The Role of Circulating Tumour Cells and Carcinoembryonic Antigen as Diagnostic Tool for Metastatic Colorectal Cancer in Indonesia

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ABSTRACT

Background: *Patients with metastatic colorectal cancer (CRC) have a poor prognosis, with a 5-year survival rate of only 14%. Early detection and early intervention may improve outcome/. Both circulating tumour cells (CTC) and serum carcinoembryonic antigen (CEA) have been suggested as diagnostic biomarkers for metastatic CRC. This study explored the performance of CTC and CEA as tools for the detection of metastatic colorectal cancer in Indonesia.*

Methods: *This study was conducted from December 2024 to April 2025 on metastatic colorectal cancer patients from three hospitals in Jakarta. CTC was analysed using the gradient density method and flow cytometry. CEA was analysed using chemiluminescent microparticle immunoassay.*

Results: *This study recruited 160 patients with colorectal cancer patients of whom 45% were known to have metastatic colorectal cancer. The median age was 57 (47—66) years and analysis was done at one time point only. The area under the curve (AUC) for CTC, CEA, and the combination of both CTC and CEA in diagnosing metastatic colorectal cancer was 0.579, 0.811, and 0.703, respectively. CTC showed 56.94% sensitivity and 50.00% specificity in detecting metastatic colorectal cancer. Meanwhile, CEA showed higher sensitivity (72.22%) and specificity (72.72%). Combination of both CTC and CEA increased sensitivity to 91.67%, but with a lower specificity of 37.50%. The optimal cut-off for CTC and CEA were 34.5 cells/3 ml and 18.31 ng/ml, respectively.*

Conclusion: *CEA showed a better performance than CTC in diagnosing metastatic colorectal cancer. Combination of CTC and CEA showed promising potential as a valuable surveillance tool for detecting metastasis in colorectal cancers, but not as a primary diagnostic tool.*

Keywords: *Carcinoembryonic Antigen, Cancer Surveillance, Circulating Tumour Cell, Metastatic Colorectal Cancer*

ABSTRAK

Latar Belakang: *Pasien kanker kolorektal (KKR) metastatik memiliki prognosis yang buruk dengan angka ketahanan hidup 5-tahun 14%, menunjukkan pentingnya deteksi dan tatalaksaana secara dini. Circulating tumour cells (CTC) dan carcinoembryonic antigen (CEA) telah diusulkan sebagai biomarker diagnostik untuk KKR metastatik. Studi ini mengeksplorasi performa CTC dan CEA sebagai alat diagnostik pada KKR metastatik di Indonesia.*

Metode: *Studi ini dilaksanakan dari Desember 2024 hingga April 2025 pada pasien KKR metastatik dari tiga rumah sakit di Jakarta. CTC dianalisa menggunakan metode densitas gradien dan flow cytometry. CEA dianalisa menggunakan chemiluminescent microparticle immunoassay.*

Hasil: *Kami merekruit 160 pasien KKR dengan 45% diantaranya memiliki KKR metastatik. Usia median adalah 57 (47—66) tahun. Area under the curve (AUC) untuk CTC, CEA, dan kombinasi CTC dengan CEA untuk mendiagnosis KKR metastatik secara berurutan adalah 0,579, 0,811, dan 0,703. CTC memiliki sensitivitas 56,94% dan spesifisitas 50,00% dalam mendeteksi KKR metastatik. CEA menunjukkan sensitivitas (72,22%) dan spesifistas (72,72%) yang lebih tinggi. Kombinasi CTC dengan CEA meningkatkan sensitivitas ke 91,67%, namun memiliki spesifisitas yang lebih rendah, yaitu 37,50%. Cut-off optimal untuk CTC dan CEA secara berurutan adalah 34,5 sel/3 ml dan 18,31 ng/ml.*

Kesimpulan: *CEA menunjukkan performa yang lebih baik dibandingkan CTC dalam mendiagnosis KKR metastatik. Kombinasi CTC dengan CEA menunjukkan potensi yang menjanjikan sebagai alat skrining untuk membedakan KKR metastatik dari nonmetastatik, namun tidak sebagai alat diagnostik.*

