CORRELATION OF TUMOR-STROMA RATIO (TSR) WITH HISTOLOGICAL GRADING AND STAGING IN COLORECTAL ADENOCARCINOMA

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ABSTRACT

Background: Colorectal carcinoma ranks the third-largest in the world, and second as the cause of death from cancer. Risk factors include precursor lesions such as adenomas and dysplasia and diets of high in calorie and animal fat accompanied by lack of physical activity. In addition to grading and histological staging, the prognosis is also influenced by the reaction between the tumor and the stroma (Tumor-Stroma Ratio / TSR) based on a comparison between the percentage of carcinomas and stroma, which is one of the most recent prognostic factors and is still not widely used.

Objective: To analyze the correlation between Tumor-Stroma Ratio (TSR) with histological grading and staging in colorectal adenocarcinoma.

Methods: 40 histopathological slide samples from the operating tissue of patients with a diagnosis of colorectal adenocarcinoma with hematoxylin-eosin staining assessed staging, grading and TSR scores. If the carcinoma component ≤30% (TSR-Low), carcinoma 40%-60% (TSR-Intermediate) and carcinoma ≥70% (TSR-High).

Results and discussion: Most histological grading distributions are moderately differentiated, most staging is stage II, and the highest TSR score is Intermediate TSR. Based on the Spearman correlation test, it was found that there was a strong correlation between TSR scores with grading ($p = 0.0001$, $r = +0.747$) and a weak correlation between TSR and staging ($p = 0.012$, $r = +0.395$).

Conclusions: TSR is a prognostic factor in colorectal adenocarcinoma patients and should be reported in the diagnosis of an Anatomic Pathologist.

Keywords: Tumor-Stroma Ratio, colorectal prognosis.

INTRODUCTION

Colorectal carcinoma is defined as a malignant tumor of the large intestine (colon and rectum) originating from the epithelial, there must be an invasion of tumor cells between the stroma of the mucosal muscular layer to the submucosal layer. Most colorectal malignancies (90%) are adenocarcinomas. The high incidence of colorectal carcinoma is associated with several risk factors, such as a diet with a high-calorie diet and animal fat (Western type) accompanied by a lifestyle with low physical activity. The pathogenesis of colorectal adenocarcinoma is largely preceded by a history of precursor lesions such as adenomas and dysplasia [1-3]. According to Global Burden Cancer (GLOBOCAN) in 2018, colorectal carcinoma ranks third in the world, with more than 1.8 million new cases, and ranks second as a cause of cancer deaths, with around 881,000 cases per year, or about 1 in 10 cases of death [4]. In Indonesia data obtained from GLOBOCAN in 2018, colorectal cancer ranks fourth, after breast, cervical and lung cancer, with a new case incidence rate of 30,017 (8.6%) [5]. From the medical record data at H. Adam Malik Central General Hospital Medan in 2015, 2016 and 2017, there was an increase in the number of colorectal carcinoma cases, with 75 cases each (2015), 83 cases (2016) and 98 cases (2017) [6]

Histopathological grading of colorectal adenocarcinoma was assessed based on the extent of presentation of gland formation divided into 4 criteria, grade 1 (well differentiated), grade 2 (moderately differentiated), grade 3 (poorly differentiated), and grade 4 (undifferentiated carcinoma) [1]. Prognosis of colorectal carcinoma, can be assessed using TNM staging based on the classification of the American Joint Committee on Cancer (AJCC) sixth edition staging. The determination required complete clinical data in the form of tumor size, lymph involvement, and distant metastases [7].

The management of colorectal cancer so far has largely been based on clinicopathological characteristics, such as the patient's age, tumor type, malignancy, tumor size, and the presence of regional or distant metastases. Lately, many studies have been developed based on biological markers and focused on the tumor microenvironment, one of which is tumor stromal compartment, which plays an important role in the initiation and progression of cancer, in which stromal interactions with malignant cells and non- malignant at various stages of tumorigenesis [8]. A comparison between tumor and stroma, known as Tumor-Stroma Ratio (TSR), is closely related to prognostic factors. TSR assessment can be done with a simple examination using a light microscope and Hematoxylin & Eosin staining [8,9].

