Detection of methylated septin 9 DNA in blood for diagnosis, prognosis, and surveillance of colorectal cancer

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KEY MESSAGES

1. The sensitivity of methylated septin9 (mSEPT9) in blood was significantly higher than carcinoembryonic antigen (CEA) in detecting colorectal cancer (CRC) [73.9% vs 48.2%, P<0.001]. However, both were not sensitive enough for detecting colorectal adenoma (<28%).
2. In patients with colorectal cancer, increased number of positive mSEPT9 PCR reactions in plasma samples after surgery was associated with higher rates of mortality (26.3% vs 4.2%, P<0.01), recurrence (47.4% vs 14.1%, P<0.01), and metastasis (36.8% vs 8.5%, P<0.01).
3. In patients with colorectal cancer, the proportion of those with negative CEA was higher than that of those with negative mSEPT9 at 6 months (71.8% vs 55.3%, P=0.035) and 12 months (68.1% vs 48.1%, P=0.028) after surgery.

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Introduction

In Hong Kong, colorectal cancer (CRC) has surpassed lung cancer to become the most common cancer.[1] Colonoscopy is the most direct method to detect colorectal neoplasm, but it has potential risk and discomfort. Non-invasive blood test is simpler and have higher compliance, but there is no reliable blood biomarker for screening of CRC.

Aberrant methylation is a regulatory mechanism of gene expressions commonly found in tumour suppressor genes of cancers, including CRC. Various epigenetic biomarkers have been identified for diagnosis and prognosis of CRC. Methylated septin9 (mSEPT9) has high sensitivity for serological diagnosis of CRC. Detection of mSEPT9 DNA in blood has been approved by the Food and Drug Administration of the United States as a non-invasive screening test for CRC. However, no study has investigated the role of mSEPT9 on monitoring CRC patients after curative resection, particularly in comparison with carcinoembryonic antigen (CEA). The American Society of Clinical Oncology recommends that CEA be measured every 3 months for at least 3 years after surgery in patients with stage II or III CRC.

This prospective study aimed to (1) compare the diagnostic accuracy of mSEPT9 DNA in the plasma and CEA among patients with different stages of colorectal neoplasm, and (2) compare mSEPT9 with CEA in monitoring CRC patients who had undergone surgical resection of tumour.

Methods

This prospective study was approved by the Institutional Review Board of the Hospital Authority Hong Kong West Cluster and University of Hong Kong (UW 12-489). Informed consent was obtained from all patients. We prospectively enrolled symptomatic patients who were referred to have colonoscopy for bowel symptoms or diagnostic workup for iron deficiency anaemia as well as patients undergoing screening colonoscopy. We excluded patients with previous bowel resection, familial CRC syndrome, inflammatory bowel disease, or diagnosis of any other malignancy in the past.

During colonoscopy, all polyps were removed for histological examination and lesions suspicious of CRC were biopsied. Histological samples were reviewed by experienced pathologists and classified into (1) adenocarcinoma, (2) advanced adenoma, (3) non-advanced adenoma, or (4) normal colonoscopy without any polyp or adenoma. Advanced adenoma was defined as lesion with a diameter of ≥10 mm, with villous histology or the presence of high-grade dysplasia.

We prospectively recruited patients who were newly diagnosed as having adenocarcinoma of the colon or rectum and scheduled for curative resection. Blood sample was taken immediately before surgery (baseline) and after surgery at 3-month intervals for up to 2 years. We excluded patients who had received preoperative chemotherapy or radiotherapy or patients with other non-colonic malignancy. Tumour

staging was classified according to the seventh edition of the American Joint Committee on Cancer TNM Classification. Clinical relapse or recurrence was determined by history, physical examination, and relevant investigation findings. Imaging (including computed tomography or positron emission tomography–computed tomography) was arranged to confirm the presence of distant or regional recurrence. Surveillance colonoscopy after surgical resection was performed according to current recommendation.

Plasma samples were blinded to laboratory staff. mSEPT9 was determined by the Epi proColon 2.0 assay (Epigenomics AG, Berlin, Germany). Samples were analysed using real-time PCR in triplicate, and the mSEPT9 assay was considered positive when more than one PCR reactions was positive.

CEA levels were determined by enzyme-linked immunoassay. Abnormal or positive CEA level was defined as >3 ng/mL. Increased CEA level was defined as any increase in subsequent follow-up (compared with baseline) and was >3 ng/mL.

