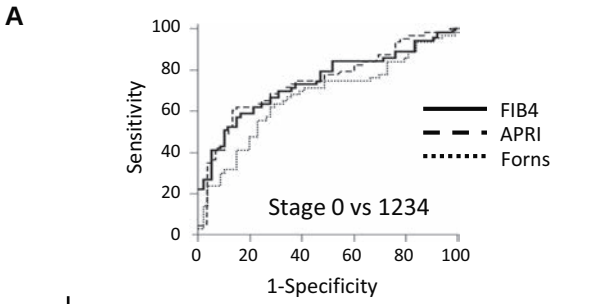
	NAFLD (125)	NAFL (62)	NASH (63)	
Age (years)	50.8 ± 15.6	46.6 ± 13.7	54.9 ± 16.3	$p < 0.01$
BMI	27.9 ± 4.1	27.7 ± 3.7	28.1 ± 4.5	n.s.
Sex (M:F)	58:67	32:30	26:37	n.s.
Histological findings				
Brunt stage (0:1:2:3:4)		(62:0:0:0:0)	(0:23:14:21:5)	
BF (+)		(−)	17	
BH (+)		(−)	49	
Fat (%)		36.2 ± 21.3	34.3 ± 17.1	n.s.
Laboratory date				
AST (U/mL)	51 ± 39	39 ± 23	62 ± 47	$p < 0.01$
ALT (U/mL)	84 ± 90	74 ± 73	94 ± 104	n.s.
gGT (U/mL)	84 ± 64	80 ± 62	88 ± 67	n.s.
TC (mg/dL)	201 ± 42	206 ± 38	194 ± 40	n.s.
Ferritin (ng/mL)	237 ± 208	224 ± 168	248 ± 239	$p < 0.01$
T4C7s (ng/mL)	5.12 ± 2.1	3.91 ± 1.23	6.09 ± 2.15	$p < 0.01$
Plt ($\times 10^4$)	223 ± 66	234 ± 53	213 ± 76	n.s.
HbA1C (%)	6.0 ± 0.9	5.7 ± 0.7	6.2 ± 1.1	$p < 0.01$
Glucose (mg/dL)	110 ± 27	104 ± 22	115 ± 29	$p = 0.016$
Insulin (mU/mL)	28.4 ± 34.2	26.1 ± 33.1	31.1 ± 35.7	n.s.
HOMA-R	8.51 ± 12.1	7.45 ± 11.57	9.67 ± 12.67	n.s.
Fibrosis score				
FIB4 index	1.54 ± 1.21	1.04 ± 0.55	2.03 ± 1.46	$p < 0.01$
APRI	0.81 ± 0.67	0.56 ± 0.38	1.05 ± 0.79	$p < 0.01$
Forns index	5.87 ± 2.22	5.24 ± 1.86	6.48 ± 2.39	$p < 0.01$

ALT alanine transaminase, *APRI* AST to Platelet Ratio Index, *AST* aspartate transaminase, *BF* bridging fibrosis, *BH* ballooned hepatocyte, *γGT* gamma-glutamyl transferase, *HOMA-R* homeostatic model assessment for insulin resistance, *NAFLD* non-alcoholic fatty liver, *NAFLD* non-alcoholic fatty liver disease, *NASH* non-alcoholic steatohepatitis, *T4C7s* type 4 collagen 7s, *TC* total cholesterol

