Majalah Kedokteran Sriwijaya
Th.56 Nomor 3 , July 2024

NEUTROPHIL INFILTRATION BASED ON CLINICOHISTOPATHOLOGICAL CHARACTERISTICS IN SEROUS OVARIAN CARCINOMA

Soilia Fertilita [1*], Aisyah Mulya Zhafirah[2], Wresnindyatsih[3], Veny Larasati[1], Riana Sari Puspita Rasyid[1]

[1]Department of Histology, Faculty of Medicine, Universitas Sriwijaya, Palembang
[2]Under Graduate Program of Medical Education, Faculty of Medicine, Universitas Sriwijaya, Palembang
[3]Department of Anatomical Pathology, Faculty of Medicine, Universitas Sriwijaya, Palembang

Email: soilia.fertilita@fk.unsri.ac.id

ABSTRACT

Ovarian cancer is the main cause of death globally and is the third most common cancer in women in Indonesia. Neutrophils play a role in response against cancer and are related to the patient's prognosis. The aim of this study was to determine differences in neutrophil infiltration based on the clinicohistopathological characteristics of serous ovarian carcinoma tissue at RSUP Dr. Mohammad Hoesin Palembang. This research used a cross-sectional method. The samples used were archived serous ovarian carcinoma tissue preparations with Hematoxylin-Eosin staining from the Anatomical Pathology Department and clinical data from the Medical Records Installation of RSUP. Dr. Mohammad Hoesin Palembang. Observations were made by identifying neutrophil cells that infiltrate the microtumor environment in the stromal and intratumoral areas. Data were obtained from 35 patients diagnosed with serous ovarian carcinoma. Most of the samples were aged 40-60 years (48.6%) with the most histological subtype (74.3%) being HGSC and 77.1% were at an advanced stage. There were no significant differences between neutrophil cell infiltration based on clinicohistopathological characteristics, including age (stromal p=0.85 and intratumoral p=0.22), subtype (stromal p=0.29 and intratumoral p=0.72), and FIGO stage (stromal p=0.35 and intratumoral p=0.38). It can be concluded that neutrophil infiltration does not differ based on clinicohistopathological (age, subtype, and stage) characteristics in serous ovarian cancer.

Keywords: *Serous ovarian carcinoma, Tumor microenvironment, Neutrophil Infiltration*

1. INTRODUCTION

Ovarian cancer is the fifth most common cause of cancer death in women worldwide and the third most common cancer in women in Indonesia with an incidence rate of 14,896 cases and a death rate of 9,581 cases in 2020.[1,2] Although in recent years there have been advances in cancer treatment, the recurrence rate for ovarian cancer still reaches 80% within 18 months after initial treatment.[2] This causes ovarian cancer to be one of the most dangerous gynecological cancers with a 5-year survival rate only 46%.[3]

Most ovarian tumors originate from epithelial tissue (85-90%), less frequently, tumours originate from the germ cells (dysgerminomas and teratomas) and the follicular cells (granulosa cell tumours). Classification of epithelial ovarian cancer (EOC) refers to the WHO classification which is divided into serous carcinoma, mucinous carcinoma, endometroid carcinoma, and clear cell carcinoma. Among the 4 types of EOC, serous carcinoma is the one with the highest prevalence.[3–7]

Serous carcinoma is graded into High-Grade Serous Carcinoma (HGSC) and Low-Grade Serous Carcinoma (LGSC) based on

its biological characteristics. HGSCs are characterized by SET features (solid, endometrioid-like, and transitional) and high frequency of *TP53* mutations. HGSC is a malignant, aggressive type of cancer, generally diagnosed at an advanced stage, and most deadly type of EOC. LGSCs are often associated with borderline or atypical proliferative serous tumors, often contain mutations in *BRAF* and *KRAS* and contain wild-type *TP53*.[4,5,8,9]

The high recurrence rate and low 5-year survival rate, especially in advanced stage patients, as well as decreased sensitivity to chemotherapy, cause an urgent need to find new therapies. One of the therapies currently being developed is immunotherapy. However, the effectiveness of this therapy is dependent on the condition of the tumor microenvironment (TME).[7]

