Original

Immunopathologia Persa

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Evaluation of the relationship between CD163 positive macrophages and prognostic factors in serous ovarian tumors

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Abstract

Introduction: Ovarian cancer is the seventh most common cancer in women and is the fifth most common cause of mortality from malignancy.
Objectives: The present study was performed to evaluate the number of CD163 positive macrophages and prognostic factors in serous ovarian tumors.
Materials and Methods: In the present cross-sectional study, 43 tissue samples obtained from patients with serous ovarian tumors were selected from the pathology ward of Al-Zahra hospital, Isfahan, Iran. Then, the patients' demographic information including age, pathological results, clinical stage of the disease, degree of tissue differentiation, the number of CD163 positive macrophages, and the five-year survival rate after the surgery were recorded and evaluated.
Results: Patient age, International Federation of Gynecology and Obstetrics (FIGO) stage, histological grade, lymphatic metastasis, and the five-year survival rate were positively and significantly associated with the number of CD163 positive macrophages ($P < 0.05$). In addition, the number of CD163 positive macrophages increased the odds of the five-year survival rate in these patients by 1.490 times ($P = 0.026$).
Conclusion: According to the results of this study, the number of CD163 positive macrophages had a significant association with the progression of ovarian cancer. Moreover, the increased number of CD163 positive macrophages increased the chance of mortality in these patients.

Introduction

Ovarian cancer is the seventh most common cancer in women and is the fifth most common cause of mortality from malignancy. Ovarian cancer has a 1-1.5% chance of occurring during a woman's lifetime (1,2). The prevalence rate of ovarian epithelial tumors is reported to be 75% in serous, 20% in mucinous, 2% in endometrioid, and less than 1% in each of clear cell carcinomas and undifferentiated carcinomas (1,2). As the symptoms of ovarian cancer are non-specific in the early stages of the disease and most patients refer to the hospital in advanced stages of the disease, the survival rate of the patients is low (3,4). Hence, the significance of its early diagnosis is unquestionable. In this regard, several studies have been performed to evaluate the pertinent risk factors and determine the factors affecting the prognosis of ovarian cancer.

According to the results of these studies, the family history of ovarian cancer can be considered as one of the risk factors of this disease. Moreover, it has been revealed that

Key point

In a study on 43 tissue samples obtained from patients with serous ovarian tumors, we found CD163 positive macrophages had a significant positive relationship with patients' age, stage of disease, histological grade, lymphatic metastasis, and five-year survival rate. We also found, the mean of CD163 positive macrophages was higher in patients with ages over 50 years, lymph node metastasis, and FIGO stages of III and IV. We concluded that the number of CD163 positive macrophages might be considered as a proper prognostic factor for the incidence and progression of serous ovarian tumor.

aging has an adverse effect on the prognosis of ovarian cancer. In addition, early onset of menstruation, delayed menopause, history of infertility and ovulation-inducing medications can be mentioned as risk factors for ovarian cancer (5-7).

In addition, CD163 positive macrophages are among the macrophages that have been recently investigated in the prognosis of ovarian cancer (8, 9). CD163 is a glycoprotein belonging to scavenger *receptor cysteine-*

rich family of proteins (8, 10). The expression of CD163 macrophages is regulated by various inflammatory mediators such as interferon-γ (gamma), interleukin 6 and 10, and glucocorticoids. Moreover, it is speculated that CD163-expressing macrophages play an important role in regulating the immune response (9,11). Recently, CD163 positive macrophage has been recognized as a hemoglobin digestive molecule that performs its function by binding and purifying haptoglobin-hemoglobin complexes (12).

Many recent studies have indicated that the distribution of CD163 positive macrophages was significantly associated with ovarian cancer and the disease stage. In contrast, another array of studies has reported a lower or non-significant sensitivity of CD163 positive macrophages as compared with other macrophages such as CD68 (13-16). The total-body M2 macrophage load can be estimated by CD163 although its biological role has not been comprehensively specified (17). CD163 positive macrophages in tumor tissue were associated with poor prognosis in patients with melanoma (18). Although a pertinent study has indicated the association between the increased level of CD163 positive macrophage and poor prognosis in tissues with ovarian cancer; however, the role of CD163 in ovarian cancer has not been thoroughly attended to (19).

Objectives
The present study aimed at determining the association between prognostic factors of patients with ovarian serous carcinoma and the number of CD163 positive macrophages.

