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Original Article

The value of FS, NLR, and CA-125 in the diagnosis of endometriosis

Gongxiang Li[1], Zhaopeng Yu[1], Ke Li[2]

[1]Department of Clinical Laboratory, People's Hospital of Linyi City, Linyi 276000, Shandong, China; [2]Department of Medical Oncology, Third Affiliated Hospital of Kunming Medical Univesity, Tumor Hospital of Yunnan Province, Kunming 650118, Yunnan Province, China

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Abstract: Numerous researches focused on the diagnosis of endometriosis (EMT) through biochemical or tumor marker. This study aimed to explore the value of NLR, CA-125, and follistatin (FS) in EMT diagnosis. 100 EMT patients, 60 benign tumor patients, and 100 healthy women were enrolled as observation group, benign tumor group, and normal control respectively. Peripheral venous blood was collected at the third day after menstruation. Neutrophils and lymphocytes were detected and their ratio (NLR) was calculated. CA-125 was tested by automatic chemiluminescence analysis system. Enzyme-linked immunosorbent assay was used to detect FS level. The sensitivity and specificity of FS, NLR and CA-125 combined detection were evaluated. FS, NLR, and CA-125 levels in observation group were significantly higher than that in benign tumor group and healthy controls ($P < 0.05$). ROC curve analysis showed that the sensitivity of FS, NLR, and CA-125 in the diagnosis of EMT were 86.7%, 72.9%, and 60.9%, respectively. While their specificities were 89.9%, 60.7%, and 89.9%, respectively. The sensitivity and specificity of FS were both better than that of NLR and CA-125. Three markers' combined detection showed obviously better sensitivity and specificity than two markers' combination, but with no significant difference to FS. Correlation analysis revealed that three markers' combined diagnosis has significant correlation with EMT ($r = 0.782$, $P < 0.001$). Single FS detection presented high sensitivity, specificity, and relevance to EMT. It has high value in clinic and combining CA-125 can provide more sufficient basis. NLR showed no significant sensitivity, specificity, and correlation to EMT diagnosis.

Keywords: Follistatin, NLR, CA-125, endometriosis

Introduction

Endometriosis (EMT) refers to the endometrium with growth function appeared out of the uterine cavity covered by mucous membrane, such as pelvic peritoneum and ovaries. It is one of the most common diseases in gynecology. Since it is featured as infiltrating growth, destroying the surrounding tissue, and distant metastasis, and often gives rise to pain and infertility, it can cause serious influence to the patient's quality of life. It may affect 8~10% women in child-bearing period, while about 30% of the primary and secondary infertility were closely related to EMT. Furthermore, the incidence of EMT related infertility increased year by year [1]. It was found that the incidence of EMT in infertile women were as much as 20%-50% [2]. Laparoscopic examination with biopsy is the gold standard for clinical diagnosis [3], and other auxiliary methods include ultrasound, clinical history and physical examination. However, laparoscopic is an invasive operation with shortcoming of complicated process, high requirements for the operator, and expensive. It is necessary to seek a more safe with high specificity and sensitivity marker for EMT diagnosis. Currently, it was thought that FS, NLR, and CA-125 have great impact on EMT diagnosis-Darai considered that NLR elevation revealed that EMT induced local and systemic inflammatory response [4]; Florio thought that FS can be used as reliable marker for accurately diagnosis of EMT [5]; Kim SK found that NLR and CA-125 have high correlation with EMT, while combined detection showed obviously diagnostic significance [6]. Single FS, NLR, or CA-125 has diagnostic significance to EMT, while there is still lack of investigation about their combined detection in EMT. Our study

Table 1. Basic information comparison (Mean ± SD)

Group	Cases	Age (y)	Height (m)	Weight (kg)	Period (d)	Menstrual cycle (d)
EMT observation group	100	35.26±3.68	1.57±0.141	51.46±1.34	5.12±1.31	29.78±2.16
Benign tumor group	60	37.41±5.25	1.57±0.364	50.02±3.21	5.69±1.33	29.66±1.95
Healthy control	100	36.58±4.23	1.59±0.268	50.58±2.54	5.07±1.47	29.35±2.05
F value		0.231	0.102	0.082	0.032	0.141
P value		0.794	0.960	0.943	0.984	0.863

Table 2. FS, NLR, and CA-125 levels comparison (Mean ± SD)

	EMT observation group (n = 100)	Benign tumor group (n = 60)	Healthy control (n = 100)
FS (ng/L)	1366.14±285.54*	418.27±58.39	77.12±36.97
NLR (×109)	2.43±0.29*	1.64±0.65	1.48±0.52
CA-125 (IU/ml)	136±34.12*	79.15±26.48	63.40±31.57

*P < 0.05, compared with benign tumor group and healthy control.

tended to explore FS, NLR, and CA-125 values in EMT diagnosis, and compare their relevance and accuracy.

