Assessment of Circulating Tumor Cells in Colorectal Cancer as an Adjunctive Non-invasive Diagnostic Method

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ABSTRACT

Background: *Colorectal cancer (CRC) is a significant contributor to cancer-related morbidity and mortality. Biopsy remains the gold standard for CRC diagnosis, but invasive testing may not be preferred as an initial diagnostic procedure. Therefore, alternative non-invasive approaches are needed. Circulating tumor cells (CTC) present in the bloodstream have great potential as a non-invasive diagnostic marker for CRC patients. This study aimed to assess the diagnostic potential of CTC in CRC as an adjunctive diagnostic method using a subjective manual identification method and laser capture microdissection at 40x magnification.* ***Methods:*** *A cross-sectional study was conducted on adult patients suspected to have CRC at Dr. Cipto Mangunkusumo National General Hospital, Jakarta, between November 2020 and March 2021. CTC analysis was performed using the negative selection immunomagnetic method with Easysep™ and the CD44 mesenchymal tumor marker. The identification and quantification of CTC were conducted manually and subjectively, with three repetitions of cell counting per field of view at 40x magnification.* ***Results:*** *Of 80 subjects, 77.5% were diagnosed with CRC, while 7.5% and 15% exhibited adenomatous polyps and inflammatory/hyperplastic polyps, respectively. The diagnostic analysis of CTC for detecting CRC (compared to polyps) using a CTC cutoff point of >1.5 cells/mL suggested sensitivity, specificity, and positive predictive value (PPV) of 50%, 88.89%, and 93.94%. Additionally, the negative predictive value (NPV), as well as the positive and negative likelihood ratio (PLR*

and NLR) were 34.04%, 4.5, and 0.56, respectively. The subjective manual identification and quantification of CTC were performed at 40x magnification using laser capture microdissection. ***Conclusion:*** *This study assessed the diagnostic potential of CTC examination in CRC as an adjunctive diagnostic method using the subjective manual identification method and laser capture microdissection at 40x magnification. Despite the limitations associated with subjective cell counting, the results showed 50% sensitivity and 88.89% specificity in diagnosing CRC. Further studies are needed to optimize the manual identification process and validate the clinical utility of CTC analysis in CRC patients.*

Keywords*: Circulating tumor cells; Colorectal cancer; Cancer; Adenoma; Diagnosis*

INTRODUCTION

Colorectal cancer (CRC) is a significant public health concern globally, contributing to a substantial morbidity and mortality rate.[1,2] Timely and accurate diagnosis is crucial for effective treatment and improved patient outcomes. The available diagnostic approaches primarily rely on the presence of CRC-related symptoms or abnormal screening results to initiate further investigations.[3–6] However, there is an increasing need for non-invasive and convenient methods to complement existing diagnostic strategies.[7–10]

Circulating Tumor Cells (CTC) have been reported as biomarkers with the potential to revolutionize cancer diagnosis and management, including CRC.[11–13] These tumor cells detach from the primary source or metastatic sites and enter the bloodstream.[14,15] CTC offers a unique opportunity for non-invasive assessment of tumor burden, molecular characterization, and monitoring treatment response.[16,17] Several studies showed their diagnostic value in various cancers, including CRC, providing insights into prognosis and treatment decision-making.[18–20]

Despite their tremendous potential, the use and standardization of CTC technologies in Indonesia remain in the nascent stage. Currently, there is a lack of established protocols and standardized approaches for CTC detection and characterization. This gap presents an opportunity to enhance the quality of investigations and explore the diagnostic potential in CRC patients.

This study aimed to assess the diagnostic potential of CTC in CRC using subjective manual identification and laser capture microdissection at 40x magnification. The results will contribute significantly to the advancement of CTC studies and the development of standardized protocols in Indonesia, ultimately enhancing patient outcomes.

METHODS

This cross-sectional study was conducted at Dr. Cipto Mangunkusumo National General Hospital in Jakarta from November 2020 to March 2021.

Ethics Statement

The study protocols adhered to the ethical principles outlined in the Declaration of Helsinki. Approval was also obtained from the Ethics Committee of Dr. Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia (Approval No. LB.02/621/0014/2020).

