Novel Liquid Biopsy Test Based on a Sensitive Methylated SEPT9 Assay for Diagnosing Hepatocellular Carcinoma

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Liquid biopsies are not used in practice for hepatocellular carcinoma (HCC). Epi proColon is the first commercial blood-based test for colorectal cancer screening based on methylated DNA testing of the septin 9 gene (SEPT9). However, Epi proColon has some disadvantages, including the requirement of a large amount of blood and lack of quantitative performance. Therefore, we previously developed a novel liquid biopsy test that can quantitatively detect even a single copy of methylated SEPT9 in a small amount of DNA. In the current study, we evaluated the application potential of this assay for diagnosing HCC. Study subjects included 80 healthy volunteers, 45 patients with chronic liver disease (CLD) without HCC, and 136 patients with HCC (stage 0, 12; stage A, 50; stage B, 31; stage C, 41; and stage D, 2), according to the Barcelona Clinic Liver Cancer staging system. For the assay, DNA was treated with methylation-sensitive restriction enzymes in two steps, followed by multiplex droplet digital polymerase chain reaction. The median copy number of methylated SEPT9 was 0.0, 2.0, and 6.4 in the healthy control, CLD, and HCC groups, respectively, with significant differences among the groups (HCC vs. healthy control, \( P < 0.001 \); HCC vs. CLD, \( P = 0.002 \); CLD vs. healthy control, \( P = 0.008 \)). Assay sensitivity and specificity were 63.2% and 90.0%, respectively (cutoff value, 4.6 copies), in detecting HCC when compared with healthy subjects. The positive rate of methylated SEPT9 increased with HCC progression (stage 0, 41.7%; stage A, 58.0%; stage B, 61.3%; stage C, 75.6%; and stage D, 100%). Conclusion: We developed a sensitive methylated SEPT9 assay that might serve as a liquid biopsy test for diagnosing HCC. (Hepatology Communications 2020;4:461-470).

Hepatocellular carcinoma (HCC) is a common malignant disease and the fourth leading cause of cancer-related deaths worldwide.\(^{[5]}\) The absolute number of primary cancer cases, of which HCC accounts for 75% to 85%, is expected to increase in the majority of 30 countries worldwide by 2030.\(^{[2]}\) To detect HCC at an early stage, international guidelines from the United States, Europe, Asia, and Japan advise HCC surveillance of at-risk populations.\(^{[3-6]}\) However, tumor biomarkers for the early detection of HCC are still lacking.\(^{[4,6]}\) Because early screening of HCC patients is reportedly related to favorable survival,\(^{[7,8]}\) a novel screening method for patients with increased risk for HCC is required.

Liquid biopsy has been suggested as a novel diagnostic tool, and circulating tumor cells and circulating DNA...
tumor DNA (ctDNA) have been recognized as potential targets in liquid biopsies. Methylated ctDNA is among the most intensively investigated liquid biopsy targets. Because methylation changes in ctDNA occur early in carcinogenesis, numerous studies have evaluated the diagnostic performance of methylated genes, including cyclin-dependent kinase inhibitor 2A (CDKN2A), Ras association domain family 1 isoform A (RASSF1A), and glutathione S-transferase pi 1 (GSTP1), in patients with HCC. However, the clinical usefulness of these methylated ctDNA tests in HCC remains limited.

Epi proColon (Epigenomics AG, Berlin, Germany), which was approved by the US Food and Drug Administration (FDA) in 2016, was the first commercial blood-based test for colorectal cancer screening based on methylated DNA testing of the septin 9 gene (SEPT9). There is only one report on the efficacy of Epi proColon in HCC patients. However, Epi proColon has some disadvantages. First, a large amount of blood plasma (>3.5 mL) is required, because the amount of cancer-specific DNA in blood is very small, and conventional DNA methylation assays require bisulfite treatment of DNA, which causes DNA degradation and loss. Second, this test does not have quantitative performance, because it is based on real-time polymerase chain reaction (PCR) plus/minus assay. To overcome these problems, we previously developed a new assay, the combined restriction digital PCR (CORD) assay, which circumvents the need for bisulfite treatment and methylated DNA immunoprecipitation. This assay is 100-plus times more sensitive than the conventional bisulfide-based methylation assay and thus overcomes the issue of limited input DNA. The CORD assay enables quantitative detection of even one copy of a methylated gene in a small DNA sample, without the need for DNA bisulfite treatment. The most unique feature of this novel assay is that it uses three methylation-sensitive restriction enzymes in combination with droplet digital PCR (ddPCR). We have reported the performance of the serum CORD assay for methylated SEPT9 in patients with colorectal neoplasia, including cancer. In this study, we evaluated the potential application of this assay for methylated SEPT9 detection in HCC patients.