Every human cancer induces an immune response in its microenvironment. In ovarian cancer, the tumor microenvironment has been reported to play a vital role in the tumorigenesis and is considered a possible therapeutic target for this cancer. Increasing evidence supports that the immune infiltration of tumours is associated with prognosis. Therefore, ovarian cancer therapies targeting tumor microenvironment is rapidly developing.[10–13]

The TME consists of various cellular and non-cellular components such as blood vessels, extracellular matrix (ECM), immune cells, and fibroblasts. Immune cells consist of macrophages, mast cells, dendritic cells, lymphocytes, myeloid-derived suppressor cells (MDSC), and neutrophils.[11,14]

Neutrophil infiltration occurs in many types of tumors including ovarian tumors. Neutrophils can play an antitumor or protumor role. Several studies have shown that neutrophils that infiltrate the tumor microenvironment play an anti-tumor role through direct cytotoxicity against tumor cells and inhibit metastasis. However, other studies have demonstrated a protumor role of neutrophils by promoting angiogenesis and stimulating tumor cell migration and invasion. This is due to the plasticity of tumor-associated neutrophils which can undergo alternative activation when exposed to various conditions in the tumor microenvironment.[14] This study aims to determine differences in neutrophil cell infiltration based on the clinicohistopathological characteristics of serous ovarian carcinoma tissue. It is hoped that this research can become a supporting theoretical basis for future research regarding the role of immune cells as targets for immunotherapy in the treatment of serous ovarian carcinoma based on clinicohistopathological characteristics.

2. METHOD

This research was an analytical study with a crosssectional design. The sample used was an archive of serous ovarian cancer tissue preparations that had been stained with hematoxylin eosin staining which was stored in the anatomical pathology department of RSUP. Dr. Mohammad Hoesin Palembang. Sampling was carried out using the total sampling method. Data taken (clinical data and tissue preparations) from January 2019 - July 2023. Patients who had relapsed and had received chemotherapy were not taken as samples.

Tissue preparations will be analyzed using a light microscope. The first magnification used was 40-100 times to view the intratumoral and stromal areas. Then the area of dense neutrophil infiltration will be determined. Next, the magnification was increased to 400 times to count the number of neutrophils in 10 fields of view in the intratumoral and stromal areas.

Data were analyzed univariately and bivariately. Univariate analysis to describe the distribution and frequency of neutrophil

infiltration in cancer tissue. Meanwhile, bivariate analysis was used to describe whether there was a difference between neutrophil infiltration and the clinicohistopathological features of serous ovarian cancer patients.

This research has received ethical approval from the Medical and Health Research Ethics Committee, Faculty of Medicine, Sriwijaya University, protocol number 140-2023.

3. RESULT

Data collection on serous ovarian carcinoma patients has been carried out at anatomical pathology department, Dr. Mohammad Hoesin General Hospital, Palembang. The data was taken from January 2019 – July 2023. During this period, 35 serous ovarian carcinoma tissue preparations were obtained in good condition and could be observed, consisting of 26 High Grade Serous Carcinoma (HGSC) preparations and 9 Low Grade Serous Carcinoma (LGSC). Of the preparations observed, the majority (48.6%) of the preparations came from patients aged 40-60 years and 77% were at an advanced stage. Data on age, stage and subtype of serous ovarian carcinoma taken as samples are shown in Table 1.

Table 1. Distribution of Research Sample Characteristics

Sample Characteristics	n	%
Age (year)		
> 60	11	31.4
40-60	17	48.6
< 40	7	20
Subtype		
HGSC	26	74.3
LGSC	9	25,7
Stage (FIGO)		
Late	27	77.1
Early	8	22.9

Neutrophils were counted if they had clear morphology. Neutrophils in areas of necrosis and within blood vessels were not included in the calculations.