Participants

The participants in this study were adult patients suspected to have CRC.

Circulating Tumor Cells (CTC) Analysis

CTC analysis was performed using the negative selection immunomagnetic method with Easysep™ and the CD44 mesenchymal tumor marker. Identification was carried out manually, while quantification was achieved through visual examination under a microscope, with three repetitions of cell counting per field of view at 40x magnification using laser capture microdissection. This method allowed for the selective isolation and enrichment of CTC from blood samples, followed by precise evaluation of their morphological and molecular characteristics. The selected method enabled the assessment of CTC presence and quantity, contributing to the understanding of their diagnostic potential in CRC.

Diagnostic Evaluation

Diagnostic study analysis was used to determine the cut-off point of CTC in detecting CRC. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) were calculated using a CTC cut-off point of >1.5 cells/mL. Subsequently, a comparison was made between CRC and inflammatory/hyperplastic polyps, as well as adenomatous polyps.

Data Analysis

The demographic and clinical characteristics of the participants were summarized using descriptive statistics. The diagnostic performance measures were calculated to evaluate the accuracy of CTC examination for diagnosing CRC.

RESULTS

Clinical Characteristics and The Relevance to Circulating Tumor Cell (CTC) Analysis in Colorectal Cancer (CRC) Detection

This study aimed to assess the diagnostic potential of CTC in detecting CRC. To gain a comprehensive understanding, the clinical characteristics of the study population were analyzed. **Table 1** presents a detailed overview of these characteristics, providing detailed insight into their relevance to the investigation of CTC in CRC detection.

The study population consisted of 80 subjects, with a mean age of 56 ± 11 years. The gender distribution showed that 53.8% were male and 46.3% were female patients. Furthermore, 77.5% were diagnosed with CRC, 7.5% had adenomatous polyps, and 15% had inflammatory/hyperplastic polyps.

The tumoral location was also examined, with 77.5% of the cases found on the left side and 22.5% on the right side. The assessment of Carcinoembryonic Antigen (CEA) levels, a commonly used marker for CRC, showed that 50.8% of participants had CEA levels below 5, while 49.2% were recorded to have levels equal to or above 5.

Data on the smoking and alcohol drinking history of participants were also collected. Based on the results, 66.3% were non-smokers, and 33.7% were current or ex-smokers. Regarding alcohol consumption, 80% were non-drinkers, while 20% were current or ex-drinkers. Body Mass Index (BMI) analysis showed that 30% were underweight, 32.5% had normal weight, 13.8% were overweight, 18.8% fell into the Obese 1 category, and 5% were categorized as Obese 2. **Table 1** shows various parameters related to age, gender, diagnosis, tumoral location, CEA levels, smoking, and alcohol drinking history, as well as BMI.

CTC Characterization and Immunofluorescence Staining

The primary objective of the invention is to address the limitations of CTC investigations

Table 1. Clinical Characteristics of the Study Population

Clinical Characteristics	Amount (%)
Total, n	80
Age in years, mean (SD)	56 ± 11
Gender, n (%)	
Male	43 (53.8)
Female	37 (46.3)
Diagnosis, n (%)	
Inflammatory/hyperplastic polyps	12 (15)
Adenomatous polyps	6 (7.5)
Colorectal cancer	62 (77.5)
Tumoral location, n (%)	
Left-sided	62 (77.5)
Right-sided	18 (22.5)
Carcinoembryonic Antigen (CEA), n (%)	
<5	33 (50.8)
≥5	32 (49.2)
Smoking history, n (%)	
Non-smoker	53 (66.3)
Smoker/ex-smoker	27 (33.7)
Alcohol drinking history, n (%)	
Non-alcohol drinker	64 (80)
Alcohol/ex-alcohol drinker	16 (20)
Body Mass Index, n (%)	
Underweight	24 (30)
Normal weight	26 (32.5)
Overweight	11 (13.8)
Obese 1	15 (18.8)
Obese 2	4 (5)

in Indonesia. The majority of existing studies primarily focused on direct molecular marker measurement, with no reports on microscopic identification. The invention comprised a centrifugation method for CTC isolation, plating for cell adhesion, and microscopic evaluation techniques. It aims to facilitate CTC assessment, enhance investigation and publications in the field, as well as provide clinical benefits including improved diagnosis and disease monitoring. The use of CTC also has great potential for prognostic evaluation and predicting clinical outcomes after radiation therapy, chemotherapy, or surgery, thereby contributing to advancements in healthcare and clinical practice. **Figure 1** shows the post-isolation results of CTC, where immunofluorescence staining was utilized to enhance detection in the isolated samples.