Materials and Methods
Study design
The present study was cross-sectional. The study population consisted of all tissue samples of patients with serous ovarian tumors that were provided by the pathology ward of Al-Zahra hospital, Isfahan, Iran from 2009 to 2013. The sample size formula was used at a 95% confidence interval, 80% test power, and the estimation of the prognostic correlation coefficient of patients with CD163 positive macrophages presented by previous studies as 0.3 (17). Therefore, 43 patients were selected by simple random sampling.

These patients had serous ovarian tumors and had not received any treatments in this regard, including chemotherapy, radiation therapy, or hormone therapy prior to the surgery. Their samples were evaluated by H&E staining and immunohistochemical staining. Clinical and pathological data as well as complete follow-up data were documented in their records. If the required information in the patient record was incomplete, that sample was excluded from the study and replaced with another sample. After obtaining the code of ethics from the Ethics Committee of the university, the samples meeting the inclusion criteria were selected from the pathology archive

unit of the hospital. Patient demographic information including age, pathological results, clinical stage of the disease, degree of tissue differentiation in patients with serous ovarian carcinoma and five-year survival rate after the surgery was examined and recorded in the data collection form.
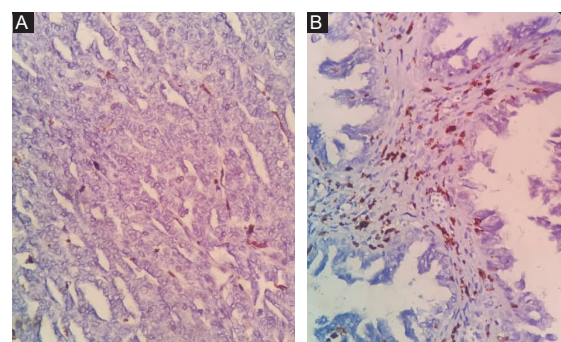
Isolation and expression of CD163
Tissues were fixed in 10% neutral-buffered formalin. Following this fixation, tissue slices were given at 4-micrometer-thick. The expression of CD163 on the edge of the tumor was determined by a two-step immunohistochemical staining. CD163 represents M2 macrophages. In the immunohistochemical staining, 4-micrometer-sections were sliced from the patients' tissue blocks. Antigen retrieval was performed using a microwave at 90°C for 15 minutes then the samples were cooled to room temperature. Non-specific binding sites were blocked with 5% bovine serum albumin (BSA) for one hour. For immunohistochemical staining, tissue sections were sequentially treated with horseradish peroxidase (HRP) using an IgG antibody (clone: LN3, ZM-0136, Zhongshan).

The antibody-binding sites were visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB; Zhongshan), and the cell nuclei were counterstained with hematoxylin.

It should be mentioned that the examinations were double-blinded and conducted by an uninformed pathologist. The required sections were reviewed as well. The nucleated cells were finally analyzed, and TAM densities were calculated as cells/mm^2 (Figure 1).

Ethical issues
This investigation was in accordance with the Declaration of Helsinki. The Ethics Committee of Isfahan University of Medical Sciences approved this study (IR.MUI. MED.REC.1398.167). This study was conducted on the relationship between CD163 positive macrophages and prognostic factors in serous ovarian tumors. Informed consent were obtained. This study also is resulted from

Figure 1. Macrophage immunoreactivity for CD163. A) Low density; ≤21 per mm2 CD163 positive macrophages (×400). B) High density; >21 per mm2 CD163 positive macrophages (×400).

the pathology residential thesis of Marjan-Sadat Hoseini-Beheshti at this university.

Statistical analysis
Finally, the collected data were entered into SPSS software (version 23). Descriptive statistics such as means, standard deviations, frequency, and percentage frequency were used. With respect to the inferential statistics, according to the results of Kolmogorov-Smirnov test indicating the abnormal distribution of data, Mann-Whitney U test, Spearman's correlation coefficient, and logistic regression were used to compare the mean number of CD163 positive macrophages, evaluate the relationship between clinical and pathological variables and CD163 positive macrophages, and assess the relationship between factors influencing the five-year survival rate, respectively. Significance level of less than 0.05 was considered in all analyses.