Kata Kunci: *Antigen Karsinoembrionik, Pengawasan Kanker, Sel Tumor yang Beredar, Kanker Kolorektal Metastatik.*

INTRODUCTION

Colorectal cancer (CRC) is a concerning public health problem that has received much attention due to its high incidence and mortality rate. According to the Global Cancer Observatory (GLOBOCAN) 2020, CRC had the third highest worldwide incidence of all cancers with 1,931,590 new cases diagnosed each year (10% of the total cancer incidence). It was the second most common cause of cancer-related death with , 935,173 recorded deaths (9.4% of the total).[1, 2] Epidemiological data in 2018 in Asia showed that China had the greatest 5-year prevalence and mortality from CRC, followed by Japan, India, Indonesia, Thailand, and Philippines. The incidence of CRC in increasing in Asia and this has been attributed to lifestyle changes, smoking, alcohol consumption, physical inactivity, obesity, diabetes, and rising elderly populations.[3] Of particular concern is the unexplained increasing incidence of early onset CRC (i.e. occurring before the age of 50). In Southeast Asia, Indonesia ranks fourth in the incidence of CRC (with 17.2 per 100,000 population) and it has the third highest mortality rate(reaching 12.9 per 100,000 population).[5]

Colorectal cancer is one of the most lethal cancers, with 90% 5-year survival rate for localized disease and 14% in metastatic disease.[4] Detecting the disease at an early stage is therefore essential to obtaining the best outcome. Similarly, early detection of recurrence could improve outcome. The National Comprehensive Cancer Network (NCCN) recommended carcinoembryonic antigen (CEA) and CT-imaging be used to monitor for recurrence and metastasis. However, even with advanced technology, imaging has limitations in detecting small primary tumors or metastases. Additionally, repeated imaging exposes patients to potentially harmful radiation and it is costly and therefor may not be affordable in some countries. There is thus a need for cheap and robust methods for surveillance in CRC. One of the emerging modalities for detection of metastatic CRC is circulating tumour cells (CTC) in blood-based liquid biopsies. The CTCs are cells that enter the bloodstream and which may originate from either primary tumour or metastatic deposits. It follows that liquid biopsy may be used to identify CTCs in peripheral blood. Several studies have explored the use of CTCs in determining the CRC recurrence and metastasis, with a reported sensitivity and specificity of more than 70% (ref). However, the cut-off for CTC remains unclear.

The CEA tumour marker in peripheral blood is widely used to assess disease progression, metastasis, and recurrence after resection. Both NCCN and the American Society of Clinical Oncology (ASCO) have recommended CEA as a reliable tumour marker. Serum CEA levels serve as an important prognostic factor, indicators of therapy efficacy, and increasing levels of serum CEA may be the first indication of recurrence in CRC patients. The utility of serum CEA monitoring is limited due to relatively poor sensitivity, specificity, and accuracy.[8] However, Chiu et al found that the combination of CTC and CEA showed better performance than CEA alone, suggesting its potential as a tool for detecting metastatic CRC.[9] Studies that explored the potential of using both CTC and CEA in the setting of metastatic CRC, especially in Indonesia, are still limited. Thus, this study aimed to explore the use of CTC and CEA as a diagnostic tool for metastatic colorectal cancer patients in Indonesia.

METHODS

Study Design and Participants

This was a cross-sectional study conducted to investigate the use of CTC and CEA as tools for detecting metastatic CRC. Patients with CRC were consecutively recruited from three hospitals in Jakarta (Dr. Cipto Mangunkusumo Hospital, Dharmais Cancer Hospital, and Fatmawati Hospital) from December 2024 to April 2025. A minimum sample of 158 subjects was required, with type I error set at 5% and absolute precision of 10%. All patients who were over 18 years old and had a biopsy-confirmed diagnosis of CRC were eligible for includsionin this study. Exclusion criteria were (i) patients that have already undergone therapy,(ii) positive history for other malignancies, (iii) active smokers, (iv) did not sign the informed consent for participating in this study. All patients underwent full standard clinical work-up including CT-imaging of chest, abdomen and pelvis. Demographics and clinical data, such as age, gender, body mass index (BMI), comorbidities, tumor location, stage, tumor cell differentiation, and histopathology results were collected. Based on the available clinicopathological data, two cohorts were collected: (i) Metastatic CRC (i.e. those with CT-imaging confirmed stage IV disease, n=72) and (ii) Non-metastatic (i.e. those with no evidence of metastatic disease after full clinical work-up and imaging, n=88) . Ethical approval for the study was received from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo Hospital (KET-1666/UN2.F1/ETIK/PPM.00.02/2024).