The staining played a crucial role in improving CTC identification, given the challenges associated with identification within post-isolation samples. Representative images expressing strong CD44 intensity are shown in **Figure 1**. This strong expression was observed in patients with stages III and IV. A positive count of 2 CTC per field of view was considered indicative of CRC.

Figure 2 shows CTC that exhibit strong CD44 expression, appearing either as single cells or clustered together with other CD44-positive or non-specific cells. On the other hand, **Figure 3** shows isolates suspected to be CTC from patients with polyps, exhibiting weak or faint staining intensity. These samples were primarily found in patients with polyps and not in those diagnosed with CRC.

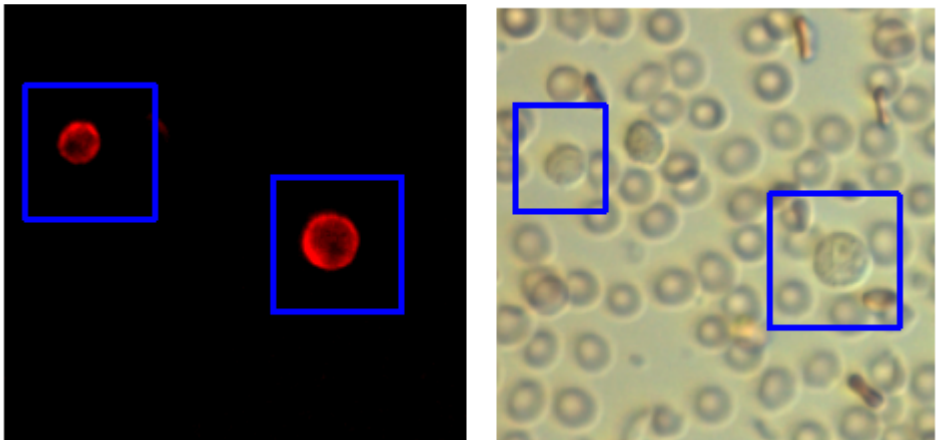

Figure 1. Immunofluorescence Staining Enhances CTC Detection in Isolated Samples: Post-Isolation Results

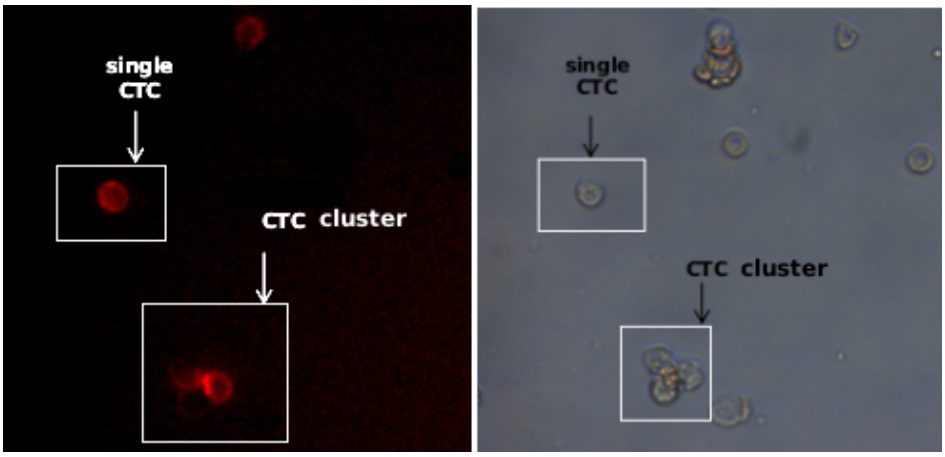

Figure 2. Representative Images of CTC with Strong CD44 Expression in Colorectal Cancer: Stage III and IV Patients

