ORIGINAL ARTICLE

Association between CD133 expression and clinicopathological profile in colorectal cancer

Imelda Rey[1], Agung Putra[2-4], Dharma Lindarto[1,3], Fauzi Yusuf [3,5]

[1]Department of Internal Medicine, School of Medicine, Universitas Sumatera Utara, Medan, [2]Stem Cell And Cancer Research (SCCR), School of Medicine, Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia, [3]Doctoral Department of Medical Science, School of Medicine, Universitas Sumatera Utara, Medan, [4]Pathology Anatomy Department, School of Medicine, Sultan Agung Islamic University (UNISSULA), Semarang, [5]Internal Medicine Department, School of Medicine, Syiah Kuala Univeristy, Banda Aceh; Indonesia

ABSTRACT

Aim To investigate CD133 expression and its relationship to clinicopathological profile in colorectal cancer (CRC) patients.

Methods This cross-sectional study was performed at the Internal Medicine Department, School of Medicine, Adam Malik General Hospital. The colorectal cancer tissue was taken from surgical resection and colonoscopy biopsy from CRC patients. Clinical profile was obtained by a questionnaire. Histopathology examination was done using hematoxylin and eosin staining. Immunohistochemistry (distribution score and intensity score) combined with ROC analysis were conducted to determine CD133 expression. An association between CD133 expression and clinicopathological profile was then analyzed.

Results Out of 118 patients, 690 (58.5%) were male. The high and low level of CD133 expression were found in 44 (37.3%) and 74 (62.7%) patients, respectively. No difference between gender, age, body mass index, hemoglobin, leucocytes, platelets, and histopathology with CD133 expression was found. There was a significant difference between CD133 and different CRC locations (p=0.002). CD133 expression was higher in the proximal colon than the rectum (p=0.002), and it was higher in the distal colon than the rectum (p=0.008), especially in terms of percentages of stained cancer cells (distribution score).

Conclusion CD133 expression was associated with the tumour location, but not with other clinicopathological factors.

Key words: carcinoma, colon, immunohistochemistry, neoplastic stem cells, rectum

Corresponding author:
Imelda Rey
Department of Internal Medicine,
School of Medicine,
Universitas Sumatera Utara
Dr Mansyur Street 5, 20155 Medan,
Indonesia
Phone: +628126500525
E-mail: imeldareyusu@gmail.com;
imeldareyŽrocketmail.com
ORCID ID: https://orcid.org/0000-0002-8651-1084

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INTRODUCTION

Colorectal cancer (CRC) is still one of the most common cancers worldwide. In global cancer statistics, approximately 1,360,600 estimated new cases of CRC were clinically diagnosed (1). In Pirngadi Hospital, Medan, Indonesia, in 25% (197 of 760) patients who underwent colonoscopy CRC was diagnosed (2). In Adam Malik hospital, Medan, Indonesia, most common CRC was rectal cancer (61.5%), followed by the left-sided colon cancer (23.1%), and the right-sided colon cancer (15.4%) (3).

Relapse, metastasis, resistance of chemotherapy and recurrence are still problems in CRC management. They contribute to higher mortality and poor survival rate (4). Cancer stem cell (CSC) was considered to be responsible for tumour progression, relapse, metastases and therapeutic resistance (5-7). Therefore, the identification of CSCs is crucial in the search for therapeutic targets and useful prognostic markers for CRC.

In CRC, putative CSCs can be identified by several markers such as CD44, CD133, CD24, EpCAM, LGR5, ALD, and many more. These markers are highly tumorigenic, chemoresistant and could affect survival rate of colorectal cancer (8,9). CD133 molecule (also known as prominin-1) is five transmembrane glycoproteins with a molecular weight of 120 kDa. It is mainly localized in membrane protrusions (10). Previous studies have shown that CD133+ tumour cells were more resistant to radiochemotherapy than CD133- cells in CRC (11). These findings showed that CD133 CSC burden in colorectal cancer was of relevance for patients' treatment outcome (11).

To date, there are no data about an expression of CD133 in CRC patient in Indonesia. Since these data are important for t patient management and prognostic predictors, we aimed to investigate the association of CD133 expression with several clinicopathological data and tumour location in CRC patients in centre at Medan, Indonesia.