The sensitivity and specificity of mSEPT9 and CEA were computed with the 95% confidence intervals. Chi-squared test or Fisher Exact test was used to compare categorical data. All statistical analyses were two-sided and a statistically significant difference was set at P<0.05. All analyses were performed by the SPSS (Windows version 21; IBM Corp, Armonk [NY], US).

Results

Of 282 patients included (62.8% male; mean age, 66.1±11.5 years), 117 had confirmed CRC, 45 had advanced adenoma, 50 had non-advanced adenoma, and 70 had normal colonoscopy. Among the 117 confirmed CRC patients, 98 had serial blood taken before and after surgical resection.

The sensitivity of mSEPT9 and CEA for different diagnoses and tumour stages is shown in Fig 1. In patients with confirmed CRC, the overall positive rate was 73.9% (95% CI=65.8%-82.0%) for mSEPT9 and 48.2% (95% CI=39.1%-57.3%) for CEA (P<0.001). The detection rate increased with higher tumour staging for mSEPT9 (P=0.003) and CEA (P=0.033). The positive rate increased from 52.6% (stage I cancer) to 100% (stage IV cancer) for mSEPT9 and from 26.3% to 100% for CEA. The positive rate differed significantly between mSEPT9 and CEA in stage II (P=0.001) and stage III (P=0.004) cancers but not in stage I and IV cancers. However, the sensitivity of both mSEPT9 and CEA was low (<28%) in advanced and non-advanced adenoma, with no significant difference in positive rate. In colonoscopy-negative subjects, the overall specificity of mSEPT9 and CEA was comparable (72.5% vs 79.3%, P=0.412).

After surgery, the proportion of patients with negative CEA was higher than the proportion of patients with negative mSEPT9 at 6 months (71.8% vs 55.3%, P=0.035), 12 months (68.1% vs 48.1%, P=0.028), 18 months (67.9% vs 53.1%, P=0.18), and 24 months (65.9% vs 30.0%, P=0.07).

Among cancer patients with positive mSEPT9 or CEA at baseline, 46.8% and 46.7% (P=1.0) turned negative at 6 months after surgery, respectively, whereas 47.2% and 39.3% (P=0.62) turned negative at 12 months after surgery, respectively. For patients with non-advanced cancer (stage I/II), 79.2% and 55.6% (P=0.013) had negative CEA and mSEPT9 at 6 months, respectively. For patients with advanced cancer stage (stage III/IV), 68.0% and 52.0% (P=0.39) had negative CEA and mSEPT9 at 6 months, respectively. For patients with no clinical recurrence, mSEPT9 or CEA was negative at 6 months (55.3% vs 72.9%, P=0.038), 12 months (49.0% vs 69.7%, P=0.033), 18 months (59.3% vs 65.9%, P=0.316), and 24 months (30.0% vs 65.9%, P=0.07), respectively.

Most patients had a decline in the number of positive mSEPT9 reactions after surgery (Fig 2). An increased number of positive reactions was associated with higher rates of mortality (26.3% vs 4.2%, P<0.01), recurrence (47.4% vs 14.1%, P<0.01), and metastasis (36.8% vs 8.5%, P<0.01).