Experimental Procedures

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, Sentohiru Hospital, and Ajisu Kyoritsu Hospital (H28-124 and H17-83).

SERUM AND TISSUE SAMPLES

We enrolled 261 participants (Fig. 1) and prospectively and retrospectively collected 221 and 40 sera, respectively, between March 2015 and August 2018 at Yamaguchi University Hospital, Sentohiru Hospital, and Ajisu Kyoritsu Hospital. The subjects consisted of 80 healthy volunteers who had neither colorectal neoplasia as determined by colonoscopy nor chronic liver disease (CLD) (healthy control group), 45 patients with CLD without HCC (CLD group), and 136 patients with HCC (HCC group). Prospective data were obtained from each patient and healthy volunteer. Blood samples in the healthy control group were collected prior to bowel preparation.
for colonoscopy. Patients in the CLD group who showed no evidence of HCC by imaging modalities within 3 months of blood sampling and 6 months later were recruited, and blood samples in the HCC group were obtained immediately before HCC treatments. HCC was diagnosed based on Japanese guidelines. We assessed HCC tumor stage using the Barcelona Clinic Liver Cancer (BCLC) staging system. A total of 35 frozen tumor tissues and 34 matched frozen nontumor tissues (one sample was lost) were collected from 35 patients who underwent surgical resection of HCC.

**SAMPLE PREPARATION AND DNA EXTRACTION**

Samples were stored at –80°C until DNA extraction. Serum samples were thawed, and 0.4 mL of each sample was used for DNA extraction with the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche, Tokyo, Japan) according to the manufacturer’s instructions. DNA was eluted in 50 μL of elution buffer. Tissue samples were thawed, and DNA was isolated using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). DNA was quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Tokyo, Japan).

**CORD ASSAY OF METHYLATED **

**SEPT9**

DNA samples were subjected to a CORD assay consisting of two-step treatments with three methylation-sensitive restriction enzymes, followed by multiplex ddPCR. In the first enzyme treatment step, 10 μL of circulating cell-free DNA (an amount of DNA equivalent to the amount in 80 μL of serum) or 10 μL of 10 ng/μL tissue DNA was digested at 37°C for 16 hours by addition of 1 μL of GeneAmp 10× PCR Buffer II, 1 μL of 25 mmol/L magnesium chloride (MgCl2), 10 units of HhaI, 10 units of HpaII, and 20 units of exonuclease 1 (Exo1) (all from Thermo Fisher Scientific, Tokyo, Japan). Exo1 was added to eliminate single-stranded DNA that would escape digestion by the restriction enzymes and to avoid PCR amplification of the undigested fraction. In the second step, DNA was further digested for 16 hours at 60°C using 10 units of BstUI (New England Biolabs, Hitchin, United Kingdom). After the restriction reaction was completed, the mixture was heated at 98°C for 10 minutes. **SEPT9** has three recognition sites for the methylation-sensitive enzymes HhaI and BstUI. When all three sites are methylated, the target DNA escapes digestion by these enzymes and is amplified by
PCR. The human telomerase reverse transcriptase gene (hTERT) has no recognition sites for these enzymes; therefore, hTERT is amplified by PCR when human DNA is present in the template DNA. Absolute copy numbers of hTERT and methylated SEPT9 were determined by multiplex ddPCR. The 20-µL PCR mixture consisted of 8 µL of enzyme-treated serum cell-free DNA (an amount of DNA equivalent to the amount in 0.04 mL serum) or 2 µL of enzyme-treated tissue DNA, 1× ddPCR Supermix for Probes (Bio-Rad, Tokyo, Japan), 0.25 µmol/L of each primer for a given target gene and an internal control, and 0.125 µmol/L of each probe for the target gene and internal control. We designed SEPT9 primers and probes within the CpG island 3 region of SEPT9 containing transcription start site of SEPT9 transcript variant 2, which is differentially methylated in colorectal cancer and is targeted by Epi proColon.(20,21) The primer and probe sequences for SEPT9 were as follows: forward primer, 5'-GCCCACCAGGCATCATGT-3; reverse primer, 5'-GTCCGAATGTCCCATCCA-3'; and probe, 5'-FAM-CCGCGGTCAACGC-MGB-3'. The PCR amplicon length was 60 base pairs (bp) (75,369,566-75,369,627 on chromosome 17; human assembly Genome Reference Consortium human genome [build 37]/human genome 19 [GRCh37/hg19]). The primer and probe sequences for hTERT were as follows: forward primer, 5'-GGGTCCCTCGCTGTGTACAG-3; reverse primer, 5'-CCTGGGAGCTCTGGGAATTT-3'; and probe, 5'-VIC-CACACCTTTGGTCACTC-MGB-3'. The amplicon length was 60 bp (1,253,375-1,253,434 on chromosome 5). Droplet generation was carried out by an automated droplet generator (Bio-Rad) and followed by PCR. PCR cycles included preheating at 95°C for 10 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds and annealing at 56°C for 60 seconds and final heating at 98°C for 10 minutes. After amplification, the PCR plate was transferred to a QX100 droplet reader (Bio-Rad), and fluorescence amplitude data were obtained using QuantaSoft software (Bio-Rad).