PATIENTS AND METHOD

Patients and study design

This cross-sectional study was conducted at Haji Adam Malik General Hospital, Medan North Sumatera, Indonesia. All patients with CRC in the period of September 2018 – July 2019 were included in the study. Colorectal cancer or adenocarcionoma was proven by biopsy of colorectal tissue obtained by either surgical resection or colonoscopy biopsy. Patients whose tissue was inadequate or did not meet the requirements for conducting histopathological examinations were excluded. Clinicopathological data included gender, age, hemoglobin (Hb) level, leucocyte, thrombocyte, body mass index (BMI), and a location of the tumour. The tumour locations were categorized into the proximal colon, distal colon, and rectum.

Ethical approvals for this study were obtained from the Institutional Ethics Committee of Universitas Sumatera Utara, Medan, Indonesia.

Methods

Histopathological examination was conducted to classify CRC based on its differentiation. After the tissue sample was processed (fixation, dehydration, clearing, impregnation) to make a paraffin block, they were stained using hematoxylin-eosin. Colorectal cancer was classified into well-differentiated adenocarcinoma, moderately-differentiated adenocarcinoma and poorly-differentiated adenocarcinoma (12).

Immunohistochemistry (IHC) was conducted to determine the presence of CD133 in the colon or rectum. Primary CD133 antibody GTX100567 (C1C2) internal (1:100-1:1000) (GeneTex International Corporation, California, USA) was used. The assessment of the CD133 expression was performed in the central and peripheral portions of the tumour, and the strongest expression results were recorded. Two blinded experienced pathologists from Anatomic Pathology Department, School of Medicine, Universitas Sumatera Utara, were assigned to assess the slides for CD133 expression. An inter-observer agreement between both pathologists was calculated using κ-statistics. The stained tumour cell was scanned by using a high-power microscope.

For CD133 expression analysis, the immunoreactivity score on the cell membrane and/or cytoplasm was calculated from the sum of both quantitative and qualitative parameters. For quantitative analysis (distribution score), the score was determined based on percentage of reactive

cells (% of the total area). The score was categorized as follows: 0 (0% of immunoreactive cells), 1 (<10% of immunoreactive cells), 2 (10%–50% of immunoreactivecells), and 3 (more than 50% of immunoreactive cells). For qualitative analysis, the immunoreactive staining intensity (intensity score) was classified as follows: 0 (no immunoreactivity), 1 (weak immunoreactivity) (Figure 1A), 2 (intermediate immunoreactivity), and 3 (strong immunoreactivity) (Figure 2B). Strong immunoreactivity staining was considered as the most intense staining observed in the positive control of respective antibody. The total scores ranged from 0 to 6 for CD133 expression (13).

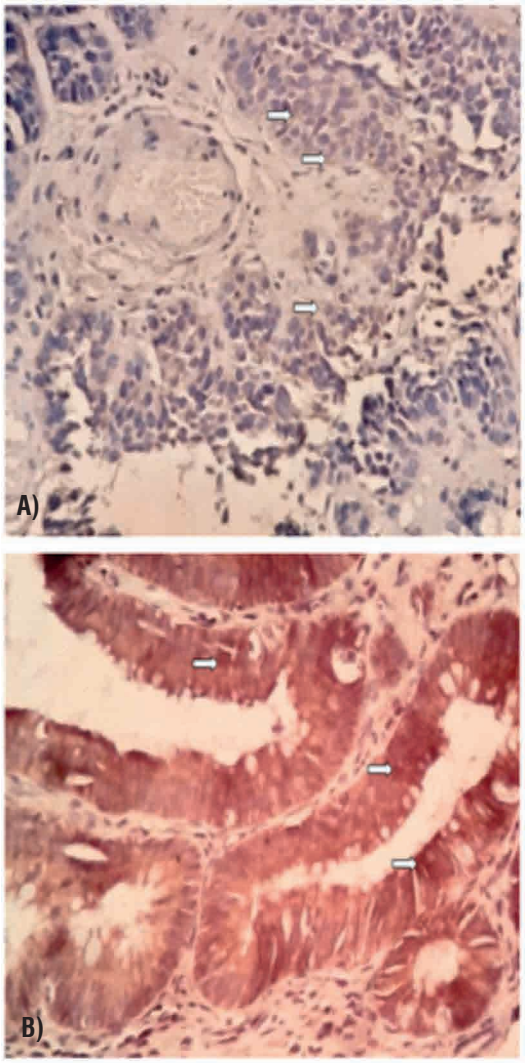

Figure 1. A) Weak immunoreactivity of CD 133 in colorectal cancer tissue. CD133 is marked by light brown colour on cytoplasm (white arrow); B) strong immunoreactivity of CD 133 in colorectal cancer tissue. CD133 is marked by dark brown colour on cytoplasm (white arrow) (200x magnification) (400x magnification) (Rey I, 2019)

