RESEARCH ARTICLE

Elevated Serum Tumor Markers (HE4 and ROMA Score) and Increased Treg Cells Distinguished Ovarian Cancer and Benign Tumor

Manuel Hutapea[1,2,*], Syamsudin[1], Iin Kurnia[3], Miswar Fattah[4]
Ujang Saeful Hikmat[4], Vinessa Gracia Putri[5]

[1]Doctoral Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Pancasila, Jl. Srengseng Sawah, Jakarta 12640, Indonesia
[2]Department of Obstetrics and Gynaecology, Soedarso Hospital, Jl. Dr. Soedarso No.1, Pontianak 78124, Indonesia
[3]Agency for Research and Inovation, Gedung B.J. Habibie, Jl. M.H. Thamrin No. 8, Jakarta 10340, Indonesia
[4]Prodia Clinical Laboratory, Jl. Kramat Raya No. 150, Jakarta, 10430, Indonesia
[5]PT Natura Nuswantara Nirmala, Jl. Jombang Raya Gang Rawa I No.18, Tangerang Selatan 15229, Indonesia

*Corresponding author. Email: manuelhtpog@yahoo.com

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Abstract

BACKGROUND: Tumor markers such as human epididymis protein 4 (HE4), cancer antigen 125 (CA-125), and risk of ovarian malignancy algorithm (ROMA) are frequently utilized for diagnostic and prognostic purposes. Lymphocytes, essential immune cells, play a significant role in eliminating cancer cells. However, the precise correlation between tumor markers and lymphocytes remains incompletely elucidated. The aim of this study was to explore the correlation between tumor markers and lymphocyte subtype profiles in differentiating ovarian cancer and benign tumors.
METHODS: This was a cross-sectional study involving 12 ovarian cancer and 17 benign ovarian tumor patients. Blood samples were collected for the characterization of T lymphocytes, B lymphocytes, natural killer (NK), and T regulatory (Treg), which were analyzed using flowcytometry. Additionally, tumor markers HE4 and CA-125 were measured from patient serum using the chemiluminescent microparticle immunoassay (CMIA) method.
RESULTS: Benign ovarian tumors and ovarian cancer can be distinguished by a significant increase in HE4 levels (p=0.004), ROMA (p=0.004), and Treg cells ($CD4^+/CD25^+/FOXP3^+$, p=0.017). Furthermore, the correlation between tumor markers and lymphocytes indicates that an increase in ROMA was weakly correlated with an increase in the percentage of T regulatory cells ($CD4^+/CD25^+/FOXP3^+$, r=0.553, p=0.006) and B lymphocytes ($CD19^+$, r=0.528, p=0.010), accompanied by a decrease in the number of T lymphocytes ($CD3^+$, r=-0.598, p=0.003), T helper lymphocytes ($CD3^+CD4^+$, r=-0.594, p=0.003), and cytotoxic lymphocytes ($CD3^+CD8^+$, r=-0.510, p=0.013).
CONCLUSION: The elevation of serum tumor markers (HE4 and ROMA) accompanied by an increase in Treg cells can distinguish between benign ovarian tumor patients and ovarian cancer patients.
KEYWORDS: tumor marker, CA-125, HE4, ROMA, subtype of lymphocytes, ovarian cancer

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Introduction

Ovarian cancer is one of the most lethal gynecologic malignancies worldwide, characterized by its late-stage diagnosis and high recurrence rates.(1-3) The identification of reliable biomarkers has been instrumental in improving early detection and monitoring treatment response.(4,5) Among the tumor markers used in ovarian cancer, human epididymis protein 4 (HE4) and cancer antigen 125 (CA-125) have emerged as potential candidates.(6) However, up to date, still little is known about how these biomarkers interact with immune cells within the tumor microenvironment.