Measurement of CTC Levels

A total of 3 mL of whole blood was collected from the peripheral veins of each participant into a heparinised tube to prevent coagulation. Within 24 hours of collection, the samples were transported to the laboratory in the Human Cancer Research Center (HCRC), Indonesia Medical Education and Research Institute (IMERI) - Faculty of Medicine Universitas Indonesia. The blood samples were diluted with phosphate-buffered saline (PBS) in a ratio of 1:1 and then transferred into a SepMate tube containing 4 ml of lymphoprep solution. During this step, the blood was added carefully to ensure that the lymphoprep solution was not disturbed. The the sample was then centrifuged at a speed of 1200 x g for 10 minutes at room temperature with the brake off centrifugation setting. Following centrifugation,3 layers were formed. The upper part (plasma) and the buffy coat were transferred into a new tube using a transfer pipette, taking care not to disturb the lymphoprep. Next, the sample was centrifuged again at 500 x g for 6 minutes. After discarding the supernatant, the pellet was gently resuspened with 1 mL PBS, then centrifuged at a speed of 500 x g for 6 minutes. The supernatant was discarded again and the pellet resuspended in 250 µL of Cytofix/Cytoperm Buffer (BD, USA). The cells were incubated at 4 °C for 20 minutes or left overnight. After incubation, the cells were washed with 1 mL Perm Wash Buffer 1x (BD, USA) and centrifuged at 500 x g for 6 minutes. The cells were then resuspended with 200 µL of Perm Wash Buffer 1x (BD, USA). The sample was split equally into two 5 ml Falcon tubes (BD, USA) and labeled as either "unstained" for control and "stained" for experimental sample. Dye conjugated antibodies were used for the detection of colonic epithelium (CK-20-PE, Santa Cruz, USA), circulating tumor cells marker (Anti-Plastin A3-FITC, Santa Cruz, USA) and lymphocytes (CD45-PerCP-Cy.5.5, BD, USA). One µL was added into the "stained" and then incubated at 4 °C for 20 minutes in the dark. The cells were then read by the flow cytometry device (BD FACSCanto) with a minimal gated event of 1,000,000 cells or until the cell volume in the tube was completely depleted, with a medium flow rate of 60 µL/minute. Using the software (BD FACSDiva), data indicating the percentage and number of cells (cell count) stained by each antibody were collected. Voltage adjustment is first carried out using an unstained control to bring all cells on scale, followed by single-stained controls to optimize the separation of negative and positive populations.

Measurement of CEA Level

Serum CEA was obtained through centrifugation at 1200-2000 g for 15 minutes. CEA was measured using the chemiluminescent microparticle immunoassay (CMIA) method performed using the ARCHITECT i2000SR immunoassay analyzer (Abott, USA). Chemiluminescence was measured in relative light units (RLUs) (Architect System). The assay results were presented as a ratio of the specimen signal to the cut-off value (S/CO). Results were considered negative if the S/CO value was < 0.8, unclear if ≥ 0.8 to < 1, and positive if ≥ 1.

Statistical Analysis

Quantitative variables were expressed in mean ± standard deviation when normally distributed, or in median with interquartile range (IQR) when not normally distributed. Categorical variables were summarized using frequencies and percentages. The area under the curve (AUC) and cut-off points for CTC and CEA were calculated based on the receiver operating characteristic (ROC) curve. Using the CTC and CEA cut-off points, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) were obtained. We also calculated the AUC, sensitivity, and specificity for the combination of CTC and CEA in diagnosing metastatic CRC. P value < 0.05 was used to indicate statistical significance. Statistical analyses were performed using SPSS 25.