In addition to colorectal carcinoma, studies to assess TSR have been carried out in many other types of cancer such as breast carcinoma, esophagus, non-small cell lung carcinoma (NSCLC), endometrium, hepatocellular carcinoma, which show a strong prognostic parameter, very easy to do and can be done in daily practice routinely by a pathologist in diagnosing [8-16]. Previous studies have found that the correlation between tumors and stroma plays an important role in the biology of carcinomas including carcinoma growth,

transformation, and progression. The wide association of stroma to tumors is often associated with a high degree of malignancy and a poor prognosis [10].

MATERIALS AND METHODS

We examined the slides of patients undergoing surgery with a diagnosis of colorectal adenocarcinoma in the Anatomic Pathology Laboratory of the Faculty of Medicine, Universitas Sumatera Utara, Medan and the Anatomic Pathology Unit of the H. Adam Malik Central General Hospital in Medan with random sampling techniques and a cross-sectional approach. Our this research was approved by the Ethics Commission for Conducting Health Research Medical Faculty of Universitas Sumatera Utara with no: 622/TGL/KEPK FK USU-RSUP HAM/2019. We chose slides that were representative of the routine coloring of Hematoxylin & Eosin and reviewing slides to determine diagnosis and grading histology then retrieve stadium data through medical record data.

After getting slides that met the inclusion and exclusion criteria, the three researchers assessed the Tumor-Stroma Ratio (TSR) score. Exclusion criteria included issues of histological preparations slides containing the mass of tumor and stroma that can not be assessed for scoring TSR, as many contain mucin, mass necrosis, and inflammatory cell lymphocytes weight.

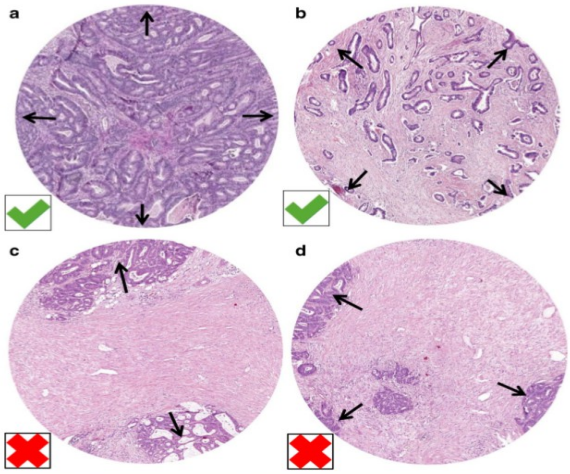

Figure 1. Example of assessing TSR in colorectal adenocarcinomas. a. stroma-low. b. stroma-high, with criteria for the presence of tumor vital cells in the four fields of view (arrow) that must be found and this is the correct scoring. c & d. When tumor cells are only seen in two or three planes, this area cannot be assessed for TSR scoring. (Image using 100x magnification) [8].

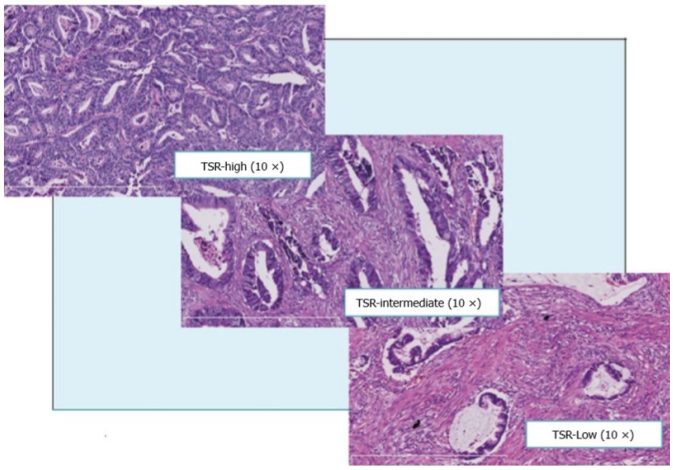

Figure 2. Example of assessing colorectal adenocarcinoma TSR by dividing according to three categories. TSR-High (Carcinoma ≥70%), TSR-Intermediate (Carcinoma 40% - 60%), TSR-Low (Carcinoma ≤30%) [9].