HE4 and CA-125 are secreted proteins that have been extensively studied in ovarian cancer.(7) CA-125, a surface antigen found on the epithelial cells of the ovarian surface, has been traditionally used as a diagnostic biomarker.(8,9). HE4, a member of the whey acidic protein four-disulfide-core (WFDC) family, has shown superior diagnostic accuracy when combined with CA-125.(10) The tumor microenvironment consists of various cell types, including immune cells, stromal cells, and extracellular matrix components. Immune cells play a crucial role in shaping the tumor microenvironment and modulating tumor growth, invasion, and metastasis. Key immune cells involved in ovarian cancer include tumor-associated macrophages, T cells, natural killer (NK) cells, and dendritic cells.(11)

Several studies have highlighted the correlation between tumor markers, such as HE4 and CA-125, and immune cells in ovarian cancer. Emerging evidence suggests that these tumor markers may affect immune cell recruitment, polarization, and function within the tumor microenvironment.(12,13) For instance, HE4 has been found to modulate the polarization of tumor-associated macrophages, promoting an immunosuppressive phenotype. CA-125, on the other hand, may influence T regulatory (Treg) cell and NK cell activity.(14,15) However, the mechanisms underlying their release, regulation, and impact on the immune system in ovarian cancer are still being unravelled.

Understanding the intricate interplay between tumor markers (HE4 & CA-125) and immune cells in the context of ovarian cancer holds immense potential for improving diagnosis, prognosis, and treatment. The aim of this study was to explore the correlation between tumor markers and lymphocyte subtype profiles in differentiating ovarian cancer and benign tumors.

Methods

Study Design and Subjects Recruitment

This was a cross-sectional study design, which study protocol had received approval from the Independent Ethics Committee (IEC) of the Faculty of Medicine, Universitas Tanjungpura/Doctor Soedarso Hospital (No. 51/RSUD/KEPK/V/2023). Subject recruitment for the study encompassed all female patients, aged 17-75 years olds, at Dr. Soedarso Hospital who were diagnosed with ovarian masses/tumors via ultrasound and assessed with The International Ovarian Tumor Analysis (IOTA) Simple Rules criteria. Subjects who had not undergone surgery to remove ovarian masses/tumors or chemotherapy were included. Subjects' data on tumor marker examinations, including CA-125 and HE4, were collected. Participation in the study was confirmed through signed consent forms. Following ultrasonography examination using IOTA criteria, subjects were categorized into suspected ovarian cancer and benign ovarian tumor subjects. The Simple Rules comprise a preoperative classification system for ovarian tumors, comprising five features typical of benign ovarian tumors (B-features) and five features typical of malignant tumors (M-features). These rules were used in diagnosing ovarian cancer in women with at least one persistent adnexal (ovarian, para-ovarian, and tubal) tumor who were deemed to require surgery. Tumors were categorized as 'Benign' if only B-features are present, as 'Malignant' if only M-features are present, or as 'Inconclusive' if no features apply or if both B- and M-features are present.(16) The ovarian cancer and benign ovarian tumor groups underwent further confirmation with histopathological results post-surgery. The study included 12 ovarian cancer and 17 benign ovarian tumor subjects.

Flowcytometer for Lymphocyte Characterization

Whole blood samples were utilized for the characterization of lymphocytes using the flowcytometry method with the BD FACSLyricTM instrument (BD Biosciences, Franklin Lakes, NJ, USA). To characterize the subset populations of T lymphocytes ($CD3^+$), T Helper lymphocytes ($CD3^+CD4^+$), T Cytotoxic lymphocytes ($CD3^+CD8^+$), B lymphocytes ($CD19^+$), and NK lymphocytes ($CD16^+CD56^+$), the BD MultitestTM 6-Color TBNK (BD Biosciences) was employed. This kit contained FITC-labeled antibodies that would bind to surface markers on lymphocytes: Cluster Differentiation/CD (Cat No. 662967; BD Biosciences) in conjunction with BD TrucountTM Tubes (BD Biosciences). Additionally, for the specific analysis of regulatory T cells ($CD4^+$, $CD25^+$, and $FoxP3^+$), the eBioscienceTM Human Regulatory T Cell Staining Kit (Cat No. 88-8999; Thermo Fisher Scientific, Carlsbad, CA, USA) was used.

The principle of the examination involves adding whole blood to the reagent, where fluorochrome-labeled antibodies in the reagent specifically bind to leukocyte surface antigens. During acquisition, these cells pass through the laser beam and scatter laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about cell size, internal complexity, and relative fluorescence intensity. Subsequently, digital data was interpreted by gating using BD FACSuiteTM Clinical software v1.1 (BD Biosciences).