**STATISTICAL ANALYSIS**

Continuous variables are presented as the mean ± SD or median (interquartile range) and were compared using a paired or unpaired t test. Categorical variables were evaluated using the chi-squared test or Fisher’s exact test. Statistical significance was defined as a P value less than 0.05. The optimal cutoff value in receiver operating characteristic (ROC) analysis was determined by the Youden index. All analyses were performed using the JMP software package v13.0 (SAS Institute, Cary, NC).

**Results**

**PATIENT CHARACTERISTICS**

Table 1 summarizes the clinical patient characteristics. The HCC group included 98 males (72%), with a mean age of 72.4 years. Etiology of HCC was hepatitis B virus (HBV) in 18 (13%), hepatitis C virus (HCV) in 58 (43%), and non-B non-C (NBNC) in 60 patients (44%). HCC tumor stage was 0, A, B, C, and D in 12 (8.8%), 50 (36.8%), 31 (22.8%), 41 (30.1%), and 2 patients (1.5%), respectively. Median tumor size and number were 3.2 cm and 1, respectively. A total of 33 patients (24%) had macrovascular invasion, and 14 (10%) showed extrahepatic spread.

**TABLE 1. CLINICAL PATIENT CHARACTERISTICS IN SERUM SAMPLES**

|                   | Healthy Control Group (n = 80) | CLD Group (n = 45) | HCC Group (n = 136) |
|-------------------|-------------------------------|-------------------|---------------------|
| Age               | 50.1 ± 8.5                    | 69.0 ± 10.4       | 72.4 ± 8.5          |
| Gender, male/female | 38/42                         | 22/23             | 98/38               |
| Etiology, HBV/HCV/NBNC | N/A                           | 12/18/15          | 18/58/60            |
| CH/Chir-Turcotte-Pugh (CTP grade A/B/C) | N/A                         | 12/33 (20/9/4)   | 68/68 (51/17/0)     |
| BCLC stage 0/A/B/C/D | N/A                           | N/A               | 12/50/31/41/2       |
| Tumor size, cm    | N/A                           | N/A               | 3.2 (2.0-5.75)      |
| Tumor number      | N/A                           | N/A               | 1 (1-3)             |
| Macrovacular invasion +/- | N/A                          | N/A               | 33/103              |
| Extrahepatic spread +/- | N/A                          | N/A               | 14/122              |

Abbreviations: CH, chronic hepatitis; CTP, Child-Turcotte-Pugh; LC, liver cirrhosis; N/A; not applicable.
The CLD group included 22 males (49%), with a mean age of 69 years. Etiology was HBV in 12 (27%), HCV in 18 (40%), and NBNC in 15 (33%) patients.