Statistical analysis

Receiver operating characteristic (ROC) analysis was conducted to determine a cut-off point of CD133 expression. Based on ROC analysis, total score of 0–3 indicated low level and a total score of 4-6 indicated high level. The associations between clinicopathological data (gender, age, BMI, Hb, leucocyte, thrombocyte, histopathology) and CD133 expression was analysed by using the $\chi 2$ test or Kolmogorov-Smirnov test. Kruskal-Wallis and Mann-Whitney test were used to assess associations between CD133 expression and CRC locations. For CD133 expression, distribution score and intensity score were first analysed separately before the total of both scores was used. The $p<0.05$ was considered as statistically significant.

RESULTS

One hundred and eighteen CRC patients were enrolled in this study. The patients' mean age was 57.17 years, with more males enrolled than females (Table 1).

Table 1. Characteristics of 180 patients with colorectal cancer

Variable	
Gender (No, %)	
Male	69 (58.5)
Female	49 (41.5)
Age (mean) (±SD) (years)	57.30 (±12.99)
Hb (mean) (±SD) (g/dL)	10.51(±2.1)
Leucocyte (median) (min.-max.) (cells/mm^3)	7,420 (1,609-34,180)
Thrombocyte (median) (min.-max.) (cells/mm^3)	286,350 (89,000-562,000)
BMI (median) (min.-max.) (kg/m^2)	22.22 (1.22-30.86)
Tumour location (No, %)	
Proximal colon	32 (27.1)
Distal colon	43 (36.4)
Rectum	43 (36.4)
Histopathology (No, %)	
Well-differentiated adenocarcinoma	55 (46.6)
Moderately differentiated adenocarcinoma	48 (40.7)
Poorly differentiated adenocarcinoma	15 (12.7)
CD133 expression (No, %)	
High Level	44 (37.3)
Low Level	74 (62.7)
Distribution score (No, %)	
0-1	43 (36.4)
2-3	75 (63.6)
Intensity score (No, %)	
0-1	86 (72.9)
2-3	32 (27.1)

min., minimum; max., maximum

There was no statistically significant difference between gender (p=0.150), age (p=0.611), BMI (p=0.995), Hb (p=0.643), leucocyte (p=0.394), and thrombocyte (p=0.227) with CD133 expressions both in distribution score, intensity score (data not shown) and total score (Table 2).

Table 2. Association of clinical characteristics and CD133 expression

Variable	CD133 expression		p
	High level	Low level	
Age (±SD) (years)	58.09 (±12.99)	56.82 (±13.06)	0.611
Gender (Male/Female)	22/22	47/27	0.150
BMI (mean) (±SD) (kg/m^2)	22.05 (±3.58)	22.05 (±4.14)	0.995
Hb (mean) (±SD) (gr/dL)	10.63 (±2.19)	10.44 (±2.06)	0.643
Leucocytes (median) (min.-max.) (cells/mm^3)	8,100 (1,609-34,180)	7,600 (4,478-21,130)	0.394
Platelets (mean) (±SD) (cells/mm^3)	321,554 (±112,588)	295,594 (±112,158)	0.227

BMI, body mass index; Hb, haemoglobin; min., minimum; max., maximum

Intra-observer agreement coefficient κ was 0.923, 1.000, and 0.927 for immunohistochemistry of the proximal colon, distal colon, and rectum, respectively.

Most tumours were located in the distal colon or rectum. Most tumours were either well-differentiated or moderately-differentiated adenocarcinoma (p=0.933) (Table 3).

Table 3. CD133 expression based on histopathology

Histopathology	CD133 expression		Total	p
	Low level	High level		
Well-differentiated	21	34	55	0.933
Moderately differentiated	17	31	48	
Poorly differentiated	6	9	15	
Total	44	74	118	

Locations of tumour were associated with different CD133 expression (p=0.002) (Table 4). The proximal and distal colon had higher CD133 expression than rectum (p=0.002 and p=0.008, respectively). In further analysis, only the distribution score was found significantly higher in the proximal and distal colon than rectum (p=0.002), while intensity score was not (data not shown). However, findings in histopathology did not relate to CD133 expression, either when scores were analysed separately (p=0.620 for distribution score and p=0.66 for intensity score, data not shown) or total score was used (p=0.933) (Table 3).