Assay for HE4 and CA-125 Analysis

In vitro examination of HE4 and CA-125 was conducted using the Chemiluminescent Microparticle Immunoassay (CMIA) method by ARCHITECT i System (Abbott, Abbott Park, IL, USA). The kit utilized for HE4 detection employs anti-HE4 antibodies (Cat. 2P54-01; Abbott), while the one for CA-125 detection utilizes anti-CA-125 antibodies (Cat. 2K45; Abbott). The Architect HE4 & CA-125 reagents constitute a two-step immunoassay for the quantitative examination of HE4 & CA-125 antigens in human serum using CMIA technology with flexible protocols referring to Chemiflex.

In the initial step, the sample and paramagnetic microparticles coated with Anti-HE4 & Anti-CA-125 were reacted. HE4 and CA-125 antigens present in the sample will bind to the Anti-HE4 and Anti-CA-125 coating the microparticles. Following washing, conjugated Anti-HE4 and CA-125 labeled with acridinium were added to the reaction mixture. After the subsequent washing cycle, pre-trigger and trigger solutions are added to generate a chemiluminescent reaction measurable as Relative Light Units (RLUs). The concentrations of HE4 (pmol/L) and CA-125 (μ/mL) in the sample are proportional to the RLUs detected by the Architect optical system (Abbott).

Risk of Ovarian Malignancy Algorithm (ROMA) Score

The ROMA score, utilized for assessing the likelihood of ovarian malignancy, was derived from a synthesis of two blood tests: CA-125 and HE4, which identify specific biomarkers indicative of ovarian cancer. Incorporating the subject's menopausal status (premenopausal or postmenopausal), the algorithm integrates these biomarker results to generate the ROMA score. Higher ROMA scores signify increased malignancy likelihood, contrasting with lower scores indicating reduced risk. Clinicians leverage this score in conjunction with other clinical factors to inform decisions regarding additional diagnostic measures and ovarian mass management.

Results

Subjects' Clinical Profiles

A total of 29 patients with ovarian tumors, consisting of 12 ovarian cancer patients (malignancy feature) and 17 benign ovarian tumor patients (benign feature) (Table 1). The clinical profiles of patients, including age, contraceptive history, family history, hematological profiles and history of endometriosis, showed no strong association with ovarian cancer occurrence. Leukocyte levels were higher in benign feature subjects compared to malignancy feature subjects, while neutrophil levels were higher in malignancy feature subjects compared to benign feature subjects.

Tumor markers, namely CA-125, HE4, and ROMA, exhibited elevated levels among patients diagnosed with ovarian cancer in comparison to those with benign ovarian tumors. Additionally, there is a significant difference in HE4 (p=0.004) and ROMA score (p=0.004) between benign ovarian tumors and malignant tumors, but not with CA-125 (p=0.690). The results of ultrasonography examination using IOTA criteria as an initial screening to determine the presence or absence of tumors showed a non-significant association with the results of histopathological examination as the gold standard for ovarian cancer diagnosis (p=0.057).

Characteristics of Lymphocyte Subtypes

Characterization of lymphocytes was performed using two approaches, namely the number of cells per microliter (cells/μL) and percentage (Table 2). Lymphocyte characterization was conducted by distinguishing subtypes. The analysis results demonstrate a diferentiation T regulation (Treg: $CD4^+/CD25^+/FOXP3^+$) in benign ovarian tumor and ovarian cancer (p=0.017). However, T Cell ($CD3^+$), T Helper ($CD3^+CD4^+$), T Cytotoxic ($CD3^+CD8^+$), B cells ($CD19^+$) and NK cells ($CD3^-/CD16^+/CD56^+$) did not show significant difference between benign ovarian tumors and ovarian cancer. Meanwhile, the observed pattern was an increase in T regulatory cells and a decrease in total T lymphocytes and subtypes of T lymphocytes, namely cytotoxic T lymphocytes and T helper cells, in ovarian cancer subjects compared to benign ovarian tumor subjects.

Correlation between Tumor Marker and Lymphocyte Subtypes

The correlation between CA-125 and lymphocyte subtypes did not show any correlation. Meanwhile, HE4 only exhibited a weak negative correlation with B lymphocytes (r=-0.416, p=0.034) and Treg cells (r=0.420, p=0.033). However, when CA-125 and HE4 were combined to form ROMA, the results indicated a weak negative correlation with total T lymphocytes (r=-0.598, p=0.003), T helper lymphocytes (r=-0.594, p=0.003), and cytotoxic lymphocytes (r=-0.510, p=0.013). Additionally, ROMA also demonstrated a weak positive correlation with the percentage of B cells (r=0.528, p=0.010) and the percentage of Treg cells (r=0.553, p=0.006) (Table 3).