**BASIC PERFORMANCE EVALUATION OF THE CORD ASSAY**

For basic performance evaluation of the CORD assay detecting hypermethylated cancer-derived DNA against a background of blood-derived DNA,\(^{(17)}\) we spiked EpiScope methylated HCT116 genomic DNA (control DNA for methylation of \(SEPT9\); Takara Bio, Inc., Japan) at ratios of 100%, 50%, 10%, 5%, 1.1%, 0.11%, and 0% into DNA extracted from leukocyte DNA (control DNA for nonmethylated \(SEPT9\)) and measured the methylation level of \(SEPT9\) in each sample. The CORD assay can quantify copy numbers of methylated \(SEPT9\) from 6.25 pg of control methylated DNA (approximately two haploid genome copies) in a background of 5,625 pg of control nonmethylated DNA (Supporting Fig. S1).

**METHYLATED \(SEPT9\) ASSAY OF SERUM SAMPLES**

The median copy number of methylated \(SEPT9\) was 0.0 (0.0-3.4), 2.0 (0.0-8.0), and 6.4 (2.6-13.3) in the healthy control, CLD, and HCC groups, respectively, with significant differences among the groups (HCC group vs. healthy control group, \(P < 0.001\); HCC group vs. CLD group, \(P = 0.002\); CLD group vs. healthy control group, \(P = 0.008\)) (Fig. 2A). We performed ROC curve analysis to set an optimal cutoff value for distinguishing the healthy control group from the HCC group. When we set 4.6 copies of methylated \(SEPT9\) as a provisional cutoff value, the area under the curve (AUC) was 0.81, with a sensitivity and specificity of 63.2% and 90.0%, respectively (Fig. 2B). On the other hand, assay sensitivity and specificity were 62.5% and 71.7%, respectively (cutoff value, 4.8 copies), in detecting HCC when compared with patients with CLD without HCC (data not shown). Therefore, 4.6 copies of methylated \(SEPT9\) was set as the optimal cutoff value to identify methylation-positive patients with HCC in the following analyses.

**FIG. 2.** Serum methylated \(SEPT9\) assay. (A) Methylated \(SEPT9\) copy numbers in each of the study groups. The median copy number of methylated \(SEPT9\) was 0.0 (0.0-3.4), 2.0 (0.0-8.0), and 6.4 (2.6-13.3) in the healthy control group, CLD group, and HCC group, respectively. (B) AUC of methylated \(SEPT9\). When we set 4.6 copies of methylated \(SEPT9\) as a provisional cutoff value, the AUC was 0.81, with a sensitivity and specificity of 63.2% and 90.0%, respectively.
CORRELATION BETWEEN HCC TUMOR STAGE AND SERUM METHYLATED SEPT9 LEVEL

The correlation between HCC tumor stage according to the BCLC staging system (18) and methylated SEPT9 copy number is shown in Fig. 3. The copy number of methylated SEPT9 increased with HCC progression. The median copy number was 2.1, 5.6, 6.6, 9.2, and 53.7 in stage 0, A, B, C, and D, respectively (HCC group stage A, B, and C vs. healthy control group, *P* < 0.001, *P* < 0.001 and *P* < 0.001, respectively; HCC group stage C vs. CLD group, HCC group stage 0, A and B, *P* = 0.002, *P* = 0.002, *P* = 0.003 and *P* = 0.008, respectively) (Fig. 3A). The positive rate of methylated SEPT9 (cutoff ≥ 4.6 copies) increased from 41.7% to 100% with HCC progression (stage 0, A, B, C, and D, 41.7%, 58.0%, 61.3%, 75.6% and 100%, respectively) (Fig. 3B). There were significant differences in background liver disease, tumor size and number, and macrovascular invasion between methylated SEPT9 < 4.6 copies and ≥ 4.6 copies. In addition, the positive rate of methylated SEPT9 was 66.7% (40 of 60), 55.6% (10 of 18), and 62.1% (36 of 58) in patients with NBNC-HCC, HBV-related HCC, and HCV-related HCC, respectively, with no significant difference among them (*P* = 0.672) (Supporting Table S1).

