学位論文（博士）

A Novel Index Including Age, Sex, *hTERT*, and Methylated *RUNX3* Is Useful for Diagnosing Early Gastric Cancer

（年齢、性別、*hTERT*、メチル化 *RUNX3* を含む新しい指標は早期胃癌の診断に有用である）

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学位論文の関連論文の研究背景及び要旨

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〔題名〕

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〔研究背景〕

本邦では 2013 年に慢性胃炎に対する *H.pylori* 除菌治療が保険適応となったのを機に、胃癌の死亡数は減少傾向に転じているが、依然として胃癌の罹患者数および死亡者数は癌の中でも上位に位置している。本邦における胃癌の 5 年生存率は Stage Ⅰでは 96.7%と非常に高い一方で、stage Ⅳでは 6.6%と進行すると予後が悪くなり、早期発見が非常に重要な癌と考えられる。現状、胃癌のスクリーニング検査としては、主に胃 X 線造影検査と上部消化管内視鏡検査が推奨されているが、前者は最近では感度が低く有意な死亡率減少を示さないと報告されており、後者は非常に侵襲・苦痛が大きく高価という欠点がある。そのため、国民生活基礎調査では胃癌検診の受診率は男女共に 50%以下で推移しており、かつ検診で指摘され切除された胃癌の 22%が進行癌であったことが報告されている。胃癌における血液検査としては、腫瘍マーカーとして CEA や CA19-9 が用いられることが多いが、喫煙や年齢等の要素に作用されるため、早期胃癌に対する感度や特異度は低く、胃癌検診には実用的ではない。他に抗 *H.pylori* 抗体やペプシノーゲンなどを用いることがあるが、これらは *H.pylori* の感染歴や胃粘膜の慢性胃炎による変化などを反映するものであり、長期的な胃癌発症の予測因子でしかないため、スクリーニングには適さない。よって、胃癌早期診断に有用な簡便・低侵襲かつ高精度なスクリーニング検査の開発が望まれており、その候補としてリキッドバイオプシーを考えた。胃癌発癌と DNA メチル化異常は密接に関係しており、我々はまず、胃癌に対する癌抑制遺伝子として作用している転写因子である *RUNX3* に注目した。*H.pylori* 感染により *RUNX3* のメチル化が惹起され、血清中の *RUNX3* メチル化 DNA(m-*RUNX3*) は胃癌のバイオマーカーとなり得ることが報告されている。ただし、m-*RUNX3* の早期胃癌に対する診断性能については不明瞭であるため、我々は高感度メチル化解析法(CORD 法)によるリキッドバイオプシー検査を開発し、血清中の m-*RUNX3* の定量解析の早期胃癌診断の有用性について検討し報告した。その検討における感度は 50.0%、特異度は 80.3%であり、m-*RUNX3* の単独使用では、感度および特異度において臨床使用には限界があった。そこで、先行研究のデータを再評価したところ、その過程で内部コントロールとして測定していた *hTERT* 遺伝子が早期胃癌群において有意に増加していることに気づき、*hTERT* 遺伝子が胃癌のリスクマーカーとなり得ると考えた。*hTERT* は *Telomerase* の触媒サブユニットであり、*Telomerase* は染色体の末端部（*telomere*）に *hTERT* を触媒としてヌクレオチドを付加していき、この反復 DNA 配列の付加により染色体の *telomere* の伸長を行っていく。*telomerase* の活性はがん細胞の不死化に重要な役割を果たしており、がんの 90%で

*telomerase*の活性化を認めると報告されている。*telomerase*の活性化は*hTERT*によって制御されており、触媒要素である*hTERT*はがんの治療標的として注目されている。そこで、その 2 つのマーカーを含む統合インデックスを作成し、胃癌早期診断のパラダイムシフトとなり得るかを検討した。