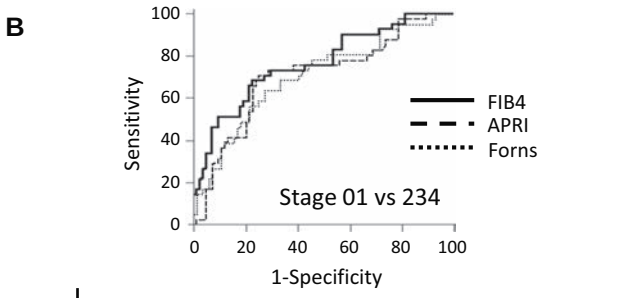
AUROCs of 0.743, 0.748, and 0.672 when the cut-off values of each formula were 1.33, 0.68, and 6.04, respectively (Fig. 2a). To distinguish the patients with Brunt stages 0–1 and 2–4, the FIB-4 index, APRI, and Forns index showed AUROCs of 0.765, 0.708, and 0.706 when the cut-off values of each formula were 1.40, 0.65, and 6.45, respectively (Fig. 2b). To determine the patients with Brunt stage 3–4 (advanced fibrosis), the FIB-4 index, APRI, and Forns index showed AUROCs of 0.781, 0.763, and 0.681 when the cut-off values of each formula were 1.62, 0.76, and 6.45, respectively (Fig. 2c). To identify the patients with Brunt stage 4, the FIB-4 index, APRI, and Forns index showed AUROCs of 0.870, 0.732, and 0.892 when the cut-off values of each formula were 1.73, 1.57, and 7.40, respectively (Fig. 2d). All formulae in each analysis showed a relatively high AUROC, high sensitivity, and high specificity. However, the cut-off value of the FIB-4 index was consistent in each analysis, while those of the other indices were inconsistent among each analysis. Therefore, we used the FIB-4 index to evaluate the serial change of fibrosis during the observation period.

No factors were associated with serial change of liver fibrosis

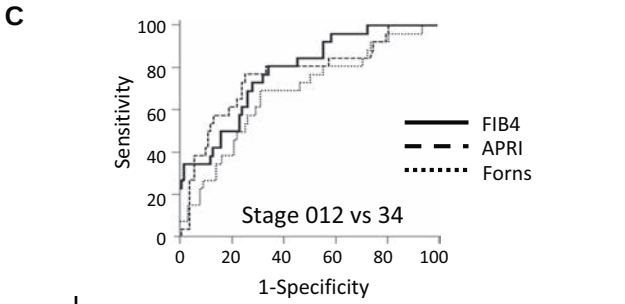
Since the FIB-4 index showed the most accurate estimation of liver fibrosis in this study, we considered that serial change of the FIB-4 index, i.e., delta FIB-4 index, might reflect fibrotic change of the liver in our study patients. To identify factors associated with the progression of liver fibrosis, linear regression analysis for the delta FIB-4 index in all patients was performed using BMI, sex, age, BW change during the observation period, the observation period, and histological findings (fat in the liver, bridging fibrosis, and ballooned hepatocytes). None of the factors was identified in this analysis (data not shown).



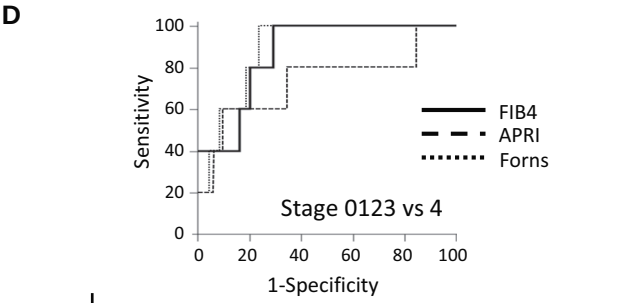
	AUROC	Cut-off	Sensitivity	Specificity	PPV	NPV
FIB4	0.743	1.33	0.57	0.85	0.80	0.66
APRI	0.748	0.68	0.57	0.85	0.80	0.66
Forns	0.672	6.04	0..62	0.73	0.69	0.65



	AUROC	Cut-off	Sensitivity	Specificity	PPV	NPV
FIB4	0.765	1.40	0.68	0.77	0.59	0.83
APRI	0.708	0.65	0.71	0.75	0.58	0.84
Forns	0.706	6.45	0.63	0.74	0.53	0.80



	AUROC	Cut-off	Sensitivity	Specificity	PPV	NPV
FIB4	0.781	1.62	0.73	0.74	0.44	0.91
APRI	0.763	0.76	0.76	0.74	0.45	0.91
Forns	0.681	6.45	0.69	0.69	0.37	0.89