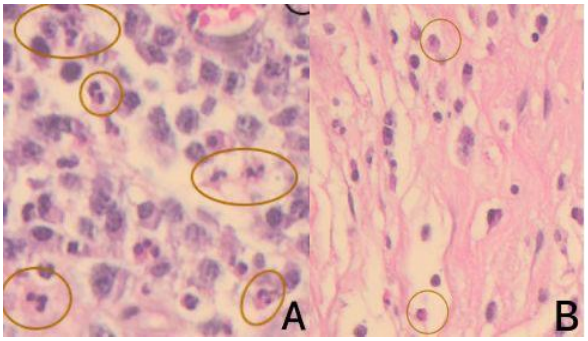

Figure 1. Neutrophils in intra-tumoral areas (A) and stroma (B) in High Grade Serous Carcinoma

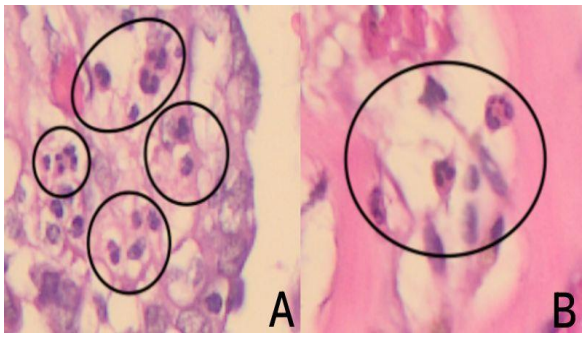

Figure 2. Neutrophils in intra-tumoral areas (A) and stroma (B) in Low Grade Serous Carcinoma

The results of the normality test with the Shapiro-Wilk test showed a p value <0.05, which means the data distribution is not normal (Table 2). Based on the test results, a non-parametric test was used using the Kruskal Wallis test and further tests used the Mann-Whitney test.

Table 2. Normality Test Result

Location	*Shapiro-Wilk*		
	Statistic	df	Sig.
Stromal	0.896	35	0.003
Intratumoral	0.924	35	0.019

*Test for normality using the Shapiro-Wilk test with p<0.05 indicating the data is not normally distributed

The results of the Kruskal-Wallis statistical test between age and neutrophil density in the stromal and intratumoral areas in this study were found to be p>0.05. This means that there is no difference in neutrophil infiltration in both the intratumor and stromal areas based on age (Table 3).

Table 3. Neutrophil Infiltration in Intratumor and Stroma Area Based on Age

Location	Age (year)	Median (min-max)	*Kruskal-Wallis* *p
Stromal	> 60	2.30 (0.4-8.6)	0.85
	40-60	2.50 (0.6-8.7)	
	< 40	2.90 (0.4-8.7)	
Intra tumoral	> 60	4.50 (1.4-10.6)	0.22
	40-60	3.40 (0.6-8.8)	
	< 40	2.60 (1.2-4.9)	

* Bivariate analysis used the Kruskal-Wallis test. significantly different if p<0.05

The results of the Mann-Whitney statistical test between grading and density of neutrophils in the stromal and intratumoral areas in this study were found to be p>0.05. there is no difference in neutrophil infiltration in both the intratumor and stromal areas based on subtype (Table 4).

Table 4. Neutrophil Infiltration in Intratumor and Stroma Area Based on Subtype

Location	Subtype	Median (min-max)	*Mann-Whitney* *p
Stromal	HGSC	2.70 (0.4-8.7)	0.29
	LGSC	2.30 (0.4-5.5)	
Intratumoral	HGSC	3.65 (0.6-10.6)	0.72
	LGSC	3.60 (0.6-10.6)	

*Bivariate analysis used the Mann-Whitney test. significantly different if p<0.05

The results of the Mann-Whitney statistical test between stage and density of neutrophils in the stromal and intratumoral areas in this study were found to be p>0.05. there is no difference in neutrophil infiltration in both the intratumor and stromal areas based on stage FIGO (Table 5).