〔要旨〕

2017 年 12 月から 2022 年 8 月に早期胃癌の診断・治療が行われた早期胃癌群(EGC)94 例、検診 EGD で胃癌を認めないコントロール群(Con)225 例の計 319 例を対象とした。EGC は内視鏡治療にて最終診断確定され、画像検査で他臓器に癌を認めず、癌の既往がない症例とした。Con は上部消化管内視鏡検査で腫瘍性病変を認めないことを確認し、癌の既往がない症例とした。早期胃癌群は治療前に、コントロール群は内視鏡検査時に血清を採取した。リキッドバイオプシー検査は、血清 0.4mL を 3 種類のメチル化制限酵素にて DNA 処理後に hTERT 遺伝子及び m-*RUNX3* をデジタル PCR にて定量測定(copies/40μL serum)した。EGC の年齢の中央値は 74 歳、Con の年齢の中央値は 65 歳であり有意に EGC で高齢であった（p<0.001）。男性比率は EGC で 78.7%、Con で 51.5%であり、EGC で有意に男性が多かった（p<0.001）。*hTERT*の中央値は EGC で 910 copies/40μL serum、Con で 218 copies/40μL serum であり有意に EGC で高値であった（p<0.001）。m-*RUNX3*の中央値は EGC で 5.2 copies/40μL serum、Con で 1.8 copies/40μL serum であり、EGC で有意に高値を示した（p<0.001）。ROC 解析による胃癌診断能は、m-*RUNX3* 単独使用では感度が 57.4%、特異度が 87.6%、AUC は 0.78 であり、既報と近い値であった。*hTERT* 単独使用では感度が 85.1%、特異度が 80%、AUC は 0.89 であり m-*RUNX3* よりも優れた結果であった（p<0.001）。多変量解析を行ったところ、年齢、性別、*hTERT*、m-*RUNX3* いずれも早期胃癌の独立した危険因子であることが示されたため、年齢、性別、*hTERT*、m-*RUNX3* を用いた統合インデックス(ASTEm-R3 index：Age, Sex, *hTERT*, and m-*RUNX3*)を作成したところ、ROC 解析による胃癌診断能は、感度が 79.8%、特異度が 91.1%、AUC0.93 と非常に良好な早期胃癌診断能を示し、*hTERT* よりも優れた結果であった（p=0.006）。ASTEm-R3 index は血液検査と問診のみでリスク度を判定でき、より簡便・低侵襲・高精度な新たな早期胃癌スクリーニング法になり得る。臨床で実用化されれば検診受診率の増加が見込め、早期発見率が増加することで、胃癌の予後の改善も期待できる。また、限られた医療資源を有効活用することで医療費の削減にもつながると考える。統合インデックス：ASTEm-R3 index は早期胃癌診断のパラダイムシフトとなり得る。

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A Novel Index Including Age, Sex, hTERT, and Methylated RUNX3 Is Useful for Diagnosing Early Gastric Cancer

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Keywords
Biomarker · Early detection · Gastric cancer · Liquid biopsy

Abstract
Introduction: As the incidence of gastric cancer (GC) is increasing in East Asia including Japan, a simple blood test for early GC is needed as an alternative to upper gastrointestinal (UGI) endoscopy. We performed this study to address this issue. Methods: We collected serum samples from 319 participants comprising 225 healthy subjects without GC (control group) and 94 patients with early GC (early GC group). After evaluating copy numbers of serum hTERT and methylated RUNX3 (m-RUNX3) using the Combined Restriction Digital PCR (CORD) assay, which we developed, we assessed the diagnostic performance of hTERT and m-RUNX3 for early GC. Results: Serum levels of hTERT and m-RUNX3 were significantly higher in the early GC group than in the control group. The area under the curve (AUC) was 0.89 for hTERT and 0.78 for m-RUNX3. Multivariate logistic regression analysis revealed age, sex, hTERT copy number, and m-RUNX3 copy number to be independent factors for early GC. We then established a prediction formula and named it the ASTEm-R3 (age, sex, hTERT, and m-RUNX3) index. The AUC of the ASTEm-R3 index was 0.93 with a sensitivity of 79.7% and specificity of 91.1%. Conclusion: We demonstrated excellent performance of the ASTEm-R3 index using the CORD assay to detect early GC. This index might be a promising alternative to UGI endoscopy.