RESULTS

Clinical Characteristics

We enrolled 166 participants in this study. Out of 166 participants, six were excluded due to not signing the informed consent form, leading to a final total of 160 patients included in this cross-sectional study. The complete participant characteristics can be viewed in **Table 1 and 2**. The median age of the subjects was 57 (47—66) years, and 55.6% were male. Almost half of them were considered normoweight (46.9%) based on their BMI and dominated by patients without significant comorbidity. However, nearly half of them were past smokers (42.5%). Positive family history for cancer was observed in a small part of our subjects (9.4% for CRCs, 23.8% for other malignancies). Most of the CRCs were left-sided (88.8%) and stage IV (45%). The histopathology results showed that most of the CRCs were well-differentiated (76.2%), with adenocarcinoma being the most common pathology (98.2%).

Compared to the non-metastatic group, patients with metastatic disease in our study were generally older (60 vs. 55 years), more likely to be male (62.5% vs. 50.0%), and more likely to present with left-sided tumors (91.7% vs. 86.4%) and poorly differentiated histology (13.9% vs. 9.1%). Hypertension was also more common in the metastatic group (29.2% vs. 23.9%) while diabetes was more common in the non-metastatic group (20.5% vs. 13.9%). The prevalence of those with risk factors, such as drinking alcohol or smoking, was also slightly higher in the metastatic group (drinking history: 22.2% vs. 18.2%; former smoker: 45.8% vs. 39.8%). Of note, family history of CRC was slightly higher in the non-metastatic group (11.4% vs. 6.9%).

Table 1. Clinicopathological Characteristics of Study Participants

Variable	Metastatic Colorectal Cancer (n=72)	Nonmetastatic Colorectal Cancer (n=88)	Total (n=160)
Age, median (IQR) years	60 (49.50 – 66.0)	55 (46.25 – 66)	57 (47 – 66)
Sex			
Male	45 (62.5)	44 (50.0)	89 (55.6)
Female	27 (37.5)	44 (50.0)	71 (44.4)
Tumor location, n (%)			
Left-sided tumor	66 (91.7)	76 (86.4)	142 (88.8)
Right-sided tumor	6 (8.3)	12 (13.6)	18 (11.2)
CRC stage, n (%)			
I	0 (0.0)	15 (17.0)	15 (9.4)
II	0 (0.0)	21 (23.9)	21 (13.1)
III	0 (0.0)	52 (59.1)	52 (32.5)
IV	72 (100.0)	0 (0.0)	72 (45.0)
Cancer differentiation, n (%)			
Well-differentiated	55 (76.4)	67 (76.1)	122 (76.2)
Moderately differentiated	7 (9.7)	13 (14.8)	20 (12.5)
Poorly differentiated	10 (13.9)	8 (9.1)	18 (11.3)
Histopathology result, n (%)			
Adenocarcinoma	70 (97.2)	87 (98.9)	157 (98.2)
Spindle cell carcinoma	0 (0.0)	1 (1.1)	1 (0.6)
Squamous cell carcinoma	1 (1.4)	0 (0.0)	1 (0.6)
Undifferentiated carcinoma	1 (1.4)	0 (0.0)	1 (0.6)