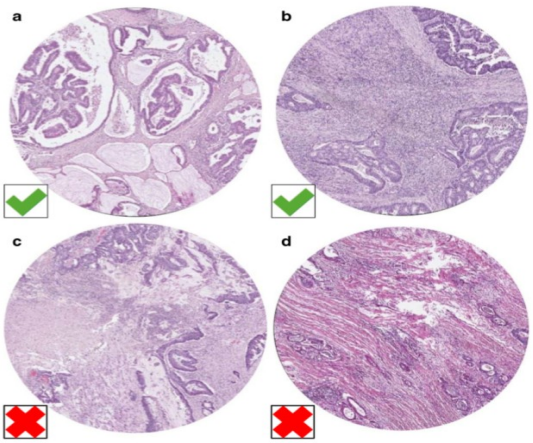

Figure 3. Histological problems. a. Example assessing mucinous adenocarcinoma, where the mucin area was not assessed. b. Infiltration of inflammatory cells. c. Field of view that shows the area of necrosis. d. Smooth muscle tissue, both (c and d) cannot assess the TSR score [8].

RESULTS

In this study 40 samples of colorectal adenocarcinoma were found that met the inclusion and exclusion criteria at RSUP Adam Malik Medan, which aimed to analyze the correlation between tumor-Stroma Ratio (TSR) grading and histological staging. The frequency distribution of grading, staging and TSR values in adenocarcinomas of the colorectal can be seen in Tables 1 to 3. The analysis of the Tumor-Stroma Ratio (TSR) correlation with grading and histological staging can be seen in Tables 4 to 7.

Table 1. Distribution of colorectal adenocarcinoma samples based on histological grading.

Grading	Amount (n)	Percentage (%)
Well differentiated	8	20
Moderately differentiated	24	60
Poorly differentiated	8	20
Total	**40**	**100**

The distribution of colorectal adenocarcinoma samples in this study was based on WHO histological grading of 8 well-differentiated cases (20%), moderately differentiated in 24 cases (60%), and poorly differentiated in 8 cases (20%).

Table 2. Distribution of colorectal adenocarcinoma samples based on histological staging.

Grading	Amount (n)	Percentage (%)
Well differentiated	8	20
Moderately differentiated	24	60
Poorly differentiated	8	20
Total	**40**	**100**

In this study, colorectal adenocarcinoma sample distribution based staging histology most are stage II as many as 20 cases (50%), followed by stage III 10 cases (25%) and stage IV 7 cases (17.5%), while for least stage I is 3 cases (7.5%)

Table 3. Distribution of colorectal adenocarcinoma samples based on Tumor-Stroma Ratio (TSR) assessment.

Tumor-Stroma Ratio (TSR)	Amount (n)	Percentage (%)
High	7	17,5
Intermediate	20	50
Low	13	32,5
Total	**40**	**100**

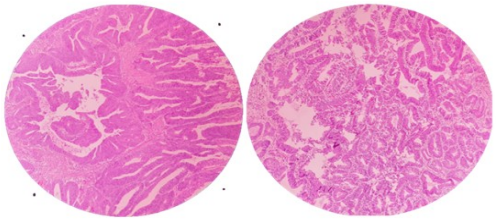

Figure 4. TSR High (Carcinoma ≥70%)

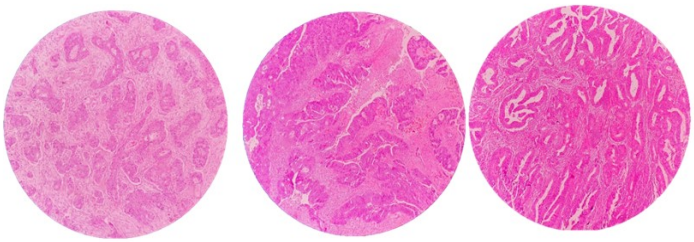

Figure 5. TSR Intermediate (Carcinoma 40%, 50%, 60%)

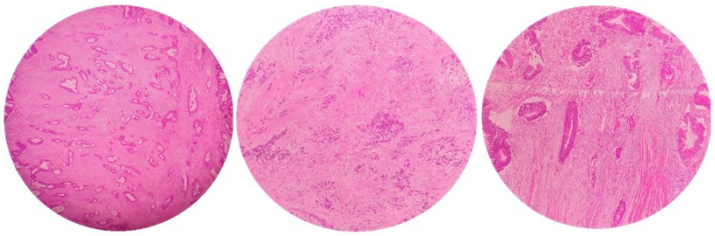

Figure 6. TSR Low (Carcinoma ≤30%)

Comparison of tumors with stroma (TSR) in colorectal adenocarcinoma in this study, the most common is Intermediate TSR found in 20 cases (50%), followed by TSR Low found in 13 cases (32.5%), and only 7 cases with TSR High (17.5%).