COMBINATION OF TUMOR MARKER AND SERUM CORD ASSAY OF METHYLATED SEPT9

We set 20 ng/mL and 4.6 copies as cutoff values for AFP and methylated SEPT9, respectively. The positive rates for both methods, AFP alone, and methylated SEPT9 alone were 27.2% (37 of 136), 11.0% (15 of 136), and 36.0% (49 of 136), respectively (Fig. 4A). The combination of AFP and our assay resulted in 74.3% sensitivity (101 of 136). In HCC patients with a positive methylated SEPT9 alone (49 patients), BCLC stages 0 and A, the

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**FIG. 3.** Correlation between HCC stage according to the BCLC staging system and serum methylated SEPT9. (A) The median copy number was 2.1, 5.6, 6.6, 9.2, and 53.7 in stage 0, A, B, C, and D, respectively. (B) The positive rate of serum methylated SEPT9 (cutoff value = 4.6 copies) increased from 41.7% to 100% with HCC progression.
so-called very early and early stages, accounted for 57.1% (28 of 49) in 4 and 24 patients, respectively, whereas 11, 9, and 1 patients were in HCC stage B, C and D, respectively (Fig. 4B). Therefore, adding the serum CORD assay for methylated \textit{SEPT9} enabled us to detect HCC patients, including those with very early and early HCC stages.

**METHYLATED \textit{SEPT9} ASSAY OF TISSUE SAMPLES**

Supporting Table S2 summarizes clinical characteristics for 35 patients with surgically resected HCC. Etiology of HCC was HBV, HCV, and NBNC in 3 (8.6%), 16 (45.7%), and 16 (45.7%) patients, respectively. HCC stage was 0, A, B, and C in 2 (5.7%), 18 (51.4%), 7 (20.0%), and 8 (22.9%) patients, respectively. Histological findings revealed well differentiated HCC, moderately differentiated HCC, poorly differentiated HCC, undifferentiated carcinoma, and combined hepatocellular and cholangiocarcinoma in 6 (17.1%), 23 (65.7%), 3 (8.6%), 1 (2.9%), and 2 (5.7%) patients, respectively. The median copy number of methylated \textit{SEPT9} was 34 (10.85-51.5) in nontumor tissues (n = 34), and 2,360 (130-5,860) in tumor tissues (n = 35) (Fig. 5A,B). The median copy number of serum methylated \textit{SEPT9} (n = 35) was 6.2 (2.6-10.0), which was significantly lower than that in tumor tissues ($P < 0.001$) (Fig. 5C). We subjected 34 paired nontumor and tumor tissue samples to the CORD assay. Nearly all tumor tissues had a significantly higher copy number of methylated \textit{SEPT9} than nontumor tissues ($P < 0.001$) (Supporting Fig. S2).

**Discussion**

In the present study, we showed that a novel liquid biopsy test targeting methylated \textit{SEPT9} is useful for diagnosing HCC. ctDNA is one of the targets in liquid biopsies and carries information on tumor-specific genetic or epigenetic alterations. DNA methylation is one of these epigenetic alterations, and several studies have investigated ctDNA methylation in various genes.$^{(9-11)}$ In this study, we focused on \textit{SEPT9} methylation. \textit{SEPT9} acts as either an oncogene or a tumor-suppressor gene.$^{(22)}$ \textit{SEPT9} overexpression occurs in human liver tumors.$^{(23)}$
and rat hepatocarcinogenesis, whereas SEPT9 hypermethylation is associated with liver carcinogenesis and colorectal carcinogenesis. Epi proColon, which targets methylated SEPT9, was the first liquid biopsy test to be approved by the US FDA for colorectal cancer screening and has been reported to be useful for diagnosing HCC. As an advantage over the Epi proColon assay, the CORD assay requires only a small amount of serum (0.4 mL), because it circumvents bisulfite treatment. Furthermore, the CORD assay is easier to perform, and the methylation level can be evaluated quantitatively (Supporting Table S3). Therefore, we believe the CORD assay is useful for liquid biopsy targeting methylated genes.

The CORD assay uses methylation-sensitive restriction enzymes and ddPCR. Although there are a few reports on the use of methylation-sensitive restriction enzymes or ddPCR for methylation assays, this is the first study to combine both methods for liquid biopsy for HCC. In addition, because a single methylation-sensitive restriction enzyme may cause incomplete digestion of nonmethylated DNA, which may lead to false PCR amplification, we used three methylation-sensitive restriction enzymes so that nonmethylated DNA would be completely digested.