Table 5. Neutrophil Infiltration in Intratumor and Stroma Area Based on Stage

Location	Stage (FIGO)	Median (min-max)	*Mann-Whitney* *p
Stromal	Late	2.30 (0.4-8.6)	0.35
	Early	3.20 (1.4-8.7)	
Intra tumoral	Late	3.60 (0.6-10.6)	0.38
	Early	3.80 (2.2-9.0)	

*Bivariate analysis used the Mann-Whitney test. significantly different if p<0.05

4. DISCUSSION

This study evaluated 35 serous ovarian cancer tissue preparations. Most of the patient are diagnosed with serous ovarian cancer at the age of over 40 years (48.6% at the age 40-60 years and 31.4% at the age over 60 years). Study by Wentzensen, et al., 2016 analyzed data on ovarian cancer patients from 21 prospective cohorts studies from North America, Asia, and Europe found that the median age of patients diagnosed with serous ovarian cancer is 67 years.[8] Study by Rasyid et al in the pathology department of RSUP Dr. Mohammad Hoesin Palembang also showed that 63.3% of cases of malignant ovarian tumors were diagnosed at the age of more than 45 years.[15]

Epidemiological data shows that the average age at which women develop ovarian cancer varies in each region. Average age also varies based on ethnicity

within a country. However, in general, ovarian cancer is rarely found in people under 40 years of age and more than 90% of cancers in women over the age of 40 are epithelial cancers.[16] This happens because as age increases, there will be an accumulation of genetic mutations, long-term exposure to hormones, and genetic damage that can occur, increases the risk of ovarian cancer in the elderly population.[6]

Based on the subtype of serous ovarian cancer, it was found that the majority (74.3%) of samples were of the HGSC subtype. Research by Kalińska, et al., 2020, also found that the majority (74.1%) of patients have HGSC subtype, which can be caused by genetic predisposing factors such as BRCA gene mutations, especially in patients of a certain age at diagnosis.[17] Another study by Valencia et al, from 3 anatomical pathology laboratories in the city of Padang, Indonesia showed that 82.2% of cases were of the HGSC subtype.[18]

Various studies report that HGSC is the EOC subtype with the highest prevalence. HGSCs account for more than 60% of epithelial-type ovarian cancers and more than 70% of all ovarian cancer deaths. Most cases of HGSC are diagnosed at an advanced stage, which causes the five-year survival rate to be low.[5]

The sample distribution of this study found that most patients were diagnosed at an advanced stage (77.1%). Study by Kalinska et al also found 69.9% patient was diagnosed at FIGO stage III and IV.[17] This can be due to the lack of early diagnosis methods and the absence of specific early warning symptoms. As is known disease symptoms usually develop at very late stage when the cancer cells have spread. This cause the prognosis becomes worse and mortality rate is high.[11,19,20]

There was no significant difference in neutrophil infiltration density based on age in this study. However, neutrophil density tends to be high in the 40-60 years age group. Research by Puga et al., 2021 on lung cancer also showed an increase in neutrophil density in elderly patients compared to young people.[21] In the HGSC and LGSC subtypes, there were also no significant differences in neutrophil infiltration either intratumorally or in the stroma. Research by Mayer et al showed that of 232 ovarian cancer tissues in which neutrophil infiltration was detected, there were no differences in the number of infiltrating neutrophils based on subtype. However, neutrophil infiltration in HGSC was slightly higher than in LGSC.[19]

Neutrophil infiltration also did not differ significantly based on stage. However, if we look at the median value, early-stage serous ovarian cancer has higher neutrophil density than late stage. Research by Sagiv et al., 2015 found that anti-tumor neutrophils are dominant in early-stage cancer, but as the tumor progresses, neutrophils can turn pro-tumor. This transition process depends on TGF-β and is influenced by chemokines or cytokines in the TME.[22] Research by Matsumoto et al., 2017 on cervical cancer shows that the density of pro-tumor neutrophils tends to increase at advanced stages. Pro-tumor neutrophils can work through immunosuppressive mechanisms in the TME, suppress T cell activity, and play a role in cancer development.[23] Other study by Meyer et al which analyzed neutrophil infiltration in ovarian cancer tissue found that the amount of neutrophil infiltration is not related to disease severity or histological subtype.[19]

In this study, variable neutrophil infiltration was found in both the stromal and intratumor areas of HGSC and LGSC, this can be seen from the data which was not normally distributed. This finding is in line with research by Meyer et al which found the distribution of neutrophils in each sample was not homogeneous. There are

tumors with neutrophil infiltration that looks dense, rare or even absent at all.