	AUROC	Cut-off	Sensitivity	Specificity	PPV	NPV
FIB4	0.870	1.73	1.00	0.71	0.13	1.00
APRI	0.732	1.57	0.60	0.90	0.21	0.98
Forns	0.892	7.40	1.00	0.73	0.15	1.00

Fig. 2 Diagnostic accuracy of the Fibrosis 4 (FIB-4) index, aspartate transaminase to platelet ratio index (APRI), and Forns index according to the Brunt stage. **a**–**d** Diagnostic accuracy of the FIB-4 index, APRI, and Forns index was assessed using the receiver operating characteristic (ROC) curve method, and the results are expressed as the area under the ROC curve (AUROC). The cut-off value was estimated by the Youden index. Using each cut-off value, sensitivity, specificity, the positive predictive value (PPV), and negative predictive value (NPV) were estimated. Each graph reveals the ROC curve for Brunt stage 0 versus 1–4 (**a**), 0–1 versus 2–4 (**b**), 0–2 versus 3–4 (**c**), and 1–3 versus 4 (**d**)

Longer observation period and presence of ballooned hepatocytes were associated with the progression of liver fibrosis

Considering the natural progression of NAFLD, NASH will eventually become the more progressive type of liver fibrosis. Therefore, linear regression analysis of the delta FIB-4 index was performed separately in the NAFL and NASH groups. Because the NAFL group did not show histological findings associated with liver fibrosis, bridging fibrosis and ballooned hepatocytes were not included in this analysis. The observation period was negatively associated with the delta FIB-4 index in the NAFL group (Table 2; $t = -2.621$, $p = 0.011$). Regarding the NASH group, linear regression analysis of the delta FIB-4 index was analyzed using BMI, sex, age, BW change during the observation period, the observation period, and histological findings (fat in the liver, bridging fibrosis, and ballooned hepatocytes). The presence of ballooned hepatocytes was negatively associated with the delta FIB-4 index in the NASH group (Table 2; $t = -2.371$, $p = 0.023$). To confirm the relationship between ballooned hepatocytes and liver fat, the liver fat volume was compared with the presence of ballooned hepatocytes or the grading of ballooned hepatocytes. Neither the presence of ballooned hepatocytes nor the grading of ballooned hepatocytes was associated with the liver fat volume (Supplemental Fig. 1A, B).

Discussion

The clinically significant findings of this study were as follows: (1) the FIB-4 index was the best formula for estimating liver fibrosis in patients with biopsy-proven

Table 2 Multivariate analysis for delta FIB4 using linear regression analysis to the NAFL and the NASH groups

NAFL	*t*	*p*
Sex	1.193	
Age	− 1.239	
BMI	− 0.610	
BW change	0.533	
Duration (year)	− 2.621	0.011
Fat	1.080	
BH	–	
BF	–	
NASH	***t***	***p***
Sex	− 0.123	
Age	− 1.541	
BMI	− 0.228	
BW change	− 0.873	
Duration (year)	− 0.655	
Fat	0.437	
BH	− 2.371	0.021
BF	− 0.311	

BF bridging fibrosis, *BH* ballooned hepatocyte, *BMI* body mass index, *BW* body weight, *NAFL* non-alcoholic fatty liver, *NASH* non-alcoholic steatohepatitis

NAFLD and (2) presence of ballooned hepatocytes predicted the progression of liver fibrosis in the future.

The prevalence of NAFLD is increasing among individuals in the developed countries [1]. The aggressive form of NAFLD, NASH, leads to the progression of liver fibrosis and results in liver cirrhosis [2]. Although the malignant potential of NASH has been recognized, the adequate approach for treating patients with NAFLD remains unclear. As the first step in establishing the treatment strategy for NASH, patients with NAFLD who show progression of liver fibrosis need to be identified. In this study as well as in previous studies [16, 17], the FIB-4 index showed accurate estimation of liver fibrosis in patients with NAFLD. Therefore, the FIB-4 index will be useful for assessing liver fibrosis when a general physician evaluates patients with NAFLD using laboratory data without imaging findings.