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Introduction

Gastric cancer (GC) was the fifth leading cause of morbidity and fifth of mortality worldwide in 2022. A significantly higher incidence of GC occurs in East Asia including Japan [1]. At 96.7%, the 5-year relative survival rate for early GC in Japan is very high. However, it drops drastically to 6.6% for stage IV GC [2]. Therefore, early detection of GC is very important to reduce the mortality rate of this disease. In Japan, either contrast radiography or upper gastrointestinal (UGI) endoscopy is performed as a population-based screening test for GC [3]. Contrast radiography has a very low sensitivity for diagnosing early GC [4]. In contrast, UGI endoscopy is the most sensitive test for diagnosing early GC [5]. However, almost half of the people in Japan do not undergo UGI endoscopy due to its invasiveness and high cost [2]. Therefore, a simple, inexpensive, and non-invasive test to screen for early GC as an alternative to UGI endoscopy is urgently needed.

Among blood tests, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are widely used as tumor markers for GC. Nevertheless, no current blood test can diagnose early GC due to the low sensitivity of conventional tumor markers [6]. The risk of developing GC depends on the underlying condition, namely Helicobacter pylori infection and gastric atrophy [7, 8]. In Japan, a combined test of serum anti-H. pylori antibody titer and serum pepsinogen levels has been performed as an alternative blood test for assessing GC risk [9, 10]. Serum anti-H. pylori antibody titer reflects previous or current infection with H. pylori. Contrastingly, serum pepsinogen reflects the degree of gastric atrophy [11]. However, the combined test of serum anti-H. pylori antibody titer and serum pepsinogen levels is not applied to GC screening as it is only a predictor of GC development over the long term [12].

One strategy to address these problems is a liquid biopsy that targets circulating tumor cells or circulating cell-free DNA from tumors in the blood. Although there are many reports on liquid biopsy for the detection of GC [13], liquid biopsy has not been used in practice for GC screening. To address this issue, we developed a new methylation assay, the Combined Restriction Digital PCR (CORD) assay. After applying it to a liquid biopsy of early GC targeting methylated RUNX3 (m-RUNX3) in serum, we found a sensitivity of 50.0% and specificity of 80.3% [14]. However, as a single use of m-RUNX3 had limitations in sensitivity and specificity for clinical use, we re-evaluated the data from the previous studies and found that copies of serum hTERT, measured as an internal control, were dramatically increased in the early GC group compared to the non-GC group. Therefore, we speculated that hTERT might also be a potential biomarker for early GC. Further, we also hypothesized that the development of a prediction formula based on logistic regression analysis with variables including hTERT, m-RUNX3, and other factors might improve the diagnostic performance for early GC. To prove these speculations, we conducted this study to evaluate the diagnostic performance of hTERT in combination with m-RUNX3 for early detection of GC.

Methods

Serum Samples

This was a retrospective study in which serum samples from 319 participants were collected prior to any medical treatments the participants would undergo between November 2016 and June 2022 at Yamaguchi University Hospital, St. Hill Hospital, and Ajisu Kyoritsu Hospital in Japan. To avoid artificial contamination of the blood samples by cancer cells, blood collection was performed at least 3 weeks after the biopsy if it had been performed. Serum samples were stored at −80°C until DNA extraction. DNA was extracted at 1–6 months after blood collection. The participants comprised 225 healthy subjects and 94 patients with early GC with tumor invasion limited within the submucosal layer. Their clinicopathologic characteristics are shown in Table 1. Twelve patients with gastric adenoma were excluded from this study. The healthy subjects did not have GC on UGI endoscopy and did not have a previous history of GC. The diagnosis of early GC was confirmed pathologically after endoscopic submucosal dissection and/or surgical resection, with no evidence of cancer shown in other organs by imaging modalities. The depth of tumor invasion was classified as mucosal (m) and submucosal (sm). Well or moderately differentiated tubular adenocarcinoma and papillary adenocarcinoma were classified as differentiated carcinoma. Poorly differentiated adenocarcinoma and signet-ring cell carcinoma were classified as undifferentiated carcinoma. Lesions containing both differentiated and undifferentiated carcinomas were classified on the basis of which was predominant microscopically [15]. This study was conducted in compliance with the ethical principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Boards of Yamaguchi University Hospital, St. Hill Hospital, and Ajisu Kyoritsu Hospital (H28-124 and H17-83).