Results
The present study involved 43 patients with serous ovarian tumors. Regarding the age range of the patients, 15 (34.9%) patients were less than or equal to 50 years old while 28 (65.1%) patients were older than 50 years old. Moreover, the mean age of the patients was 56.05 ± 10.00 years. Around 44.2% of patients had lymphatic metastasis, since the mean number of CD163 positive macrophages was 19.51 ± 5.15 (Table 1).

The mean number of CD163 positive macrophages was significantly associated with patients' higher age range, FIGO stage, histological grade, lymphatic metastasis and five-year survival rate ($P<0.05$). The mean number of

Table 1. Clinical and pathological data of patients with serous ovarian tumors

Variables	No. (%) or Mean ± SD
Age (y)	56.05±10.00
≤50	15 (34.9)
>50	28 (65.1)
FIGO stage	
I+II	12 (27.9)
III+IV	31 (72.1)
Histological grade	
Moderately and poorly differentiation	16 (37.2)
Well differentiated	27 (62.8)
Lymphatic metastasis	
Yes	19 (44.2)
No	24 (55.8)
Five-year mortality	
Yes	27 (62.8)
No	16 (37.2)
Number of CD163 positive macrophages[a]	19.51±5.15
Low density (≤21 per mm2)	22 (51.2)
High density (>21 per mm2)	21 (48.8)

[a] The criterion for determining CD163 density was the median value.

CD163 positive macrophages in patients over 50 years old, with lymphatic metastasis, and FIGO (International Federation of Gynecology and Obstetrics) stage (III + IV) was higher than that of the patients less than 50 years old, without lymphatic metastasis, and FIGO stage (I + II) (Table 2).

Finally, the evaluation of factors affecting the five-year survival rate revealed that FIGO stage, histological grade, lymphatic metastasis, and the number of CD163 positive macrophages increased the odds of the five-year survival rate by 5750, 9680, 11 900, and 1490 times, respectively ($P<0.05$; Table 3).

Discussion
The findings of the present study revealed that CD163 positive macrophages had a significant positive relationship with the patient age, stage of disease, histological grade, lymphatic metastasis, and five-year survival rate. The mean of CD163 positive macrophages was higher in patients with ages over 50 years, lymph node metastasis and FIGO stages of III and IV.

It must be considered that factors such as FIGO stage, histological grade, lymph node metastasis, and the number of CD163 positive macrophages can increase the chance of mortality. Since stage of disease and severity of disease progression have a significant role in mortality and survival rate of these patients, however CD163 macrophage can also have a significant role in mortality rate.

Consistent with the objectives of the present study, the study performed by Yafei et al also addressed the role of CD163 macrophages on the prognosis of ovarian cancer patients. They indicated a significant relationship between the distribution of CD163 positive macrophages and the incidence of ovarian cancer and the stage of disease (13).

In fact, recent evidence in this regard indicates that inflammation is associated with many types of cancers. Actually, ovulation-induced chronic inflammation is one of the carcinogenic mechanisms in ovarian cancer, therefore immune cells can play a role in the initiation of ovarian cancer by producing reactive oxygen species and promoting genetic instability (20-22).

No et al revealed that the level of soluble CD163 was associated with the presence of positive malignant cells in the lesion fluid and advanced stage of ovarian cancer. The mentioned finding indicated a relationship between tumorigenesis and CD163 (23). This finding was associated with the role of macrophages in enhancing tumor initiation and proliferation of metastatic cells (24).

In addition, the results of an animal model indicated that macrophage colony-stimulating factor increased the distant and nodal metastasis with a degree of an increase in tumor burden (25).

Another study showed that pelvic peritoneal tissue in patients with ovarian cancer as compared to peritoneal tissue in patients with benign pelvic diseases, showed a significant permeability to macrophages (26). Given

Table 2. Determination and comparison of the mean and association of CD163 positive macrophages with clinical and pathological findings in patients with serous ovarian tumors

| Variables | The number of CD163 positive macrophages | | | Correlation | P value |
	Total	Low density (≤21 per mm²)	High density (>21 per mm²)		
Age (y)					
≤50	17.07±6.24	10 (45.5%)	5 (23.8%)	0.358	0.047
>50	20.82±3.99	12 (54.5%)	16 (76.2%)		
FIGO stage					
I+II	15.58±5.21	10 (45.5%)	2 (9.5%)	0.436	0.016
III+IV	21.03±4.31	12 (54.5%)	19 (90.5%)		
Histological grade					
Moderately and poorly differentiation	15.69±4.59	14 (63.6%)	2 (9.5%)	0.575	<0.001
Well differentiated	21.78±4.04	8 (36.4%)	19 (90.5%)		
Lymphatic metastasis					
Yes	22.79±2.66	4 (18.2%)	15 (71.4%)	0.579	0.001
No	16.92±5.19	18 (81.8%)	6 (28.6%)		
Five-year mortality					
Yes	22.11±3.59	8 (36.4%)	19 (90.5%)	0.646	<0.001
No	15.13±4.36	14 (63.6%)	2 (9.5%)		