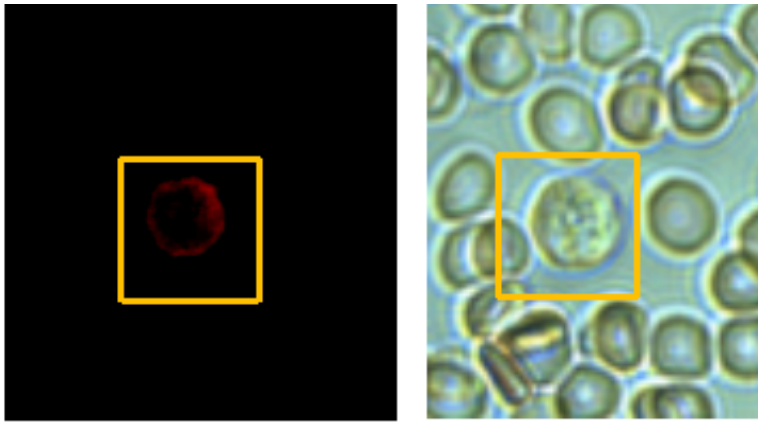

Figure 3. Differential CD44 Expression in CTC in Polyp Samples

Exploring the Diagnostic Potential of Circulating Tumor Cell (CTC) in Colorectal Cancer (CRC): Comparative Analysis and Cut-Off Point Evaluation

The diagnostic potential of CTC in detecting CRC was assessed by comparing with inflammatory/hyperplastic polyps and adenomatous polyps. The area under the curve (AUC) value for this comparison was determined to be 72.5%, indicating a moderate level of diagnostic accuracy. At a CTC cut-off point of 1.5 cells/mL, the sensitivity was 50%, meaning that CTC analysis correctly identified half of CRC cases. The specificity was 88.89%, suggesting a high rate of correctly identifying non-cancerous cases. Furthermore, the Positive Predictive Value (PPV) was 93.94%, implying a high probability of correctly identifying CRC cases among positive results. NPV was 34.04%, suggesting a moderate probability of correctly ruling out CRC among negative results. PLR of 4.5 indicated a moderate increase in the likelihood of CRC, while NLR of 0.56 implied a modest decrease in the likelihood of disease.

CTC analysis was performed to distinguish between inflammatory/hyperplastic polyps, adenomatous polyps, and CRC. The AUC value for this discrimination was found to be 66.7%, which was considered a fair level of diagnostic accuracy. At the same CTC cut-off point of 1.5 cells/mL, the sensitivity was 45.59%, implying CTC analysis correctly identified approximately 45.59% of colorectal neoplasms in the sample. A high rate of correctly identifying non-neoplastic cases was also observed according to the specificity value of 83.33%. PPV was 93.34%, suggesting a high probability of correctly identifying colorectal neoplasms among positive results. NPV was 21.28%, indicating a lower probability of correctly ruling out colorectal neoplasms among negative results. PLR of 2.74 suggested a small increase in the likelihood of colorectal neoplasms, while the NLR of 0.65 implied a modest decrease.

The cut-off point for CTC analysis in detecting CRC including inflammatory/hyperplastic polyps and adenomatous polyps compared with CRC, was determined using Receiver Operating Characteristic (ROC) curve analysis. The resulting AUC value was calculated as 72.5%, showing the overall diagnostic performance. The ROC curve, presented in **Figure 4**, visually represents the relationship between sensitivity and specificity at various cut-off points, aiding in the interpretation of the diagnostic potential of CTC analysis in CRC detection.

Circulating Tumor Cell (CTC) Analysis Shows Variations in Different Subject Groups

CTC analysis among various subject groups, including polyps and CRC provided valuable insights into the relationship between CTC presence and different pathological conditions. As shown in **Table 2**, there were detectable levels of CTC in both inflammatory/hyperplastic and adenomatous polyps. The mean number was higher in the inflammatory/hyperplastic (0.75 ± 0.96) compared to the adenomatous polyps group (0.33 ± 0.51), suggesting a potential association between CTC presence and the pathological characteristics of polyps.