Table 1. Characteristics of subjects.

Characteristics	Benign Ovarian Tumor (n=17)	Ovarian Cancer (n=12)	*p*-value
Age (years), median (CI 95%)[a]	43.00 (38.28-49.24)	47.50 (37.41-52.58)	0.842
Contraception, n (%)[b]			
No contraception	12 (70.59%)	8 (66.66%)	0.617
Hormonal	4 (23.53%)	2 (16.66%)	
Intraurine devices (IUD)	1 (5.88%)	2 (16.66%)	
Family history, n (%)[b]			
Yes	2 (11.76%)	12 (100.00%)	0.218
No	15 (88.24%)	0 (0.00%)	
Endometriosis history, n (%)[b]			
Yes	1 (5.88%)	11 (91.67%)	0.798
No	16 (94.12%)	1 (8.33%)	
Ultrasonography examination (IOTA), n (%)[b]			
Benign feature	13 (76.47%)	5 (41.67%)	0.057
Malignancy feature	4 (23.53%)	7 (58.33%)	
Hemoglobin (g/dL), median (CI 95%)[a]	11.00 (9.88-11.76)	10.60 (9.58-12.41)	0.965
Thrombocyte (10^3/µL), median (CI 95%)[a]	357.00 (304.29-475.20)	348.00 (221.74-634.25)	0.116
Leucocyte (10^3/µL), median (CI 95%)[a]	7.61 (6.82-13.79)	8.06 (6.03-13.06)	0.690
Basophil (%), median (CI 95%)[a]	0.50 (0.09-2.11)	0.40 (0.24-0.55)	0.737
Eosinophil (%), median (CI 95%)[a]	1.90 (0.73-2.52)	1.4 (0.35-12.11)	0.155
Neutrophil (%), median (CI 95%)[a]	67.10 (57.91-78.06)	74.40 (56.17-87.70)	0.929
Limphocyte(%), median (CI 95%)[a]	23.90 (15.63-33.11)	15.40 (9.06-25.73)	0.877
Monocyte (%), median (CI 95%)[a]	5.15 (4.36-6.78)	5.30 (3.10-8.37)	0.465
CA-125 (U/mL), median (CI 95%)[a]	121.90 (14.82-944.59)	167.70 (42.51-1597.42)	0.626
HE4 (pmol/L), median (CI 95%)[a]	47.40 (38.10-96.64)	74.10 (27.29-957.95)	0.004**
ROMA (%), median (CI 95%)[a]	752.00 (408.15-3056.01)	2082.00 (465.72-9917.32)	0.004**

**Significant if $p<0.05$. [a]Tested with Mann-Whitney analysis. [b]Tested with Pearson Chi-square analysis.

Discussion

The initial phase involved ultrasound examination using IOTA criteria for preliminary screening to determine the status of non-tumor, benign features, and malignancy features. The IOTA criteria have not been able to differentiate between the benign feature, and malignancy feature groups as this heavily relies on the subjectivity of the operator conducting the examination. This is also demonstrated by the patients' clinical history and hematologic profiles.

CA-125, initially identified as a large transmembrane glycoprotein through the use of the OC125 monoclonal antibody on human ovarian carcinoma cell lines.(17) The results of this study indicate that CA-125 is unable to differentiate between the ovarian cancer patients and benign ovarian tumor patients. This aligns with prior studies indicating the limited specificity of CA-125 as a biomarker for early ovarian cancer detection; nonetheless, it shows strong correlation with the response to initial therapy in most cases of epithelial ovarian cancers.(18) The prevailing treatment protocol for advanced-stage ovarian cancer comprises aggressive surgical cytoreduction succeeded by combination chemotherapy based on platinum agents. This regimen leads to clinical remission in almost 80% of patients, marked by the normalization of CA-125 levels and imaging findings.(19) Following primary therapy, individuals exhibiting sustained elevation in CA-125 levels (CA-125 >35 µ/mL) are susceptible to symptomatic recurrence and disease advancement within six months, presenting a heightened mortality risk.(20) Additionally, in patients who attain clinical remission with level of CA-125 <35 µ/mL, their overall survival and progression-free survival seem to vary inversely with the absolute CA-125

Table 2. Differential analysis of lymphocyte subtypes between patients with benign ovarian tumors and ovarian cancer.