Table 2. Comorbidities and Lifestyle Factors of Study Participants

Variable	Metastatic Colorectal Cancer (n=72)	Nonmetastatic Colorectal Cancer (n=88)	Total (n=160)
Age, median (IQR) years	60 (49.50 – 66.0)	55 (46.25 – 66)	57 (47 – 66)
Sex			
Male	45 (62.5)	44 (50.0)	89 (55.6)
Female	27 (37.5)	44 (50.0)	71 (44.4)
Body mass index, mean (SD)	21.14 (3.88)	21.46 (3.79)	21.32 (3.81)
Normoweight	39 (54.2)	36 (40.9)	75 (46.9)
Underweight	16 (22.2)	23 (26.1)	39 (24.4)
Overweight	11 (15.2)	12 (13.6)	23 (14.4)
Obesity degree I	3 (4.2)	16 (18.2)	19 (11.9)
Obesity degree II	3 (4.2)	1 (1.2)	4 (2.4)
Diabetes mellitus, n (%)			
Yes	10 (13.9)	18 (20.5)	28 (17.5)
No	62 (86.1)	70 (79.5)	132 (82.5)
Hypertension, n (%)			
Yes	21 (29.2)	21 (23.9)	42 (26.2)
No	51 (70.8)	67 (76.1)	118 (73.8)
Smoking history, n (%)			
Former smoker	33 (45.8)	35 (39.8)	68 (42.5)
Never	39 (54.2)	53 (60.2)	92 (57.5)
Family history of CRC			
Yes	5 (6.9)	10 (11.4)	15 (9.4)
No.	67 (93.1)	78 (88.6)	145 (90.6)
Family history of other cancer			
Yes	21 (29.2)	17 (19.3)	38 (23.8)
No.	51 (70.8)	71 (80.7)	122 (76.2)
Drinking history, n (%)			
Yes	16 (22.2)	16 (18.2)	32 (20.0)
Never	56 (77.8)	72 (81.8)	128 (80.0)

CTC Diagnostic Value

The median CTC value for metastatic CRC was higher than non-metastatic CRC (42.5 vs. 34.5 cells/3mL, **Table 3**). Using ROC analysis, we acquired an AUC of 57.9% (95% CI 0.488—0.669; p=0.087) for CTC in differentiating between metastatic and nonmetastatic CRC with an optimal cut-off value of 34.5 cell/3mL. The sensitivity, specificity, PLR, NLR, PPV, and NPV were 56.94% (95% CI 44.73—68.57%), 50.00% (95% CI 39.15—60.85%), 1.14 (95% CI 0.85—1.52), 0.86 (95% CI 0.61—1.21), 48.24% (95% CI 41.09—55.46%), and 58.67% (95% CI 50.31—66.56%), respectively **(Figure 1-2 and Table 4).**

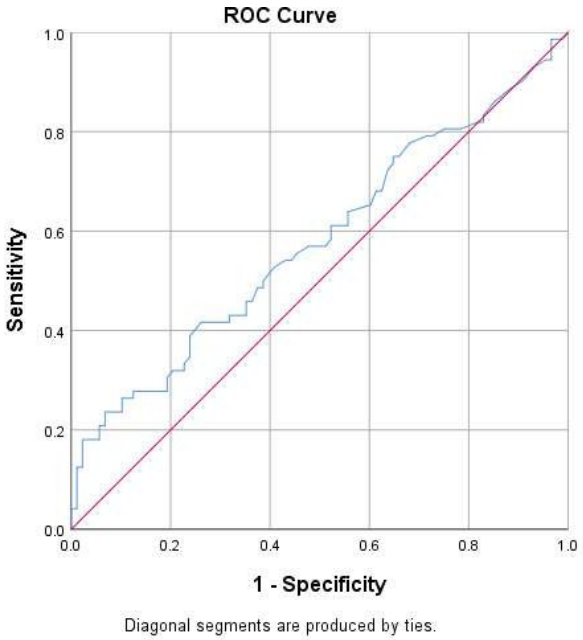

Figure 1. ROC Curve for CTC in Diagnosing Metastatic Colorectal Cancer

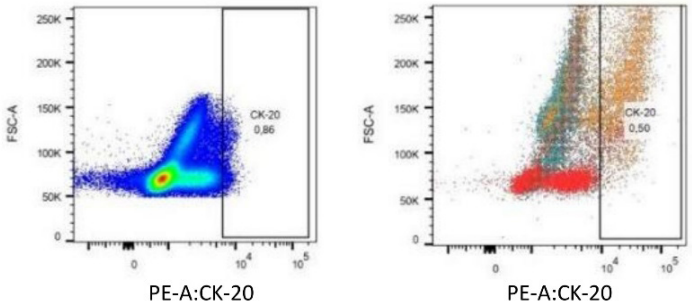

Figure 2. Scatter Plot of Flowcytometry for CTC

Table 3. Median Value for CTC and CEA

Variable	Metastatic CRC (n=72)	Non-Metastatic CRC (n=88)	Total (n=160)
Median CTC (95% CI)	42.5 (20.5 – 113.25)	34.5 (16.25 – 59.75)	37.5 (18.25 – 72.0)
Median CEA (95% CI)	86.35 (16.42 – 755.80)	5.4 (2.80 – 19.58)	16.45 (4.13 – 86.67)

CEA: Carcinoembryonic antigen; CI: Confidence interval; CRC: Colorectal cancer; CTC: Circulating tumour cells.