Table 1. Clinicopathologic characteristics of the subjects

Characteristics	Cancer (n = 94)	Control (n = 225)	p value
Age, years, median (range)	74 (34–90)	65 (34–88)	<0.0001
Sex			
Male	74	116	<0.0001
Female	20	109	
Tumor size, median (range), mm	12.5 (3.0–65.0)	NA	NA
Depth of tumor invasion			
m	82	NA	NA
sm	12	NA	
Tumor differentiation			
Differentiated	90	NA	NA
Undifferentiated	4	NA	
Anti-H. pylori antibody titer			
≥10 U/mL	25	NA	NA
3–9.9 U/mL	31	NA	
<3 U/mL	38	NA	
hTERT, median (range), copies/40 μL serum	910 (156–9,900)	218 (5–1,780)	<0.0001
Methylated RUNX3, median (range), copies/40 μL serum	5.2 (0–32)	1.8 (0–10.6)	<0.0001
CEA			
>6 ng/mL	8	NA	NA
≤6 ng/mL	86	NA	
CA19-9			
>37 U/mL	3	NA	NA
≤37 U/mL	91	NA	

NA, not available; m, mucosa; sm, submucosa.

Analysis of hTERT and Methylated RUNX3 Using CORD Assay

The serum samples preserved at −80°C were thawed. Then, 0.4 mL of each serum sample was used for DNA extraction with a MagNa Pure Compact Nucleic Acid Isolation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. DNA was eluted in 50 μL of elution buffer. DNA was treated in a two-step procedure using three methylation-sensitive restriction enzymes (HhaI, HpaII, and BstUI) and exonuclease I as previously reported [7–10], followed by multiplex droplet digital PCR. The primer and probe set sequences for RUNX3 were sense primer, 5′-TATGCGTATTCCCGTAGACCC-3′; anti-sense primer, 5′-GCTGTTCTCGCCCATCTTG-3′; and probe, 5′-FAM-TCCCCGGCCTTCCCCTGCGG-TMARA-3′ [14]. Those for hTERT were sense primer, 5′-GGGTCCTCGCCTGTGTACAG-3′; anti-sense primer, 5′-CCTGGGAGCTCTGGGAATTT-3′; and probe, 5′-VIC-CACACCTTTGGTCACTC-MGB-3′ [16]. The PCR reaction solution consisted of 8 μL of enzyme-treated DNA, 1× ddPCR Supermix for Probes (Bio-Rad, Hercules, CA, USA), 0.25 μmol/L of each primer, and 0.125 μmol/L of each probe in a total volume of 20 μL. After droplets were generated by an automated droplet generator (Bio-Rad), PCR was performed. PCR cycling conditions included preheating at 95°C for 10 min followed by 40 cycles of denaturation at 94°C for 30 s and annealing at 56°C for 60 s. Final heating was performed at 98°C for 10 min. Following amplification, the PCR plate was transferred to a QX200 droplet reader, and fluorescence amplitude data were obtained using QuantaSoft software (both, Bio-Rad).

CEA and CA19-9

Serum levels of CEA and CA19-9 were, respectively, measured using a "TOSOH" II CEA commercial immunoassay kit and an "ARCHITECT CA19-9XR" CA19-9 commercial immunoassay kit (both from Tosoh, Tokyo, Japan). An AIA-2000 automated immunoassay analyzer (Tosoh) was used for CEA, and an ARCHITECT i2000 automatic immunoassay analyzer (Abbot Corporation, Tokyo, Japan) was used for