The serum methylated SEPT9 CORD assay revealed significantly higher copy numbers in the HCC group than in the healthy control and CLD groups and displayed a sensitivity and specificity of 63.2% and 90.0%, respectively, for diagnosing HCC. Epi proColon has a sensitivity and specificity for HCC of 88.7% and 89.8%, respectively, based on a cutoff value of at least two positive triplicates. The proportions of tumor stages differed between our study and this previous Epi proColon report: BCLC stages 0 and A accounted for 45.6% (62 of 136) in our study and for 32.3% (31 of 96) in the previous report. Therefore, we cannot directly compare the clinical performance of our assay with that of Epi proColon 2.0 CE. Furthermore, because Epi proColon is currently not available in Japan, further investigations will be required to compare the clinical performance of the two assays.

We also determined methylated SEPT9 levels in tissue samples. The median copy number was 2,360 in the tumor tissues and 34 in adjacent nontumor tissues. This result was consistent with findings in an earlier report. Because the SEPT9 methylation level was significantly different between tumor tissues and adjacent nontumor tissues, we assume that methylated SEPT9 detected in the serum may be derived primarily from tumor tissue. However, because low levels of methylated SEPT9 were found in adjacent nontumor
tissues, serum methylated SEPT9 may be partly derived from nontumor hepatic cells associated with CLD.

AFP is the most commonly used surveillance marker for HCC, and the American Association for the Study of Liver Diseases (AASLD) recommends semiannual HCC surveillance using ultrasound imaging with or without AFP assay.(3) We also studied the combination of AFP and methylated SEPT9 assays for HCC diagnosis. We used 20 ng/mL as a cutoff value of AFP, as suggested by the AASLD for HCC screening.(3) AFP was detected in 39.0% (52 of 136) of patients with HCC, and the assay enabled detecting HCC patients with BCLC stage 0 or A in 8.1% of cases (11 of 136). In contrast, methylated SEPT9 was detected in 63.2% (86 of 136) of patients, and the assay enabled detecting HCC of BCLC stage 0 or A in 25.0% (34 of 136) of patients. In particular, the positive rate of methylated SEPT9 alone was 36.0% (49 of 136), and we could diagnose 28 out of 49 patients based on positive methylated SEPT9 alone (57.1%) as having BCLC stage 0 or A. Therefore, the combination of both assays improved the detection of early HCC over the AFP assay alone. The sensitivity of the AFP assay for HCC diagnosis is low, and our assay can complement this weakness. In addition, there was no significant difference in the positive rate of methylated SEPT9 among patients with NBNC-HCC and viral-related HCC \((P = 0.672;\) Supporting Table S1), indicating the same clinical performance of our assay in patients with NBNC-HCC, including nonalcoholic fatty liver disease–related HCC. Therefore, our assay may be useful for NBNC-HCC screening for which adequate surveillance systems are currently lacking.\(^3-6\) However, because the sample size was small, further validation in a large population will be required to draw a conclusion.

It has been recently reported that methylated DNA marker panel testing is an effective liquid biopsy to diagnose HCC in an early stage.\(^31,32\) The panel developed by Xu et al.\(^31\) had a sensitivity of 83.3% to 85.7% and a specificity of 90.5% to 94.3%, whereas the panel developed by Kisiel et al.\(^32\) had a sensitivity of 95% and a specificity of 92%. Both panel tests display high sensitivity and specificity when compared with our assay. However, these panels appear expensive because multiple markers are used, and more than 1 mL of plasma is required. Therefore, we expect that these panels will not be commonly used for HCC screening.

There are several limitations to the present study. First, patients were collected from a few centers, and the cohort size was limited. Therefore, further investigations and a validation study in a large population size are necessary. Second, SEPT9 is not a specific methylation biomarker for HCC,\(^25\) and hypermethylation of SEPT9 is associated with various cancers, including colorectal cancer, head and neck squamous cell carcinoma, and breast cancer.\(^22,33\) Thus, an increase in serum methylated SEPT9 may suggest the presence of other types of cancer. We have reported that serum methylated SEPT9 is significantly increased in patients with advanced colorectal adenoma and cancer.\(^17\) In the current study, colonoscopy was performed in all of the controls but not in all patients with CLD and with HCC, because it is difficult to perform a colonoscopy in patients who have no symptoms in relation to bowel movement. We suggest that colonoscopy should be indicated for patients with CLD who have elevated methylated SEPT9 levels, to rule out colorectal neoplasms.

In conclusion, the sensitive methylated SEPT9 assay has potential as a liquid biopsy for diagnosing HCC.

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Supporting Information

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