Subtype of Limphocytes	*p*-value
T lymphocyte (cell/μL)	0.790
T helper (cell/μL)	0.825
T cytotoxic (cell/μL)	0.400
B cell (cell/μL)	0.757
NK cell (cell/μL)	0.690
Regulatory T cell (Cell/μL)	0.063
T helper/cytotoxic ratio	0.757
T lymphocyte (%)	0.790
T helper (%)	0.894
T cytotoxic (%)	0.825
B cell (%)	0.595
NK cell (%)	0.452
Treg cell (%)	0.017**

**Significant if $p<0.05$. Tested with Mann–Whitney analysis.

levels.(21) Unfortunately, most of these individuals will eventually face disease recurrence within a period ranging from 2 to 5 years.(22)

Meanwhile, HE4 as an independent marker is able to differentiate between benign features and malignancy features. This indicates that in the early stages of ovarian cancer diagnosis, HE4 becomes a promising marker. Considering that ovarian cancer is manageable when diagnosed as early as possible, compared to late-stage diagnosis which indicates a poor prognosis. Meanwhile, if anatomical pathology examination is performed as the gold standard, it is more invasive and requires longer time. HE4 can be an alternative marker to avoid unnecessary surgery. Therefore, it is reasonable that several studies have suggested that HE4 is superior to CA-125. Specifically, this study also demonstrates HE4's ability to differentiate between benign and malignant diseases (*i.e.*, its sensitivity) is superior to CA-125 in ovarian cancer detection. Furthermore, when CA-125 and HE4 are combined into the ROMA score, it shows a relatively similar ability as the HE4 value.

In addition to testing established tumor markers (CA-125, HE4, and ROMA), this study also added lymphocyte subtype examinations. It is known that lymphocytes play a role in the elimination of cancer cells. This study demonstrates that Treg cells ($CD4^+/CD25^+/FOXP3^+$) provide new insights into the early diagnosis process of ovarian cancer to differentiate between patients with benign

Table 3. Correlation between tumor markers and subtype of lymphocytes based on the ROMA Score, CA-125, and HE4.

Subtype of Limphocytes	*r*	*p*-value
ROMA SCORE		
T lymphocyte (cell/μL)	-0.598*	0.003
T helper (cell/μL)	-0.594*	0.003
T cytotoxic (cell/μL)	-0.510*	0.013
B cell (cell/μL)	0.235	0.280
NK cell (cell/μL)	-0.175	0.425
Regulatory T cell (Cell/μL)	-0.050	0.822
T helper/cytotoxic ratio	0.050	0.820
T lymphocyte (%)	-0.365	0.087
T helper (%)	-0.236	0.277
T cytotoxic (%)	-0.310	0.151
B cell (%)	0.528*	0.010
NK cell (%)	-0.105	0.632
Treg cell (%)	0.553*	0.006
CA-125		
T lymphocyte (cell/μL)	0.084	0.524
T helper (cell/μL)	0.000	1.000
T cytotoxic (cell/μL)	0.099	0.453
B cell (cell/μL)	-0.116	0.378
NK cell (cell/μL)	-0.089	0.499
Regulatory T cell (Cell/μL)	-0.020	0.881
T helper/cytotoxic ratio	-0.067	0.612
T lymphocyte (%)	0.113	0.388
T helper (%)	-0.010	0.940
T cytotoxic (%)	0.108	0.409
B cell (%)	-0.123	0.348
NK cell (%)	-0.160	0.223
Treg cell (%)	0.000	1.000
HE4		
T lymphocyte (cell/μL)	-0.083	0.552
T helper (cell/μL)	-0.077	0.582
T cytotoxic (cell/μL)	-0.071	0.612
B cell (cell/μL)	0.028	0.843
NK cell (cell/μL)	-0.237	0.090
Regulatory T cell (Cell/μL)	0.194	0.165
T helper/cytotoxic ratio	0.012	0.930
T lymphocyte (%)	0.132	0.343
T helper (%)	0.071	0.612
T cytotoxic (%)	0.046	0.741
B cell (%)	0.071	0.612
NK cell (%)	-0.416*	0.034
Treg cell (%)	0.420*	0.033

*Weak correlation ($r<0.6$), **Strong correlation ($r>0.6$). All data were calculated using Spearman's rank correlation coefficient.