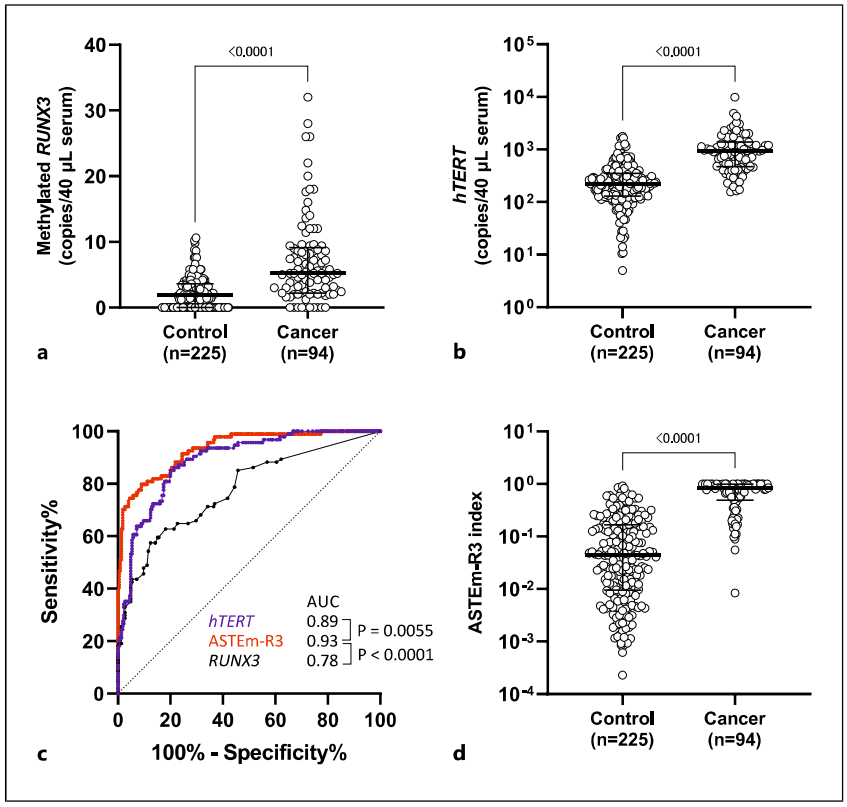

Fig. 1. Diagnostic performance. The distributions of methylated RUNX3 copy numbers (a), hTERT copy numbers (b), and ASTEm-R3 index (d) for each group are shown. Each sample is indicated by an open circle. The horizontal lines indicate the median and interquartile range. Receiver operating characteristic curve analyses between the control group and the early GC group show the areas under the curve (AUCs) of hTERT, methylated RUNX3 (RUNX3), and the ASTEm-R3 index (c).

CA19-9 in the Division of Laboratory, Yamaguchi University Hospital. In accordance with the manufacturer's instructions, the cutoff values were 6 ng/mL for CEA and 37 U/mL for CA19-9.

Serum Anti-H. pylori Antibody Titer

Serum anti-H. pylori antibody titer was measured using the LZ test "Eiken" for H. pylori antibody (Eiken Chemical, Tokyo, Japan) on a BM9130 automatic clinical chemistry analyzer (JEOL, Tokyo, Japan) at LSI Medience Corporation, a clinical laboratory testing service in Tokyo, Japan. The antibody titer cutoffs were set at 3 U/mL and 10 U/mL to evaluate H. pylori infection. An antibody titer <3 U/mL is regarded as a negative infection or possible past exposure to H. pylori, 3–9.9 U/mL indicates a current or past infection, and ≥10 U/mL indicates a current infection [17].

Statistical Analysis

The Mann-Whitney U test was used to compare differences in numerical variables between two groups. Categorical variables were analyzed using the χ^2 test. The optimal cutoff value was determined by the Youden index method from the receiver operating characteristic (ROC) curve. Multivariate logistic regression analysis was performed to determine independent factors that contribute to the diagnosis of early GC. Statistical significance was defined as a p value <0.05. For statistical analysis, we used GraphPad Prism ver. 9 (GraphPad Software, San Diego, CA, USA), StatFlex ver. 7.0 (Artec Corporation, Osaka, Japan), and JMP Pro (JMP Statistical Discovery, Cary, NC, USA).

Results

CEA, CA19-9, and Serum Anti-H. pylori Antibody Titer in the Early GC Group

Among the 94 patients with early GC, the number of patients with positive CEA and CA19-9 results was 8 (8.5%) and 3 (3.2%), respectively, and that of patients with serum anti-H. pylori antibody titers of ≥3 U/mL and <3 U/mL were 56 (59.6%) and 38 (40.4%), respectively. Anti-H. pylori antibody titers of ≥10 U/mL were found in 26.6% (25/94) of the patients with early GC.

Table 2. Logistic regression analysis

Variables	β	SE (β)	z value	p value	OR (95% CI)
(Intercept)	−15.2	2.0700			
Age, years	0.138	0.0227	6.08	<0.001	1.15 (1.10–1.20)
Sex (male)	1.360	0.4250	3.19	0.001	3.88 (1.69–8.92)
Methylated RUNX3 (copies)	0.138	0.0684	2.01	0.044	1.15 (1.00–1.31)
hTERT (copies)	0.004	0.0006	6.43	<0.001	1.00 (1.00–1.01)

CI, confidence interval; OR, odds ratio; SE, standard error.