Table 3. Results of logistic regression evaluating the factors affecting the five-year survival rate in patients with serous ovarian tumors

Factors	B	Standard error	Odds ratio (95% CI)	P value
Age (y)				
≤50	-	-	Reference	
>50	0.614	0.657	1.847(0.510-6.695)	0.350
FIGO stage				
I+II	-	-	Reference	
III+IV	1.749	0.737	5.750(1.356-24.388)	0.018
Histological grade				
Moderately and poorly differentiation	-	-	Reference	
Well differentiated	2.270	0.732	9.680(2.304-40.669)	0.002
Lymphatic metastasis				
No	-	-	Reference	
Yes	2.477	0.855	11.900(2.229-63.524)	0.004
The number of CD163 positive macrophages	0.399	0.114	1.490(1.192-1.863)	0.026

the peritoneal propensity for seeding to act as a central mechanism in ovarian cancer metastasis, these findings may identify and justify the association between sCD163, advanced disease stage, and poor prognosis. However, the functional role of CD163 positive macrophages in epithelial ovarian cancer requires further clarification.

Kawamura et al expressed that the number of CD163 positive macrophages in tumor tissues was associated with the degree of malignancy in epithelial ovarian cancer (27). Increased shedding of sCD163 was due to inflammatory stimuli, and sCD163 was a valuable marker for macrophage activation. However, there was no evidence that sCD163 originated from tumor-associated macrophage (TAM) in tumor tissue of cancer patients. In addition, a preliminary study showed no association between macrophage infiltration and serum sCD163 levels in melanoma patients, which would indicate the association of sCD163 levels with infectious co-morbidity (18).

No et al stated that sCD163 in ovarian cancer patients originated from TAM because serum sCD163 levels were not associated with either white blood cell count or the percentage of segmented neutrophils (23). Although the role of sCD163 in ovarian cancer has not yet been elucidated, the association between TAM and sCD163 may be an explanation for the prognostic effect of sCD163 in ovarian cancer patients. Cytokines and growth factors released by tumor cells may play a role in elevating sCD163 in patients with advanced stage tumors. The relationship between poor prognosis and elevated serum sCD163 levels in ovarian cancer requires further examinations.

Although the present study did not evaluate the mean serum level of sCD163 in ovarian cancer patients as compared with healthy subjects, many previous studies have considered the serum level of this macrophage in ovarian cancer patients to be the same as that of the healthy controls (28,29). Accordingly, it may be argued that the serum sCD163 acts as a screening marker in the diagnosis of ovarian cancer and the elevated sCD163 levels are associated with a prognosis in patients with ovarian cancer. However, further studies on the importance of

sCD163 in ovarian cancer seem to be of great value.

Conclusion

According to the results of this study, the number of CD163 positive macrophages was directly and significantly associated with the progression of ovarian cancer, histological grade, and lymphatic metastasis, hence this factor could be significantly related to metastasis and disease progression. In addition to other factors affecting the mortality rate, an increase in the number of CD163 positive macrophages can also significantly increase the chance of mortality in these patients. Therefore, this factor may be considered as a proper prognosis in relation to the incidence and progression of serous ovarian tumor. Moreover, this factor can be even taken into consideration in the prevention of mortality among the target patients.

Limitations of the study

The limitation of the present study was relatively small sample size. Further investigation on this subject suggests.

Authors' contribution
Conception and design; MH and MSHB. Literature search and Data acquisition; MSHB. Drafting the manuscript; MD. Analysis and interpretation of data, critical revision of the manuscript for important intellectual content; MH, MSHB and MD. All authors read and approved the final paper.

Conflicts of interest
The authors declare that there are no conflicts of interest regarding the publication of this article.

Ethical considerations
Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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