ovarian tumors and ovarian cancer patients. The results indicate an increase in Treg levels in patients with ovarian cancer compared to those with benign ovarian tumors. This is because Tregs have an immunogenic role that allows for immune escape from cancer cells. Ovarian cancer, as one of the immunogenic cancer types, can modulate immune cells, including recruiting Tregs to the cancer tissue. (23) Recruitment of Tregs to the cancer tissue suppresses other pro-tumor lymphocytes, especially cytotoxic T cells ($CD3^+CD8^+$). However, in subjects at early stages of cancer such as those in the study, there was no significant decrease in cytotoxic T cells observed.

In addition, this study demonstrates a correlation between the ROMA score (CA-125 and HE4 ratio) and lymphocyte subtypes. Despite falling within the weak category, this correlation may warrant further investigation. Initial findings indicate that the ROMA score correlates with elevated levels of Tregs ($CD4^+/CD25^+/FOXP3^+$) and B cells ($CD19^+$), alongside a reduction in total T lymphocytes ($CD3^+$), T helper cells ($CD3^+CD4^+$), and cytotoxic T cells ($CD3^+CD8^+$). These findings suggest potential clinical applications as alternative markers for distinguishing between benign ovarian tumors and ovarian cancer. Moreover, they highlight the possibility of a poorer prognosis in ovarian cancer patients due to immune system failure in eradicating tumor cells. However, additional studies incorporating control subjects and a larger sample size are necessary to validate these observations.

The initial findings of this study offer a new perspective in distinguishing between benign ovarian tumors and ovarian cancer, which previously necessitated invasive surgery with pathological examination and longer processing time. Further research is still warranted using larger sample sizes and adding healthy controls, as this study is limited by a small sample size, resulting in a weak correlation and the absence of healthy control subjects. With further research, the aim is to avoid unnecessary surgeries.

Conclusion

The results of this study indicate that HE4 and ROMA levels are higher in ovarian cancer patients, along with an increase in Treg cell count ($CD4^+/CD25^+/FOXP3^+$), compared to those with benign ovarian tumors. Furthermore, the correlation between tumor markers and lymphocyte subtypes in ovarian cancer suggests that increased ROMA levels weakly associate with elevated Treg ($CD4^+/CD25^+/FOXP3^+$) and B cells ($CD19^+$), coupled with a decrease in total T lymphocytes ($CD3^+$), T helper cells ($CD3^+CD4^+$), and cytotoxic T cells ($CD3^+CD8^+$), albeit statistically weakly. These findings hint at the need for further investigation, presenting intriguing preliminary indications.

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Authors Contribution

MH was involved in conceptualization, design, data collection, and served as one of the operators for ultrasonographic examination using IOTA Simple Rules. S played a critical role in revising the manuscript, providing final approval, and ensuring the integrity and accuracy of the work. IK and MF participated in manuscript drafting, revision, and final approval, taking responsibility for the integrity and accuracy of their contributions. USH and VGP contributed to interpreting the data, critically revising the manuscript, and ensuring its integrity and accuracy. All authors made contributions to the article and endorsed the final version for submission.

References

1. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: A review. Cancer Biol Med. 2017; 14(1): 9-32.
2. Evans DG, Wisely J, Clancy T, Lalloo F, Wilson M, Johnson R, *et al.* Longer term effects of the Angelina Jolie effect: increased risk-reducing mastectomy rates in BRCA carriers and other high-risk women. Breast Cancer Res. 2015; 17: 143. doi: 10.1186/s13058-015-0650-8
3. Prayudi PKA, Winata IGS, Budiana ING, Saspriyana KY, Mahendra INB, Suwiyoga K. Preoperative Level of insulin-like growth factor binding protein 2 predicts the suboptimal outcome after primary debulking surgery in patients with advance ovarian cancer. Indones Biomed J. 2023; 15(6): 429-36.
4. Ledermann JA, Raja FA, Fotopoulou C, Gonzalez-Martin A, Colombo N, Sessa C. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013; 24 (Suppl 6): vi24-32.
5. Yuane E, Dewanto A, Widad S. High expression of PR-A and low expression of PR-B is correlated with inflammation in endometrioma cases. Indones Biomed K. 2023; 15(1): 85-93.