Methylated RUNX3 and hTERT

For m-RUNX3, the median copy number was 1.8 copies/40 μL serum in the control group and 5.2 copies/40 μL serum in the early GC group ($p < 0.0001$, Fig. 1a). The median hTERT copy number was 218 copies/40 μL serum in the control and 910 copies/40 μL serum in the early GC groups ($p < 0.0001$, Fig. 1b). For m-RUNX3, the area under the curve (AUC) by ROC analysis was 0.78 (Fig. 1c). The optimal cutoff by the Youden index was 5.0 copies/40 μL serum, resulting in a sensitivity of 57.4% (54/94) and specificity of 87.6% (197/225) for early GC. The AUC for hTERT was 0.89 (Fig. 1c). Using 400 copies/40 μL serum as the optimal cutoff by the Youden index, the sensitivity and specificity were 85.1% (80/94) and 80.0% (180/225), respectively, for diagnosing early GC.

Integrated Index Including Age, Sex, hTERT, and m-RUNX3 for Diagnosing Early GC

Logistic regression analysis showed age, sex, hTERT, and m-RUNX3 to be independent factors contributing to the diagnosis of early GC (Table 2). From the regression model, we derived the following formula and named it the ASTEm-R3 (age, sex, hTERT, and m-RUNX3) index.

$$\text{ASTEm-R3 index} = \frac{1}{\left[1+e^{\left\{-\left[-15.20+0.138\,(\text{Age})+1.360\,(\text{Sex})+0.138\,(\text{Methylated RUNX3})+0.004\,(\text{hTERT})\right]\right\}}\right]}$$

The median value of the ASTEm-R3 index was 0.04 in the control group and 0.82 in the early GC group (Fig. 1d). The ASTEm-R3 index showed the best statistically significant diagnostic accuracy with an AUC of 0.93 for early GC versus those of 0.78 for m-RUNX3 alone ($p < 0.0001$) and 0.89 for hTERT alone ($p = 0.0055$, Fig. 1c). Using an optimal cutoff of 0.35, as determined by the Youden index, the sensitivity of the ASTEm-R3 index for early GC was 79.8% (75/94) with a specificity of 91.1% (205/225).

Discussion

In this study, the sensitivity of CEA and CA19-9 for early GC was quite low: 8.5% for CEA and 3.2% for CA19-9, consistent with a previous report [3]. Therefore, it is seemingly impossible to use CEA and/or CA19-9 for the diagnosis of early GC. In the present study, the liquid biopsy test using the CORD assay of m-RUNX3 resulted in a ROC-AUC of 0.78 with a sensitivity of 57.4% (54/94) and specificity of 87.6% (197/225) for the detection of early GC. This result was almost in line with the AUC of 0.70 obtained in our previous study [10]. For hTERT, the ROC-AUC was 0.89 with a sensitivity of 85.1% (80/94) and specificity of 80.0% (180/225) for the detection of early GC. Furthermore, a multivariable logistic regression analysis showed hTERT and m-RUNX3 to independently contribute to the diagnosis of early GC even after adjustment for age and sex. Therefore, we established the novel integrated ASTEm-R3 index. The AUC of the ASTEm-R3 index was 0.93 with a relatively high sensitivity of 79.8% (75/94) and a considerably high specificity of 91.1% (205/225). In addition, the AUC of the ASTEm-R3 index was significantly better than that of a single use of either mRUNX3 ($p < 0.0001$) or hTERT ($p = 0.0055$). This is the first report to show the clinical utility of the ASTEm-R3 index for the detection of early GC.

The rate of positivity for ≥3 U/mL of anti-serum H. pylori antibodies in patients with early GC was 59.6% (56/94) and that for ≥10 U/mL was only 26.6% (25/94) (Table 1). Although anti-serum H. pylori antibodies have been used as a marker for GC [18], the number of patients positive for H. pylori has decreased due to the widespread use of H. pylori eradication therapy and subsequent decrease in the number of H. pylori-infected patients [19, 20]. In Japan, GC is often detected in patients even after H. pylori eradication therapy, and the serum antibody titers decrease after successful anti-H. pylori chemotherapy [21–23]. Therefore, it may be unreliable to use serum anti-H. pylori antibody titers as an indication for endoscopic screening, although H. pylori infection remains an important etiological factor for GC.