Table 4. Distribution of characteristics of the correlation between TSR values and histological grading of colorectal adenocarcinomas.

No.	Tumor-Stroma Ratio (TSR)	Histological grading						Total
		Well differentiated		Moderately differentiated		Poorly differentiated		
		n	%	n	%	n	%	
1.	**High**	5	71,4	2	28,6	0	0	7
2.	**Intermediate**	3	15	17	85	0	0	20
3.	**Low**	0	0	5	38,5	8	61,5	13
	Total	8		24		8		40

Table 5. Correlation test of TSR values with histological grading of colorectal adenocarcinomas.

Variable	Histological grading		
	n	r	p
TSR Category	40	+0,747	0,0001

Correlation test results showed that the correlation between histological grading of colorectal adenocarcinomas and TSR categories had a significant correlation with p = 0,0001 (p <0.05). Spearman's correlation coefficient shows a strong correlation category with r = +0.747 (r = 0,61-0.80) and in a positive direction (increasing colorectal adenocarcinoma grading will worsen the TSR category, and vice versa).

Table 6. Distribution of correlation characteristics between TSR values and histological staging of colorectal adenocarcinomas.

No.	Tumor-Stroma Ratio (TSR)	Histological staging								Total
		Stage I		Stage II		Stage III		Stage IV		
		n	%	n	%	n	%	n	%	
1.	**High**	3	42,9	4	57,1	0	0	0	0	7
2.	**Intermediate**	0	0	10	50	6	30	4	20	20
3.	**Low**	0	0	6	46,2	4	30,8	3	23,1	13
	Total	3		20		10		7		**40**

Table 7. Correlation test of TSR values with histological adenocarcinoma staging.

Variabel	Histological staging		
	n	r	p
TSR category	40	+0,395	0,012

Correlation test results showed that the correlation between histological colorectal adenocarcinoma staging with the TSR category had a significant correlation with p = 0.012 (p <0.05). Spearman's correlation coefficient shows a weak correlation category with r = +0.395 (r = 0,21-0.40) and in a positive direction (worsening colorectal adenocarcinoma staging will worsen the TSR category, and vice versa).

DISCUSSION

Based on the histological grading of colorectal adenocarcinomas the most is moderately differentiated, which is 60 % of cases, followed by well-differentiated and poorly differentiated as many as 20% of cases. Based on research conducted by Scheer et al and Mesker et al, grading colorectal adenocarcinoma is more often diagnosed as moderately differentiated, and followed by poorly differentiated and well-differentiated [9-17].

Research conducted by Nasution in H. Adam Malik Central General Hospital Medan in 2015-2017, found that the most colorectal adenocarcinoma grading was well-differentiated in 44.4% of cases, followed by moderately differentiated in 35.8% of cases and poorly differentiated in 19,8%.[6] This difference can be caused because the sampling in this study is not limited by time and randomly taken.

Staging histology of adenocarcinoma colorectal this study found that most in stage II by 50%, followed by stage III, stage IV and the least stage I. Scheer et al found that the most stage was stage III by 55.6% followed by stage II and stage I [9]. Research conducted by Huijbers et al only examined stage II and III colorectal adenocarcinoma samples, where the most staging was stage III [15]. According to a study by Nasution, most cases of colorectal adenocarcinoma sufferers were in stage III with 42% of cases followed by stage II with 40.7% cases, stage IV with 13.6% cases and stage I with 3.7% cases [6]. A large number of patients treated and classified in this advanced stage is due to lack of information and health education that makes patients arrive late for early detection and seek treatment so that the disease continues to develop and the prognosis is poor [18].

Grading and staging are some parameters to determine the prognosis of colorectal adenocarcinoma. As grade and stage increases, the patient's prognosis will get worse. In well-differentiated, 5 years survival rate is 99.3%, moderately differentiated is 86%, and in poorly differentiated is 68% [19]. For staging assessment, an Anatomic Pathologist can provide information to the clinician in assessing the patient's staging based on histopathological preparations sent by the clinician and the clinician must also completely write down the T, N, and M status in the patient's medical record to design what treatment should be given to patients and determine the patient's prognosis, for example, stage I and II undergo partial or total colectomy, while about two-thirds with stage III colorectal carcinoma (also some with stage II disease) receive chemotherapy in addition to colectomy to reduce the risk of recurrence. Stage II and III rectal carcinomas are treated with neoadjuvant chemotherapy plus radiation [46]. The prognosis based on the Duke's system that has been modified by Astler-Coller, 5 years survival rates for stage 1 is still very good at >90%, while for stage 2 it is 70-85%, stage 3 is 20-65%, and at stage 4 is only 5% [21].