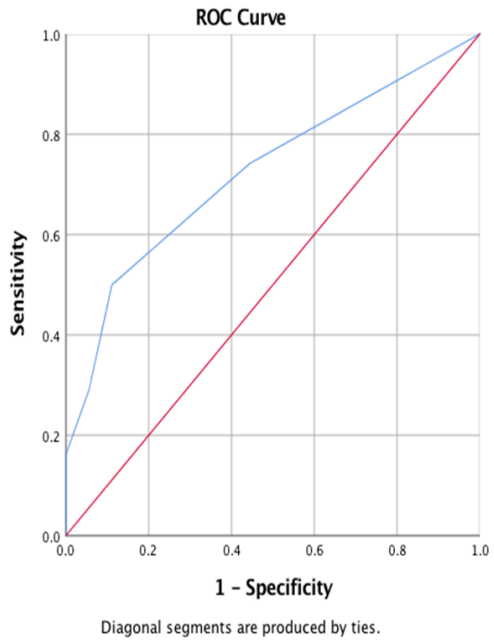

Figure 4. ROC Curve of CTC for Detecting CRC

CRC group exhibited a higher mean number of CTC (1.97 ± 2.1) compared to the polyp group. Furthermore, the non-metastatic CRC subgroup had a slightly lower mean number of CTC (1.63 ± 1.57). These varying levels of CTC indicate its potential as a diagnostic tool to distinguish between polyps and cancerous conditions.

The analysis explored the relationship between CTC and different stages of CRC. Stage I exhibited the lowest mean number of CTC (0.6 ± 0.89), while II and III showed higher mean values of 1.8 ± 1.9 and 1.76 ± 1.58, respectively. The metastatic CRC (Stage IV) subgroup had the highest mean number (2.74 ± 2.9). These results suggested a correlation between CTC levels and progression as well as the metastatic potential of CRC.

Association of Variables with Circulating Tumor Cell (CTC) Levels

The association between CTC levels and various variables was also analyzed. The distribution of individuals with CTC levels above or below 1.5 cells/mL and the corresponding p-values for each variable are presented in Table 3.

Age did not demonstrate a significant association with CTC levels (p = 0.617). Among participants aged ≤60, 23 (44.2%) had CTC levels above 1.5 cells/mL, while 29 (55.8%) exhibited levels below or equal the cut-off value. Similarly, in the group aged >60, 10 (35.7%) had CTC levels above 1.5 cells/mL, and 18 (64.3%) were found to have levels below or equal to the cut-off value.

The analysis showed no significant difference in CTC levels based on gender (p = 0.573). Among males, 16 (37.2%) had CTC levels above 1.5 cells/mL, while 27 (62.8%) exhibited levels below or equal to the cut-off value. Meanwhile, among females, 17 (45.9%) had CTC levels above 1.5 cells/mL, and 20 individuals (54.1%) showed levels below or equal to 1.5 cells/mL.

Smoking history also did not indicate a significant association with CTC levels (p = 0.81). Among non-smokers, 26 (49.1%) had CTC levels above 1.5 cells/mL, while 27 (50.9%) exhibited levels below or equal to the cut-off value. In the smoker/ex-smoker group, 7 individuals (25.9%) had CTC levels above 1.5 cells/mL, and 20 (74.1%) had levels below or equal to the cut-off value.

Table 2. CTC Values in Each Group of the Subjects

Subjects Groups	N	Mean ± SD	Range (Median)
Polyps	18	0.61 ± 0.85	0-3 (0)
Inflammatory/hyperplastic polyps	12	0.75 ± 0.96	0-3 (0.5)
Adenomatous polyps	6	0.33 ± 0.51	0-1 (0)
Colorectal cancer (CRC)	62*	1.97 ± 2.1	0-12 (1.5)
Non-metastatic CRC	43	1.63 ± 1.57	0-6 (1)
Stage I CRC	5	0.6 ± 0.89	0-2 (0)
Stage II CRC	5	1.8 ± 1.9	0-5 (1)
Stage III CRC	33	1.76 ± 1.58	0-6 (2)
Metastatic CRC (stage IV)	19	2.74 ± 2.9	0-12 (2)

*CTC level in CRC was significantly higher than in polyp/non-CRC group (p = 0.003)

Alcohol drinking history did not show a significant association with CTC levels (p = 0.23). Among non-alcohol drinkers, 29 individuals (45.3%) had CTC levels above 1.5 cells/mL, while 35 (54.7%) had levels below or equal to the cut-off value. In the alcohol/ex-alcohol drinkers group, 4 (25%) showed CTC levels above 1.5 cells/mL, and 12 (75%) had levels below or equal to the cut-off value.