Methylation of cell-free DNA has been identified as a promising marker for GC. For example, Ren et al. reported that cell-free DNA MCTA-Seq, which utilizes 153 mCGCGCGG-CpG sites, had a sensitivity of 44% for stage I GC and a specificity of 92% [24]. Kandimalla et al. developed the EpiPanGIDx model based on 50 differentially methylated regions, which showed an AUC value of 0.90 [25]. Qi et al. [26] developed a GC detection model using 21 differentially methylated regions, resulting in a sensitivity of 96.48% and specificity of 91.05% for detecting in situ and early GC. These reports indicate the potential of cfDNA methylation as an effective tool for early cancer detection. However, the above assays may be difficult to use in clinical practice due to procedural complexity and/or the large number of target regions. In contrast, the ASTEm-R3 index targets only methylated RUNX3 and hTERT, which can be counted simultaneously. Thus, the ASTEm-R3 index is simpler and less labor intensive.

This study has several limitations. Cell-free DNA, also known as circulating tumor DNA, is known to increase in patients with cancer, not just GC [27]. As the hTERT copy number may reflect the amount of cell-free DNA, hTERT would not be specific for GC. Indeed, we have observed an increase in serum hTERT copies in patients with colorectal cancer, hepatocellular carcinoma, and pancreatic cancer (unpublished data not shown). Similarly, RUNX3 is frequently hypermethylated in several types of cancer, including lung, breast, and pancreatic cancer [28, 29]. Thus, m-RUNX3 is also not specific to GC. Although the present study showed the ASTEm-R3 index to be useful for the detection of GC, additional studies will be needed to clarify its diagnostic performance in GC compared to other cancers. Further, although the optimal cutoff value of the ASTEm-R3 index was set by the Youden index, it is unclear whether this is the best value. If we continue to improve the sensitivity for detecting early GC, it may become less specific. Conversely, if specificity is improved, the sensitivity will deteriorate. Because clinical laboratory tests require a delicate balance of sensitivity, specificity, and cost, the best cutoff value should be determined in a prospective study.

Conclusion

We demonstrated the excellent performance of the ASTEm-R3 index using the CORD assay to detect early GC. Compared to the conventional markers, the ASTEm-R3 index showed better diagnostic performance for early GC than serum CEA and CA19-9. In addition, unlike UGI endoscopy, the ASTEm-R3 index is non-invasive. Thus, it could potentially be used for the screening of GC as a possible alternative test to UGI endoscopy. We also anticipate that the ASTEm-R3 index may be useful for the surveillance of individuals at very high risk for GC, such as those with H. pylori infection and atrophic gastritis. Confirmatory prospective studies will be needed to support our speculations.

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Statement of Ethics

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of Yamaguchi University Hospital (H28-124 and H2019-053). Written informed consent was obtained from all participants.

Conflict of Interest Statement

Y.S. and T.Y. received grant support from Eiken Chemical Co., Ltd. All other authors declare no conflicts of interest.

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Author Contributions

Katsuhiko Nakamura, Yutaka Suehiro, Koichi Hamabe, Shinichi Hashimoto, Isao Sakaida, and Takahiro Yamasaki designed the study. Yuki Kunimune, Naoko Okayama, Tomohiro Fujii, Yukiko Nakahara, Mitsuaki Nishioka, and Yutaka Suehiro performed sample processing. Katsuhiko Nakamura, Koichi Hamabe, Atsushi Goto, Shingo Higaki, Ikuei Fujii, and Chieko Suzuki performed data curation. Katsuhiko Nakamura, Yutaka Suehiro, Koichi Hamabe, Akiyo Ishiguro, and Takahiro Yamasaki performed statistical analysis. Katsuhiko Nakamura, Koichi Hamabe, Yutaka Suehiro, and Takahiro Yamasaki wrote the original draft of the manuscript. Jun Nishikawa and Taro Takami reviewed and edited the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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