Another assessment that can be used to determine the prognosis of colorectal adenocarcinoma patients currently being studied is to assess Tumor-Stroma Ratio (TSR). This assessment is needed to observe how the tumor-stromal compartment, by looking at the interaction of the tumor with the stroma that plays an important role in the initiation and progression of cancer. The assessment technique is very easy so that it is easy to apply

in the practice of routine diagnosis by an Anatomic Pathologist just by looking at what percentage of the tumor and its stroma. In this study, it was found scoring TSR-Intermediate is the most widely encountered These results are consistent with studies conducted Scheer et al, who found that cases of adenocarcinoma of colorectal most common with a value of TSR-Intermediate, which is associated that the prognosis is a little more worse, followed by TSR -Low is said to be a poor prognosis, and TSR High is said to be a good prognosis. Some other studies using a cut-off value for TSR is 50%, where TSR High is said to be a tumor area with less stroma, ie tumors >50%, which means good prognosis, and if TSR Low is said to be a tumor area that is less than the stroma, ie tumors <50% mean poor prognosis [8-16].

For tumor cells to attack normal tissues, loss of interaction between one cell and another must occur, and attack and integrate tumor cells surround and enter the normal structure. Normal tissue is designed to fight the action of these tumor cells, making the invasion of active processes on the part of cancer cells. Cancer-Associated Fibroblasts (CAF) have been shown to assist in the proliferation and development of cancer through the production of growth factors and chemotaxis factors, angiogenesis factors, and molecules that cause the invasion and spread of cancer cells. Angiogenesis requires active repair and integration of new cells into existing structures. To facilitate restructuring, fibroblasts, macrophages, and extracellular matrix from the stroma and secreting MMPs. The secretion of these molecules by stromal cells is the result of complex cross-tumor stromal communication, involving many ligands and signals, all of which play a vital role in local growth, invasion, and migration of cancer cells [22,23].

Malignant epithelial tumors from patients with a poor prognosis show a high proportion of stroma, whereas tumors with abundant carcinoma tissue is associated with a better prognosis [8-16]. According to research conducted by Hansen et al, TSR values can also predict the recurrence of colon cancer patients treated with neoadjuvant chemotherapy [24].

CONCLUSION

Tumor-Stroma Ratio (TSR) has a strong correlation with grading histology of colorectal adenocarcinoma and in a positive direction, ie, if there is an increase in the coloration of colorectal adenocarcinoma grading, will worsen the TSR category, and vice versa. There is a significant correlation between Tumor-Stroma Ratio (TSR) with histological staging of colorectal adenocarcinoma, although the correlation is categorized as weak, and with a positive direction that if there is a worsening of colorectal adenocarcinoma staging will worsen the TSR category, and vice versa. Here it can be concluded that Tumor-Stroma Ratio (TSR) can be in a prognostic factor in colorectal adenocarcinoma cases and should be reported in the regular reports anatomic pathology diagnostic results.

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REFERENCES

1. Hamilton Hospital, Bosman TF, Boffeta P, M Ilyas, Morreau H, N akamura SI, et al. 2010. Tumors of Colon and Rectum. In: Bosman TF, Carnerro F, HRruban HR, Theise DN, editors. World Health Organization Classification of Tumors Pathology and Genetics of Tumors of the Digestive System. 4 th ed. France. IARC Press. pp103-44.
2. Fleming M, Ravula S, Tatishchev SF, Wang HL. 2012. Colorectal Carcinoma: Pathological Aspects. Journal of Gastrointestinal Oncology Vol 3. pp153-73.
3. Colorectal Cancer Facts & Figures 2017-2019. 2017. American Cancer Society. Atlanta. pp30.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. 2018. Global Cancer Statistics 2018. GLOBOCAN Estimates Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. pp396-419.
5. International Agency for Research on Cancer. Indonesia, Source: Globocan 2018. Available from: http://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf.
6. Nasution, Novia. 2018. Characteristics of Colorectal Cancer Patients in H. Adam Malik General Hospital Medan 2015-2017. [cited 2019 apr 24]. Available from: http://repositori.usu.ac.id/handle/123456789/11025.
7. Edge SB and Chompson CC. 2016. American Joint Committee on Cancer, Cancer Staging Manual Eight Edition. Chapter 20. pp251-74.
8. van Pelt GW, Kjaer-Frifeldt S, van Krieken JHJM, Al diphtheria, Morreau H, M. Tollenaar, et al. 2018. Scoring the tumor-stroma ratio in colon cancer: procedure and recommendation. [cited 2018 Oct 26] Available from: https://doi.org/10.1007/s00428-018-2408-z
9. Scheer R, Baidoshvii A, Zoidze S, Elferink MAG, Berkel AEM, Klaase JM, et al. 2017. Tumor-stroma ratio as prognostic factor for survival in rectal adenocarcinoma: A retrospective cohort study. W orld Journal of Gastrointestinal Oncology. [cited 2018 Oct 8] Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5740087/pdf/WJGO-9-466.pdf.