Tumoral location analysis indicated no significant difference in CTC levels between left-sided and right-sided tumors (p = 0.967). Among individuals with left-sided tumors, 25 (40.3%) exhibited CTC levels above 1.5 cells/mL, while 37 (59.7%) had levels below or equal to the cut-off value. For those with right-sided tumors, 8 (44.4%) had CTC levels above 1.5 cells/mL, and 10 (55.6%) were found to have levels below or equal to the cut-off value.

CTC analysis levels in different stages of CRC did not indicate a significant association (p = 0.475). In stage I, 1 individual (3.2%) had CTC levels above 1.5 cells/mL, while 4 (12.9%) showed levels below or equal to the cut-off value. For stage II CRC, 2 individuals (6.5%) had CTC levels above 1.5 cells/mL, and 3 (9.7%) exhibited levels below or equal to 1.5 cells/mL. Furthermore, in stage III CRC, 17 individuals (54.8%) were found to have CTC levels above 1.5 cells/mL, and 16 (51.6%) showed levels

Table 3. Clinical Characteristics of the Subjects with CTC Value

Variables	CTC >1.5 cells/mL	CTC ≤1.5 cells/mL	P Value
Age, n (%)			0.617
≤60	23 (44.2)	29 (55.8)	
>60	10 (35.7)	18 (64.3)	
Gender, n (%)			0.573
Male	16 (37.2)	27 (62.8)	
Female	17 (45.9)	20 (54.1)	
Smoking history, n (%)			0.81
Non-smoker	26 (49.1)	27 (50.9)	
Smoker/ex-smoker	7 (25.9)	20 (74.1)	
Alcohol drinking history, n (%)			0.23
Non-alcohol drinker	29 (45.3)	35 (54.7)	
Alcohol/ex-alcohol drinker	4 (25)	12 (75)	
Tumoral location, n (%)			0.967
Left-sided	25 (40.3)	37 (59.7)	
Right-sided	8 (44.4)	10 (55.6)	
Colorectal cancer staging, n (%)			0.475
I	1 (3.2)	4 (12.9)	
II	2 (6.5)	3 (9.7)	
III	17 (54.8)	16 (51.6)	
IV	11 (35.5)	8 (25.8)	
Tumoral size, n (%)			0.86
<5 cm	9 (28.1)	23 (71.9)	
≥5 cm	24 (50)	24 (50)	
Cancer differentiation, n (%)			*0.005
Well-differentiated	19 (39.6)	29 (60.4)	
Poorly-differentiated	12 (85.7)	2 (14.3)	
CEA, n (%)			1
<5	15 (45.5)	18 (54.4)	
≥5	15 (46.9)	17 (53.1)	
Body Mass Index, n (%)			0.337
Underweight	13 (39.4)	11 (23.4)	
Normal weight	12 (36.4)	14 (29.8)	
Overweight	3 (9.1)	8 (17)	
Obese 1	4 (12.1)	11 (23.4)	
Obese 2	1 (3)	3 (6.4)	

*Statistical test using Chi-square showed that the cancer differentiation variable had a significant value (p<0.05) with CTC value.

below or equal to the cut-off value. For stage IV (metastatic) CRC, 11 (35.5%) had CTC levels above 1.5 cells/mL, and 8 (25.8%) exhibited levels below or equal to the cut-off value.

Tumoral size did not show a significant association with CTC levels (p = 0.86). Among individuals with tumors less than 5 cm, 9 (28.1%) were found to have CTC levels above the cut-off value, while 23 (71.9%) had levels below or equal to this value. For those with tumors measuring 5 cm or larger, 24 (50%) had CTC levels above 1.5 cells/mL, and 24 individuals (50%) exhibited levels below or equal to the cut-off value.