The immune response to tumors is very complex, immune cells can respond well to tumor growth, however, on the other hand, immune cells can also increase tumor cell growth and angiogenesis.[24] Neutrophils play a role in the first line immune response to tumor cells. Neutrophils display plasticity with the ability to adapt their function in different inflammatory condition. In the tumor microenvironment, neutrophils have varied functions and have been classified using different terms including N1/N2 neutrophil, tumor-associated neutrophil (TAN), and polymorphonuclear-myeloid derived suppressor cell (PMN-MDSC).[25] Therefore, the role of neutrophils in the tumor microenvironment must be analyzed comprehensively both from the aspect of infiltrating neutrophil density and neutrophil subtype.

5. CONCLUSION

Neutrophil infiltration does not differ based on clinicohistopathological characteristics (age, grading, and stage) in serous ovarian cancer.

ACKNOWLEDGEMENTS

The author would like to thank all parties who have helped us during this research, all staff of Histology Department, Faculty of Medicine, Universitas Sriwijaya and Head of KSM Anatomical Pathology, RSUP Dr Mohammad Hoesin, Palembang.

FUNDING

This research was funded by “Sains Teknologi dan Seni (SATEKS)” scheme research funding Universitas Sriwijaya.

CONFLICT OF INTERESTS

The authors reported no potential conflicts of interest

REFERENCE

1. Direktorat Jenderal Pelayanan Kesehatan. Mengenal Kanker Ovarium, The Silent Killer [Internet]. 2022. Available from: https://yankes.kemkes.go.id/view_artikel/1043/mengenal-kanker-ovarium-the-silent-killer
2. Luvero D, Plotti F, Aloisia A, Montera R, Terranova C, Carlo De Cicco Nardone, et al. Ovarian cancer relapse: From the latest scientific evidence to the best practice. Crit Rev Oncol Hematol. 2019;140(May):28–38.
3. Lheureux S, Gourley C, Vergote I, Oza AM. Epithelial ovarian cancer. Lancet [Internet]. 2019;393(10177):1240–53. Available from: http://dx.doi.org/10.1016/S0140-6736(18)32552-2
4. Berek JS, Renz M, Kehoe S, Kumar L, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum: 2021 update. Int J Gynecol Obstet. 2021;155(S1):61–85.
5. Kim J, Park EY, Kim O, Schilder JM, Coffey DM, Cho CH, et al. Cell origins of high-grade serous ovarian cancer. Cancers (Basel). 2018;10(11):1–28.
6. La Vecchia C. Ovarian cancer: Epidemiology and risk factors. Eur J Cancer Prev. 2017;26(1):55–62.
7. Gao Y, Chen L, Cai G, Xiong X, Wu Y, Ma D, et al. Heterogeneity of immune microenvironment in ovarian cancer and its clinical significance: a retrospective study. Oncoimmunology [Internet]. 2020;9(1). Available from: https://doi.org/10.1080/2162402X.2020.1760067
8. Wentzensen N, Poole EM, Trabert B, White E, Arslan AA, Patel A V., et al.

Ovarian cancer risk factors by histologic subtype: An analysis from the Ovarian Cancer Cohort Consortium. J Clin Oncol. 2016;34(24):2888–98.