Table 4. Diagnostic Performance for CTC in Detecting Metastatic CRC.

CTC	Metastatic CRC (n = 72)	Non-Metastatic CRC (n = 88)	P Value
≥ 34.5 cells/3 mL	41	44	0.474
< 34.5 cells/ 3 mL	31	44	

CRC: Colorectal Cancer; CTC: Circulating Tumour Cells

CEA Diagnostic Value

The median CEA value for metastatic CRC was higher than non-metastatic CRC (86.35 vs. 5.4 ng/mL, **Table 3**). The AUC for CEA was 81.1% (95% CI 0.742—0.879; p<0,0001) with cut-off value 18.31 ng/mL for differentiating between metastatic and nonmetastatic CRC. The sensitivity, specificity, PLR, NLR, PPV, and NPV were 72.22% (95% CI 60.41—82.14%), 72.73% (95% CI 62.19—81.68%), 2.65 (95% CI 1.83—3.83), 0.38 (95% CI 0.26—0.57), 76.19% (95% CI 68.34—82.59%), and 72.2% (95% CI 60.41—82.14%), respectively **(Figure 3 and Table 5).**

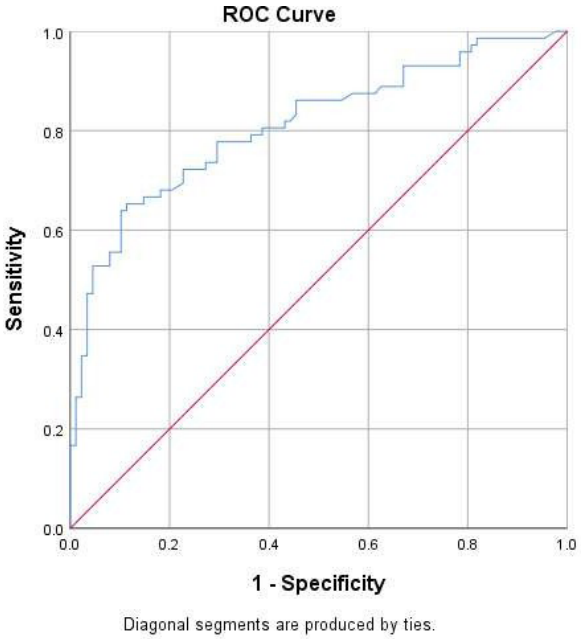

Figure 3. ROC Curve for CEA in Diagnosing Metastatic Colorectal Cancer

Table 5. Diagnostic Performance for CEA in Detecting Metastatic CRC

CEA	Metastatic CRC (n = 72)	Non-Metastatic CRC (n = 88)	P Value
≥ 18.31 ng/mL	52	24	< 0.0001
< 18.31 ng/mL	20	64	

CEA: Carcinoembryonic Antigen; CRC: Colorectal Cancer

CTC and CEA Combination Diagnostic Value

The combination of CTC and CEA for differentiating metastatic from nonmetastatic CRC yielded AUC of 70.3% (95% CI 0.622—0.784; p<0.0001). The cut-off used was based on previous results. The sensitivity, specificity, PLR, NLR, PPV, and NPV were 91.67% (95% CI 82.74—96.88%), 37.50% (95% CI 27.40—48.47%), 1.47 (95% CI 1.23—1.75), 0.22 (95% CI 0.10—0.50), 54.55% (95% CI 50.15—58.87%), and 84.62% (95% CI 70.94—92.53%), respectively **(Figure 4 and Table 6).**