Advanced fibrosis of the liver is a distinct issue because cirrhotic liver will be the cause of liver failure and/or hepatocellular carcinoma [3]. Although no factor associated with the progression of liver fibrosis was isolated from all patients with NAFLD in this study, the observation period in the NAFL group and presence of ballooned hepatocytes in the NASH group were associated with the progression of liver fibrosis. The patients with NAFL who showed normalization of the liver enzyme after nutritional intervention were followed in up this study, and those with an abnormal liver enzyme level were followed for longer periods. Thus, the observation period in the NAFL group was identified as a risk factor for the progression of liver fibrosis because of selection bias. In contrast, the presence of ballooned hepatocytes was not associated with any bias, and it is considered a clear risk factor associated with the progression of liver fibrosis.

Ballooned hepatocytes are considered a diagnostic hallmark of NASH, and they have a key role in the pathophysiology of NASH [2, 4, 18, 19]. Hepatocytes without cell death under lipotoxicity show a morphological similarity to ballooned hepatocytes [20, 21]. Furthermore, these cells secrete a fibrogenic chemokine, sonic hedgehog (SHH), which affects cell survival under an autocrine mechanism [20]. Recently, autophagic impairment was observed in a NASH model [22]. Intriguingly, the morphological likeness of ballooned hepatocytes was required with autophagic impairment [21]. Lipotoxicity, i.e., the incomplete execution of cell death and impairment of the autophagic process, may lead to the ballooning of hepatocytes. In this study, ballooned hepatocytes were not associated with liver fat, and fibrosis progressed in the liver with the ballooning of hepatocytes. Thus, ballooned hepatocytes might be a therapeutic target in patients with NASH. Further study for the interaction between ballooned hepatocytes and the pathophysiology of NASH is necessary.

We recognize that this study has limitations. The evaluation of liver fibrosis in this study was not highly accurate. However, the FIB-4 index reflected the whole liver condition because the results were calculated using laboratory data. Yet, the liver biopsy had a possibility of sampling error [23]. Although the FIB-4 index did not show a high accuracy in the histological grade of liver fibrosis, the delta FIB-4 index was the difference of each result of the FIB-4 index, and the result might have been associated with a simple change of the whole-liver condition in this study. To confirm this speculation, the delta FIB-4 index should be confirmed in a future study with other modalities, such as transient elastography or MR elastography, to evaluate liver fibrosis. We also noticed a limitation associated with the evaluation of liver fibrosis. Although transient elastography has been recognized as a useful examination for evaluating liver fibrosis [24, 25], these data are absent in this study. We are now collecting the date of transient elastography, but we do not have sufficient data regarding the use of transient elastography in this setting. Thus, we cannot confirm the degree of liver fibrosis using transient elastography. Sampling error caused by liver biopsy should be carefully considered in the histological evaluation of conditions such as fibrosis, ballooned hepatocytes, and steatosis. Although multi-sampling from the same liver

tissue would improve the accuracy of the histological evaluation, we obtained a single sample from each liver biopsy. Therefore, more accurate evaluation of ballooning based on multiple sampling may be needed to confirm our findings in the future. We also need to mention the limitation associated with the serum marker of liver fibrosis. Recently, Mac-2 binding protein glycan isomer (M2BPGi) has been reported as a useful marker for detecting liver fibrosis [26–28]. Although we need to confirm a correlation between M2BPGi and liver fibrosis in this setting, we did not evaluate this relationship because we did not keep serum samples of the subjects. To avoid subjective bias for the diagnosis of ballooned hepatocytes, the presence or absence of ballooned hepatocytes was considered in this study. Thus, the evaluation of ballooned hepatocytes was not quantitative in this study. Therefore, the meaning of the presence of ballooned hepatocytes and the pathophysiology of NASH remains unclear.

We concluded that the FIB-4 index was the best formula for estimating the progression of liver fibrosis in patients with biopsy-proven NAFLD, and the presence of ballooned hepatocytes was a risk factor for the progression of liver fibrosis.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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Notes:-

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