10. Le NH, Franken P, Fodde R. 2008. Tumor stromal interactions in colorectal cancer: converging on β-catenin activation and cancer stemness. British Journal on Cancer. pp1886-93.
11. Zhang T, Xu J, Shen H, Dong W, Ni Y, Du J. 2015. Tumor-stroma ratio is an independent predictor for survival in NSCLC. Int J Clin Pathol. [cited 2018 Oct 8] Available from: www.ijcep.com.
12. Panayiotou H, Orsi NM, Thygesen HH, Wright AI, Winder M, Hutson R, et al. 2015. The prognostic significance of tumor-stroma ratio in endometrial carcinoma. BMC Cancer 2015. [cited 2019 may 14]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26674153.
13. Wang K, Ma W, Wang J, Yu L, Zhang X, Wang Z, et al. 2012. Tumor-stroma ratio is an independent predictor for survival in esophageal squamous cell carcinoma. J Thorac Oncol. pp1457-61.
14. de Kruijf EM, van Nes JG, Van de V, Putter H, Smith VT, Liefers GJ, et al. 2011. Tumor-stroma ratio in the primary tumor is a prognostic factor in early breast cancer patients, especially in triple-negative carcinoma patients. Breast Cancer Res Treat. pp687-96.
15. Huijbers A, Tollenaar RA, v. Pelt GW, Zeestraten EC, Dutton S, McConkey, et al. 2013. The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. Ann Oncol. pp179-85. Available from: https://academic.oup.com/annonc/article/24/1/179/1746464.
16. Z Lv, X Cai, X Weng, H Xiao, C Du, J Cheng, et al. 2015. Tumor-stroma ratio is a prognostic factor for survival in hepatocellular carcinoma patients after liver resection or transplantation. Surgery. pp142-50.
17. Mesker WE, Liefers GJ, Junggeburt JM, van Pelt GW, Alberici P, Kuppen PJ, et al. 2009. Presence of a high amount of stro-ma and downregulation of SMAD4 predict for worse survival for stage I–II colon cancer patients. Cell Oncol 31(3). pp169–78. Available from : https:// doi.org/10.3233/CLO-2009-0478.
18. Panduan Penatalaksanaan Kanker Kolorektal. 2017. Komite Penanggulangan Kanker Nasional. Kementerian Kesehatan Republik Indonesia.
19. Ueno H, Mochizuki H, Hashiguchi Y, Ishiguro M, Kajiwara Y, Sato T, et al. Histological grading of colorectal cancer: a simple and objective method. 2008. Ann Surg. pp 811-8.
20. Clayton A, Evans RA, Pettit E, Hallett M, Williams JD, Steadman R. 1998. Cellular activation through the ligation of intercellular adhesion molecule-1. J Cell Sci 111. pp443–53.
21. Mohan H. 2015. Textbook of Pathology. 7th ed. pp563-74.
22. Li H, Fan X, Houghton JM. 2007.Tumor microenvironment : The role of the tumor stroma in cancer. Journal of cellular biochemistry. pp805-15.
23. Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, et al. 2003. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med 348 .pp883–90.
24. Hansen TF, Kjaer-Frifeldt S, Lindebjerg J, Raafaelsen SR, Jensen LH, Jakobsen A, et al. 2017. Tumor-stroma ratio predicts recurrence in patients with colon cancer treated with neoadjuvant chemotherapy. Acta Oncologica Vol 57. Available from : https://doi.org/10.1080/0284186X.2017.1385841.