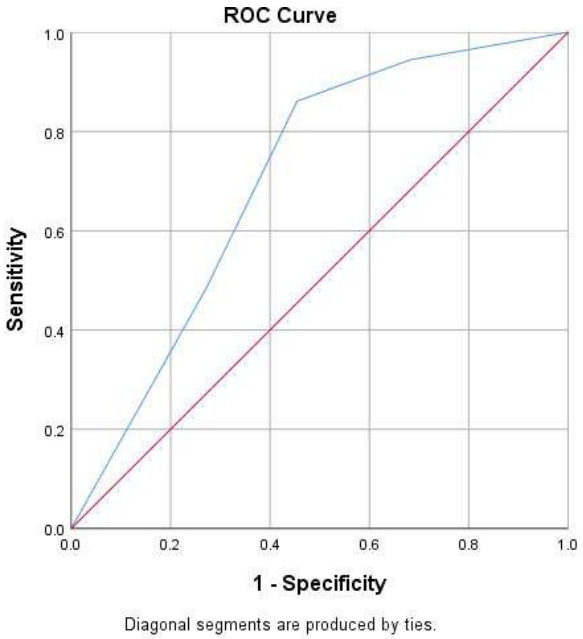

Figure 4. ROC Curve for Combination of CTC and CEA in Diagnosing Metastatic Colorectal Cancer

Table 6. Diagnostic Performance for CTC in Detecting Metastatic CRC

CTC and CEA	Metastatic CRC (n = 72)	Non-Metastatic CRC (n = 88)	P Value
Positive (either CTC or CEA or both above cut-off value)	66	55	< 0.0001
Negative (both CTC and CEA below cut-off value)	6	33	

CEA: Carcinoembryonic antigen; CRC: Colorectal cancer; CTC: Circulating tumour cells.

DISCUSSION

This is a cross-sectional case-control study which has explored the utility of CTC and CEA, both individually and in combination, in the detection of metastatic CRC. In our study, CTC counts alone showed poorer performance in diagnosing metastatic CRC compared to published studies. He et al. reported an AUC of 63.9% with 61.3% sensitivity and 61.9%.[10] Liu et al. reported even higher AUC (93.3%) and sensitivity (87.7%).[11] These differences are most likely attributable to the different methodologies used in the various studies. The most commonly used method was CellSearch, which utilizes EpCAM antibodies to detect CRC.[12] Another method, ISET (Isolation by

Size of Epithelial Tumor Cells), uses size differences to separate CTC from other blood cells[13] whilst real-time polymerase chain reaction (PCR) can be used to detect tumour epithelium gene expression to identify CTC-.[6] In contrast, taking into cognizance the reality only a low-cost solution would have any chance of adoption in Indonesia, our study used density gradient and flow cytometry. This is easier to perform and of lower cost than the more sophisticated methods but this method may produce lower sensitivity and specificity. Another confounding factor may be that other studies, such as Rahbari et al., sampled blood from the mesenteric vein and found found that CTC levels were higher when compared to the central vein Thus, evaluating CTC levels from the mesenteric vein may lead to improved CTC detection and diagnostic performance.[7] Since our long term vision is for a surveillance test for early detection of metastatic disease, the only realistic source is peripheral blood

CEA has been recommended as a biomarker in diagnosis, therapy evaluation, and monitoring for CRC recurrence. He et al. found the AUC of CEA in diagnosing metastatic CRC with a cut-off of 4.87 ng/ml was 76.1%, with a sensitivity and specificity of 74.2% and 64.3%, respectively. Similar to previous findings, our study found that CEA had an adequate performance in diagnosing metastatic CRC (sensitivity 72.22%, specificity 72.73%). Of note, the optimal cut-off value identified in our study was markedly higher than reported by He et al. (18.31 vs. 4.87 ng/ml). Several factors may account for this discrepancy. First, He et al. analyzed a substantially larger and more heterogeneous cohort of 617 patients, allowing more stable statistical modelling. In contrast, our study analyzed smaller sample sizes which could produce greater variability and shift the optimal cut-off upward. Secondly, the immunoassay platforms used in our study were not identical to those used by He et al., inter-assay differences in calibration, antibody specificity, and analytical sensitivity could also contribute to further variability in absolute CEA values.[10]