6. Van Gorp T, Cadron I, Despierre E, Daemen A, Leunen K, Amant F, *et al.* HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the Risk of Ovarian Malignancy Algorithm. Br J Cancer. 2011; 104(5): 863-70.
7. Ahmed AA, Abdou AM. Diagnostic accuracy of CA125 and HE4 in ovarian carcinoma patients and the effect of confounders on their serum levels. Curr Probl Cancer. 2019; 43(5): 450-60.
8. Scholler N, Urban N. CA125 in ovarian cancer. Biomark Med. 2007; 1(4): 513-23.
9. Muhammad S, Antonius PA, Oktavian R, Savannah A. Rapidly growing ovarian granulosa cell tumor following complete debulking for suspected ovarian cancer with histopathology result of benign ovarian cyst. Mol Cell Biomed Sci. 2023; 7(3): 162-7.
10. Hertlein L, Stieber P, Kirschenhofer A, Krocker K, Nagel D, Lenhard M, *et al.* Human epididymis protein 4 (HE4) in benign and malignant diseases. Clin Chem Lab Med. 2012; 50(12): 2181-8.
11. Anderson NM, Simon MC. The tumor microenvironment. Curr Biol. 2020; 30(16): R921-5.
12. Oltean T, Lippens L, Lemeire K, De Tender C, Vuylsteke M, Denys H, *et al.* Association of cell death markers with tumor immune cell infiltrates after chemo-radiation in cervical cancer. Front Oncol. 2022; 12: 892813. doi: 10.3389/fonc.2022.892813
13. Zhang L, Zhang H, Huang Y, Xi X, Sun Y. Expression of immune cell markers and tumor markers in patients with cervical cancer. Int J Gynecol Cancer. 2020; 30(7): 969-74.
14. James NE, Oliver MT, Ribeiro JR, Cantillo E, Rowswell-Turner RB, Kim KK, *et al.* Human epididymis secretory protein 4 (HE4) compromises cytotoxic mononuclear cells via inducing dual specificity phosphatase 6. Front Pharmacol. 2019; 10: 216. doi: 10.3389/fphar.2019.00216.
15. Anastasi E, Farina A, Granato T, Colaiacovo F, Pucci B, Tartaglione S, *et al.* Recent insight about HE4 role in ovarian cancer oncogenesis. Int J Mol Sci. 2023; 24(13): 10479. doi: 10.3390/ijms241310479.
16. Timmerman D, Testa AC, Bourne T, Ameye L, Jurkovic D, Van Holsbeke C, *et al.* Simple ultrasound-based rules for the diagnosis of ovarian cancer. Ultrasound Obstet Gynecol. 2008; 31(6): 681-90.
17. Bast RC Jr., Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest. 1981; 68(5): 1331-7.
18. Hawkins RE, Roberts K, Wiltshaw E, Mundy J, McCready VR. The clinical correlates of serum CA125 in 169 patients with epithelial ovarian carcinoma. Br J Cancer. 1989; 60(4): 634-7.
19. Lawrie TA, Winter-Roach BA, Heus P, Kitchener HC. Adjuvant (post-surgery) chemotherapy for early stage epithelial ovarian cancer. Cochrane Database Syst Rev. 2015; 2015(12): Cd004706. doi: 10.1002/14651858.CD004706.pub5.
20. Krivak TC, Tian C, Rose GS, Armstrong DK, Maxwell GL. A Gynecologic Oncology Group Study of serum CA-125 levels in patients with stage III optimally debulked ovarian cancer treated with intraperitoneal compared to intravenous chemotherapy: an analysis of patients enrolled in GOG 172. Gynecol Oncol. 2009; 115(1): 81-5.
21. Juretzka MM, Barakat RR, Chi DS, Iasonos A, Dupont J, Abu-Rustum NR, *et al.* CA125 level as a predictor of progression-free survival and overall survival in ovarian cancer patients with surgically defined disease status prior to the initiation of intraperitoneal consolidation therapy. Gynecol Oncol. 2007; 104(1): 176-80.
22. Cannistra SA. Cancer of the ovary. N Engl J Med. 2004; 351(24): 2519-29.
23. Rodriguez GM, Galpin KJC, McCloskey CW, Vanderhyden BC. The tumor microenvironment of epithelial ovarian cancer and its influence on response to immunotherapy. Cancers. 2018; 10(8): 242. doi: 10.3390/cancers10080242.