9. Schoutrop E, Moyano-Galceran L, Lheureux S, Mattsson J, Lehti K, Dahlstrand H, et al. Molecular, cellular and systemic aspects of epithelial ovarian cancer and its tumor microenvironment. Semin Cancer Biol. 2022;86(3):207–23.
10. Liu R, Hu R, Zeng Y, Zhang W, Zhou HH. Tumour immune cell infiltration and survival after platinum-based chemotherapy in high-grade serous ovarian cancer subtypes: A gene expression-based computational study. EBioMedicine. 2020;51.
11. Yang Y, Yang Y, Yang J, Zhao X, Wei X. Tumor Microenvironment in Ovarian Cancer: Function and Therapeutic Strategy. Front Cell Dev Biol. 2020;8(August):1–30.
12. Soto-Perez-de-Celis E, Chavarri-Guerra Y, Leon-Rodriguez E, Gamboa-Dominguez A. Tumor-associated neutrophils in breast cancer subtypes. Asian Pacific J Cancer Prev. 2017;18(10):2689–93.
13. Baci D, Bosi A, Gallazzi M, Rizzi M, Noonan DM, Poggi A, et al. The ovarian cancer tumor immune microenvironment (Time) as target for therapy: A focus on innate immunity cells as therapeutic effectors. Int J Mol Sci. 2020;21(9):1–24.
14. Shaul ME, Fridlender ZG. Tumour-associated neutrophils in patients with cancer. Nat Rev Clin Oncol [Internet]. 2019;16(10):601–20. Available from: http://dx.doi.org/10.1038/s41571-019-0222-4
15. Sari Puspita Rasyid R, Fertilita S, Nurwany R, Larasaty S, Phelia Zen A, Histologi B, et al. Karakteristik Ekspresi CD163 pada Tumor Ganas Ovarium. J Kedokt dan Kesehat Publ Ilm Fak Kedokt Univ Sriwij. 2023;10(1).
16. Webb PM, Jordan SJ. Epidemiology of epithelial ovarian cancer. Best Pract Res Clin Obstet Gynaecol [Internet]. 2017;41:3–14. Available from: http://dx.doi.org/10.1016/j.bpobgyn.2016.08.006
17. Millert-Kalińska S, Przybylski M, Pruski D, Stawicka-Niełacna M, Mądry R. Epithelial Ovarian Cancer—Varied Treatment Results. Healthc. 2023;11(14).
18. Valencia V, Asri A, Nizar RZ. Hubungan Ekspresi PD-L1 dengan Derajat Diferensiasi dan Densitas Til di Stroma pada Karsinoma Ovarium Serosum. Heal Med J. 2022;5(1):10–20.
19. Mayer C, Darb-Esfahani S, Meyer AS, Hübner K, Rom J, Sohn C, et al. Neutrophil granulocytes in ovarian cancer - induction of epithelial-to-mesenchymal-transition and tumor cell migration. J Cancer. 2016;7(5):546–54.
20. Jessmon P, Boulanger T, Zhou W, Patwardhan P. Epidemiology and treatment patterns of epithelial ovarian cancer. Expert Rev Anticancer Ther [Internet]. 2017;17(5):427–37. Available from: http://dx.doi.org/10.1080/14737140.2017.1299575
21. Gorría Puga T, Teixidó C, Auclin E, Gataa I, Nalda I, Reyes R, et al. 184P Association of tumor-associated neutrophils (TAN) with immunotherapy outcomes in patients in advanced non-small cell lung cancer. Ann Oncol [Internet]. 2021;32:S1462. Available from:

https://doi.org/10.1016/j.annonc.2021.10.204

22. Sagiv JY, Michaeli J, Assi S, Mishalian I, Kisos H, Levy L, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. Cell Rep [Internet]. 2015;10(4):562–73. Available from: http://dx.doi.org/10.1016/j.celrep.2014.12.039
23. Matsumoto Y, Mabuchi S, Kozasa K, Kuroda H, Sasano T, Yokoi E, et al. The significance of tumor-associated neutrophil density in uterine cervical cancer treated with definitive radiotherapy. Gynecol Oncol [Internet]. 2017;145(3):469–75. Available from: http://dx.doi.org/10.1016/j.ygyno.2017.02.009
24. Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y, et al. Role of tumor microenvironment in tumorigenesis. J Cancer. 2017;8(5):761–73.
25. Giese MA, Hind LE, Huttenlocher A. Neutrophil plasticity in the tumor microenvironment. Blood. 2019;133(20):2159–67.