It has been reported that combining CEA with other biomarkers may yield a better diagnostic accuracy[14] Thus our study evaluated the performance of combining CTC and CEA in diagnosing metastatic CRC. The results of the ROC analysis showed an AUC of 70.3%, indicating moderate diagnostic ability. In contrast Luo et al. found higher AUC value of 0.924[15] and other studies have similarly reported increased sensitivity and specificity when combining CTC with CEA . However, in our study, the increase was not significant.[9, 16] In our study, the combination of CTC ≥ 34.5 cells/3 mL and CEA ≥ 18.31 ng/mL revealed 91.67% sensitivity and 37.5% specificity. It is encouraging that our study indicated that combining the low cost methods of CTC and CEA achieved excellent senstitivey in detecting metastatic CRC. The combination of these methods also showed low specificity which was mainly to due false positives identified by flow cytometry. There may be several reasons for this such as low specificity of the antibody or possibly phagocytosis of CK20 containing exosomes by leucocytes. The low specificity limits the utility of the test as a definitive diagnostic tool and it could probably be improved by altering the thresholds for a positive call. However, since our aim is to develop a method of early detection of metastatic CRC, we believe that it is acceptable to sacrifice specificity for sensitivity – the tests are simple to perform and can easily be repeated in order to establish a trend which in turn could be used to target resources to those most at risk of tumur recurrence..

Of note, the median age for CRC in our study was 57 years old which is considerably younger than those typically reported in Western populations. In the Unites States, data from a large registry-based analysis reported a median of 66 years.[17] Similarly, studies from France and Germany also reported a median age of 71 years and 72.2 years, respectively.[18, 19] This may reflect a broader international trend for earlier CRC onset that is potentially influenced by environmental and lifestyle factors, including obesity, increased consumption of red meat and processed meat, alcohol, smoking, and low physical activity.[20] Additionally, our study also reported substantively higher proportions of well-differentiated CRCs (76.2%) compared to Western populations (5-10%).[21-23] This was also similarly reported by another study in Indonesia by Rudiman et al with 52.8% of their cohort having well-differentiated histology.[24] This discrepancy might be explained by the younger age of our cohort in whom well-differentiated tumors are more common. Interobserver variation in histopathological grading may also contribute to this difference, especially for borderline cases.

One of the advantages of this study was the relatively large number of samples included. Larger sample sizes could help improve the statistical power, better reproducibility and validity, as well as a lower risk of bias. In addition, this study not only analyzed the potential of two individual liquid biopsy biomarkers independently, but also the combination of these two biomarkers. This study also has several limitations.

Firstly, it is a cross sectional study evaluating the biomarkers at a single time-point whereas a longitudinal study would be preferable in order to validate data through replication and to identify trends associated with metastatic disease. The density gradient method and flow cytometry was used for detecting CTCs. This technique is quite commonly used due to its easy methodology and relatively low costs but it needs optimizing to identify the best antibodies and sample preparation protocol. Even with full optimization, we suspect it may yield a lower sensitivity and specificity compared to other techniques such as PCR, immunomagnetic enrichment, and microfluidic-based isolation.. Lastly, this study determined metastatic CRC based on the histopathological and computed tomography (CT) scan results. Ideally, in addition to CT scan and histopathological examination, positron emission tomography - CT (PET-CT) should also be conducted given its higher sensitivity and specificity.

CONCLUSION

We concluded that CTC performs poorly in diagnosing metastatic CRC, while CEA performs well as a diagnostic tool in metastatic CRC. The combination of CTC with CEA has an excellent performance for the detection of metastatic CRC but is prone to false positive. Of note, the combination of CTC with CEA showed great potential as a surveillance tool for disease recurrence/metastasis in CRC compared to CTC or CEA alone. This could be improved with optimization of several of the parameters and newer more extensive studies are warranted to address the limitations of this study.

Conflict of Interest

All authors declare that there are no conflicts of interest.

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Author Contribution

All authors contributed in conceptualizing the study design and methodology. SA, MA, and IH performed formal analysis and investigation. AH, IR, AF, and CM participated in data curation and validation. All authors contributed in drafting and reviewing the manuscript.

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Data Availability

Any other data not included in the manuscript could be requested from the authors by contacting the corresponding author.

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