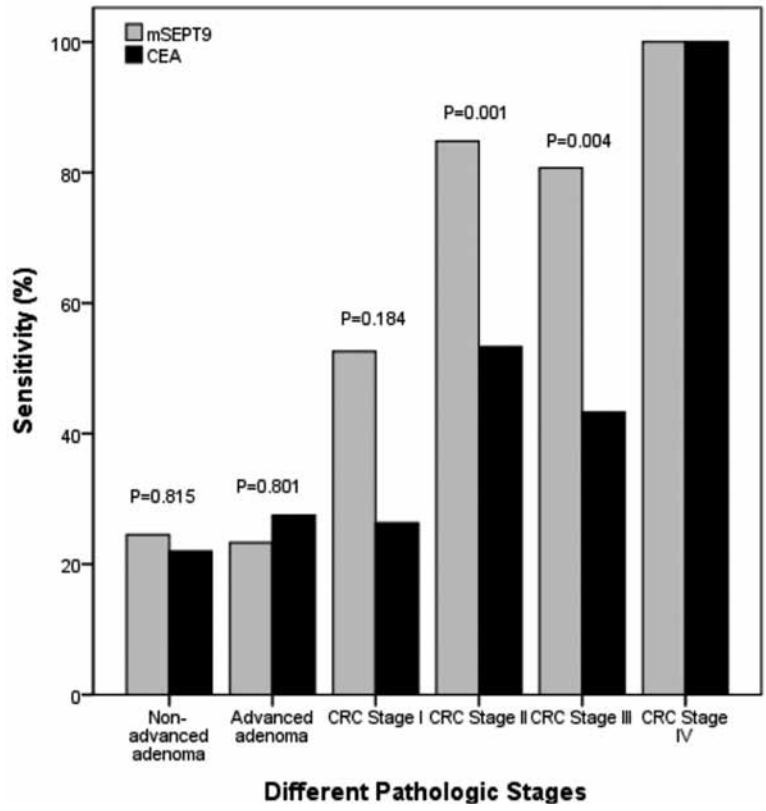

FIG 1. Sensitivity of methylated septin9 (mSEPT9) and carcinoembryonic antigen (CEA) for different diagnoses and tumour stages.

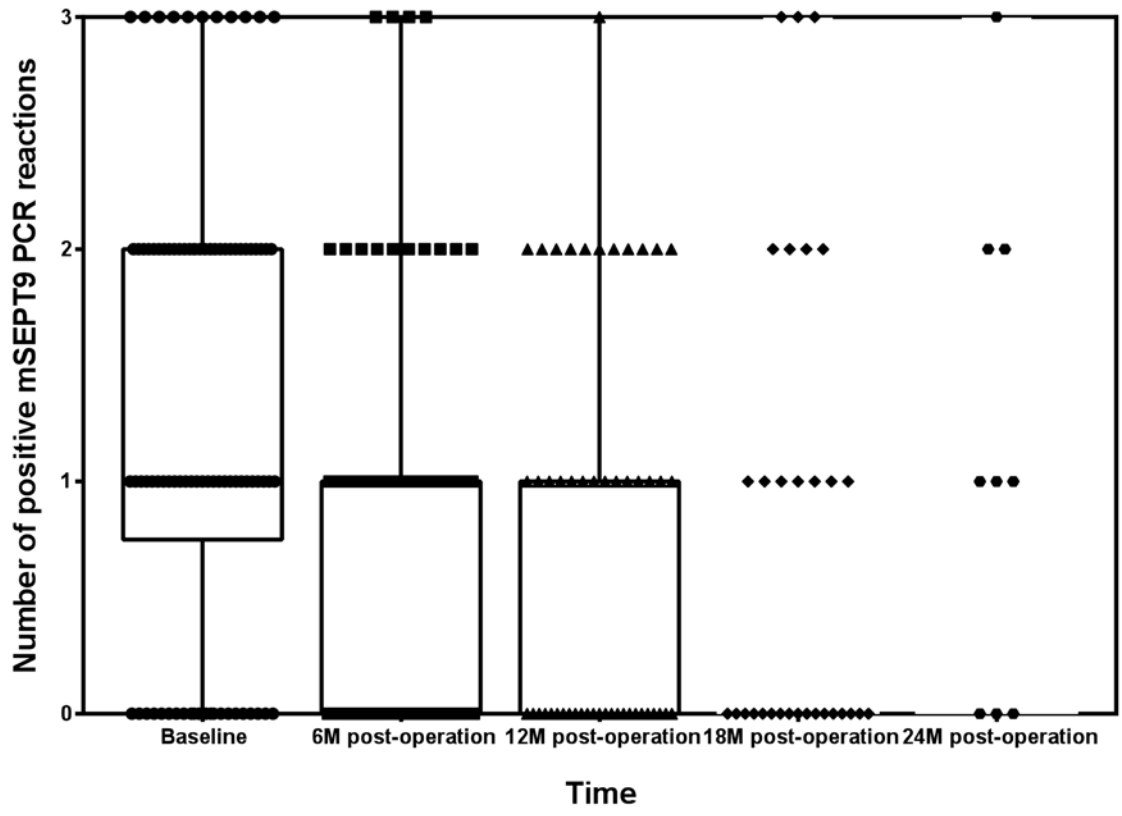

FIG 2. Distribution of number of positive methylated septin9 (mSEPT9) reactions at baseline and postoperation.

Conclusion

Sensitivity was higher for mSEPT than CEA in diagnosing CRC; sensitivity of mSEPT9 increased with higher tumour staging. An increased number of positive mSEPT9 PCR reactions in blood samples was associated with higher rates of recurrence, metastases, and mortality. After surgery, the negative rate was lower for mSEPT9 than CEA. As both mSEPT9 and CEA decline very slowly after surgery, further studies are needed to identify a more sensitive way to detect subtle changes in mSEPT9 concentration in blood for detection of early CRC recurrence.

Acknowledgements

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