Cancer differentiation showed a significant association with CTC levels (p = 0.005). Among individuals with well-differentiated tumors, 19 (39.6%) had CTC levels above 1.5 cells/mL, while 29 (60.4%) showed levels below or equal to this value. In the group with poorly differentiated tumors, 12 individuals (85.7%) were found to have CTC levels above 1.5 cells/mL, and 2 (14.3%) exhibited levels below or equal to 1.5 cells/mL.

There was no significant association between CEA and CTC levels (p = 1). Among individuals with CEA levels below 5, 15 (45.5%) had CTC levels above 1.5 cells/mL, while 18 (54.4%) indicated levels below or equal to the cut-off value. For those with CEA levels of 5 or above, 15 (46.9%) had CTC levels above 1.5 cells/mL, and 17 (53.1%) exhibited levels below or equal to 1.5 cells/mL.

The BMI did not show a significant association with CTC levels (p = 0.337). Among individuals classified as underweight, 13 (39.4%) had CTC levels above 1.5 cells/mL, while 11 (23.4%) were found to have levels below or equal to 1.5 cells/mL. In the normal weight group, 12 individuals (36.4%) had CTC levels above 1.5 cells/mL, and 14 (29.8%) exhibited levels below or equal to the cut-off value. Among individuals classified as overweight, 3 (9.1%) had CTC levels above 1.5 cells/mL, and 8 (17%) showd levels below or equal to this value. For individuals classified as obese 1 or 2, the proportion of those with CTC levels above 1.5 cells/mL was 12.1% and 3%, respectively, while 23.4% and 6.4% exhibited levels below or equal to the cut-off value.

Patients who had poorly differentiated CRC also had a higher number of CTC (>1.5 cells/mL) compared with those with well-differentiated CRC. Other variables did not have a statistically significant value with CTC value.

DISCUSSION

Isolation and Identification of CTC

This study employed various methods for the isolation and identification of CTC in CRC patients. CTC isolation methods used were size-based filtration and immunomagnetic separation. These methods have been widely used due to their ability to isolate rare CTC from a complex background of blood cells. After isolation, immunofluorescence staining was used to enhance the detection in the isolated samples.

Immunofluorescence staining played a crucial role in improving the identification of CTC within post-isolation samples.[21] The focus was directed towards the expression of CD44, a cell surface marker associated with cancer stem cells and metastasis.[22,23] Representative images of CTC exhibiting strong CD44 expression were observed, particularly in patients with stage III and IV CRC. A positive count of 2 CTC per field of view was considered indicative of CRC, indicating the diagnostic significance of CD44 expression in CTC.

Diagnostic Potential of CTC

The results obtained regarding the diagnostic potential of CTC in CRC were consistent with previous investigations. ROC analysis also yielded an AUC value of 72.5%, categorizing CTC as a good diagnostic tool.

Tsai et al.[19] reported an AUC value of 75.5% for CTC in diagnosing CRC, which was consistent with the results in this study. The focus of the study was primarily on CRC-clinically suspected patients. Baek et al. achieved a higher AUC value of 90.6%, while Yu et al. reported a value of 90.4%.[18,24] The higher AUC values in these two studies were attributed to the recruitment of CRC-already-diagnosed patients compared to controls, which may have influenced the results.

CTC Cut-Off Values and Diagnostic Performance

This study used a cut-off value of >1.5 cells/mL, which provided valuable insights into the diagnostic performance of CTC in CRC. With this cut-off, the values obtained included 50% sensitivity, 88.89% specificity, 93.94% PPV, 34.04% NPV, 4.5 PLR, and 0.56 NLR.