Table 4. CD133 expression based on colorectal cancer (CRC) location

	CRC location (median, min. – max.)			p
	Proximal colon	Distal colon	Rectum	
CD133 expression (immunoreactivity score)	4 (2-6)*	3 (2-6)†	2 (2-6)	0.002

*p=0.002 (compared with rectum); †p=0.008 (compared with rectum)

DISCUSSION

In this study, we did not find significant differences in age, gender, and BMI with CD133 expression. This is in line with a previous study (14-16). The previous study on Medan showed that the risk of CRC increased with age until 6th decade of life (2). However, it seems that the expression of CD133 depended on factors other than age and gender. Some factors are known to be involved in CRC, such as sex hormone, genetic profile (17), and also epigenetic factor (18) that is affected by the environment and lifestyle (19-20). These confounding factors were not assessed in this study.

The results of this study have shown that CD133 expression was higher in the proximal colon than in the rectum, and higher in the distal colon than the rectum, especially in terms of percentages of stained cancer cells (distribution score). This is in line with the previous study where CD133 expression was very low in the rectum (21): CD133 expression was detected in 28% cases in the right colon, in 53.8% in the left colon, and in 18.2% cases in the rectum, considering CD133 positivity as >5% of stained cancer cells (21). The result of our study is different from the study in which CD133 expression was higher in the rectum than in the colon (15). Several other studies reported that there was no significant difference between positive and negative CD133 and tumour location (16,22). However, in both studies (16,22) cut-off used was different from our study. For instance, Nosrati et al. stated that <50% staining was considered negative, and >50% staining was considered positive for CD133 (16).

We suspected that the difference of our results was due to several reasons. There was a variability of cut-off used to define low and high level of CD133 between studies. A different cut-off could produce a different result. In addition, since studies are conducted in several different countries, differences could be caused by different geographical area or race. Therefore, the result should be applied specifically for each country. Moreover, a study divided the CRC locations into 2 parts, the left and right colon (21), while our study divided the CRC locations into 3 parts: the proximal colon, distal colon and rectum.

We also analysed relationship between CD133 expression and tumour histopathology. Our study has shown that CD133 expression did not affect

CRC histopathology. This result is in concordance with almost all previous studies (15-16, 21-23). Kojima et al. study concluded that CD133 occurred mainly in well- to moderately-differentiated adenocarcinomas; however, well- and moderately-differentiated adenocarcinoma were categorized into one group, thus the difference was significant compared to poorly-differentiated adenocarcinoma (24). CD133 was known to be elevated in CRC than benign colorectal epithelium (25). But it seems its contribution to the patient's prognosis does not go through histopathological changes of colon (15-16, 21-23).

The findings of this research could impact several things in the management of CRC in Medan, Indonesia. We found a high level CD133 expression in a relatively high number of patients (37.3%). This marker should be considered in the management of CRC since CD133 could reduce the effectivity of chemotherapy (11). In this study, we found CD133 expression was highest in the proximal colon. Both proximal and distal colon have a higher amount of CD133 expression than rectum. The tumour location had impact on the survival of CRC patients in the previous study (26, 27). Three- and 5-year survival rates were 87.6% and 81.6% for the right-sided CRC group and 91.5% and 84.5% for the left-sided CRC, respectively; univariate and multivariate analysis showed that the risk of death was increased in the right-sided CRC location (26). Another study found that rectal cancer had a better survival rate than colon cancer (27). Studies assessing the role of CSCs in CRC patients also found that patients with high level CD133 expression had poor survival rates (14,28). In our study, since CD133 expression was associated with the tumour location, it is interesting to further investigate about this effect also to the survival rate of colorectal cancer in Medan.

The results of the presented study have not shown statistically significant differences between haemoglobin level and CD133 expression. Anaemia has an important role in response of first line 5FU-based chemotherapy (29). In advance CRC patients, the group with higher haemoglobin showed a better response rate, slower progressivity, and higher survival rates than the group with anaemia (29). The previous study indicated that anaemia could affect CD133 expression through hypoxia, but the results were conflicting (30-32). This area needs to be assessed in further research.

In conclusion, CD133 expressions were associated with the tumour location. This marker is a potential to be used as a CRC prognostic predictor in Medan, Indonesia.

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TRANSPARENCY DECLARATION

Conflicts of interest: none to declare.

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