Compared to other studies, Tsai et al. reported a sensitivity of 63% and a specificity of 82% using a CTC cut-off value of >2 cells/2 mL for diagnosing CRC.[19] Baek et al. also recorded higher values with a cut-off value of ≥5 cells/7.5 mL, including sensitivity, specificity, PPV, and NPV of 75%, 100%, 100%, and 58.5%, respectively.[24] Moreover, Haijiao et al. achieved a sensitivity of 83.05% and specificity of 100% with a cut-off of 2 CTC/3.2 mL, and the combination of CTC with CEA increased the sensitivity to 91.53%.[18] These comparisons demonstrated the variability in cut-off values and their impact on diagnostic performance, influenced by factors such as subject recruitment and CTC analysis techniques.

Significance of CTC Levels in CRC

The results showed significantly higher CTC levels in CRC-diagnosed patients compared to polyp or non-CRC groups ($p = 0.003$). The mean ± SD CTC level in the CRC group was 1.97 ± 2.1 cells/mL, with a range (median) of 0-12 (1.5) cells/mL. The metastatic CRC (stage IV) exhibited higher CTC levels compared to non-metastatic, potentially due to late-stage presentation, although the difference was not statistically significant ($p = 0.153$).

Consistent with other studies, the results indicated significantly higher CTC values in CRC compared to normal and polyp groups ($p<0.001$). The linear regression analysis of CTC values across the progression from the normal group to polyps, non-metastatic, and metastatic CRC showed a statistically significant increase ($p = 0.001$).[19,24] These results reinforced the clinical relevance of CTC levels in CRC and their potential as a biomarker for disease progression.

Association of CTC with Tumor Differentiation

The results showed that subjects with poorly differentiated CRC had higher CTC values (>1.5 cells/mL) compared to those with the well-differentiated type, as also reported by previous studies.[19,20,25] The association between cellular differentiation in CRC and tumoral metastasis into the bloodstream suggests the potential role of CTC in reflecting the aggressiveness of the disease.

In general, this study reinforced the diagnostic potential of CTC, the significance of specific levels in distinguishing CRC from other conditions, and the association between CTC and tumor differentiation. The results provided valuable insights and emphasized the need for further studies to optimize the diagnostic utility of CTC in CRC.

Limitation and Prospects

Although this study demonstrated the diagnostic potential of CTC in CRC, several limitations affected the results. The small sample size and potential selection bias may limit the generalizability, necessitating larger studies to validate the results. Additionally, the variability in CTC isolation methods and analysis techniques across studies necessitates the need for standardized protocols to ensure comparability and reliability.

The lack of a specific marker for CRC among CTC remains a challenge. CD44 expression as a potential biomarker provides valuable insights, but further investigation is required to identify more specific and sensitive markers for improved CTC detection and characterization in CRC.

Future studies should explore the combination of CTC analysis with established biomarkers, such as CEA, to enhance diagnostic accuracy and predictive value in CRC. Integration of advanced technologies, including next-generation sequencing and liquid biopsy platforms, has great potential for unraveling the genomic and molecular characteristics of CTC, facilitating personalized treatment strategies and better monitoring of disease progression. These efforts will contribute to the advancement of CRC diagnosis and management.

CONCLUSION

This study showed the diagnostic potential of CTC in differentiating CRC cases from

inflammatory/hyperplastic polyps and adenomas. CTC identification, particularly through CD44 expression, provided valuable insights into the metastatic potential of these cells and their association with advanced stages of CRC. Despite limitations in sample size and the lack of a specific marker, the results contributed to the growing body of evidence supporting the use of CTC as a non-invasive diagnostic tool for CRC. Further investigations with larger cohorts, standardization of methods, as well as the exploration of additional biomarkers and advanced technologies would advance the field and enhance the accuracy of CTC-based CRC diagnosis and prognosis.

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COMPETING INTEREST

The authors declare no competing interests relevant to the content of this article.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Saskia Aziza Nursyirwan, Murdani Abdullah, Dimas Ramadhian Noor, Agustinus Wiraatmadja, Wifanto Saditya Jeo, Nur Rahadiani, and Diah Rini Handjari. The first draft of the manuscript was written by Saskia Aziza Nursyirwan, Andri Sanityoso Sulaiman, Ikhwan Rinaldi, Dadang Makmun, Marcellus Simadibrata, Agustinus Wiraatmadja, and Hamzah Shatri. All authors commented on previous versions of the manuscript as well as read and approved the final manuscript.

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