Materials and methods

General information

100 EMT patients, 60 benign tumor patients, and 100 healthy women in People's Hospital of Linyi City between Jul 1, 2013 and Jan 1, 2015 were enrolled as observation group, benign tumor group, and normal control. Exclusion criteria include: (1) patients with severe underlying disease (severe heart, liver, kidney dysfunction); (2) patients with severe endocrine disorder; (3) patients complicated with acute inflammation, malignant tumor, and uterine leiomyoma or uterine glandular myopathy; (4) patients received hormone or other endocrine therapy in three months. This study had been approved by the clinical research ethics committee of People's Hospital of Linyi City. All patients or family members had signed the informed consent. The basic information in three groups showed no significant difference (P > 0.05) (**Table 1**).

Methods

Blood specimen collection and detection: 5 ml blood was extracted from the elbow vein at the third day after menstruation and centrifuged at 1000 rpm for 5 min. The serum was stored at -80°C. PENTRA60ABX globulimeter was applied to detect neutrophils and lymphocytes for calculation of the ratio (NLR). Automatic chemiluminescence analysis system was used to test CA-125. ELISA was performed to determine FS (FS detection kit, Beijing Biofine Biological Science and Technology Company). The value exceeded the normal reference was considered positive. Any one value larger than reference in combined diagnosis was considered positive.

Statistical analysis

All statistical analyses were performed using SPSS19.0 software (Chicago, IL). Numerical data were presented as means and standard deviation (Mean ± SDS), while enumeration data were presented as percentage. Differences between multiple groups were analyzed by one-way ANOVA with post hoc detection, or Student's t test. P < 0.05 was considered as significant difference. Receiver operator characteristic (ROC) curve was used to compare sensitivity and specificity of EMT diagnosis. Area under the curve (AUC) represented the ability to distinguish EMT from control (benign tumor group and healthy control), and AUC = 0.5 indicated randomized distinguish. Spearman analysis was used for correlation analysis.

Results

As shown in **Table 1**, basic information comparison showed that no significant difference was found regarding age, height, weight, period, and menstrual cycle (*P* > 0.05).

FS, NLR, and CA-125 levels comparison among three groups

FS, NLR, and CA-125 levels in EMT observation group were obviously higher than that in benign

Table 3. Comparison of three markers single or combined detection

Item	Sensitivity (%)	Specificity (%)	AUC (95% CI)
FS	86.7	89.9	0.854 (0.837-0.882)
NLR	72.9	60.7	0.618 (0.588-0.652)
CA-125	60.9	75.4	0.723 (0.663-0.762)
FS+NLR	77.5	67.1	0.756 (0.547-0.782)
FS+CA-125	88.5	65.3	0.852 (0.821-0.885)
NLR+CA-125	73.1	62.1	0.682 (0.632-0.712)
FS+NLR+CA-125	92.1	86.2	0.812 (0.737-0.865)

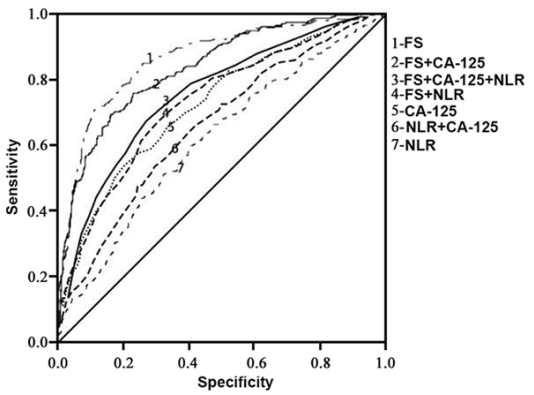

Figure 1. ROC curve of three markers single or combined detection.

Table 4. Correlation analysis of FS, NLR, and CA-125 single or combined detection in EMT diagnosis

	r	*P*
FS	0.815	< 0.001*
NLR	0.215	0.126
CA-125	0.674	< 0.001*
Combined	0.782	< 0.001*

*Indicates significance.

tumor group and healthy control ($P < 0.05$). No significant difference was observed between benign tumor group and healthy control ($P > 0.05$) (**Table 2**).

Sensitivity and specificity analysis of FS, NLR, and CA-125 in EMT diagnosis

ROC curve analysis showed that the sensitivity of FS, NLR, and CA-125 in EMT diagnosis were 86.7%, 72.9%, and 60.9%, respectively. While the specificity were 89.9%, 60.7%, and 89.9% respectively. During combined detection, related marker value was multiplied to the other according to the method reported by Fernando M. Reis et al. [7]. Following the increase of combined detection project, the sensitivity was increased, while the specificity was reduced. FS showed better sensitivity and specificity than NLR and CA-125. Three markers combined detection presented significantly higher sensitivity and specificity than any two markers combined detection in EMT diagnosis, but showed no obvious difference compared with FS (**Table 3**; **Figure 1**).

Correlation analysis of FS, NLR, and CA-125 single or combined detection

The results revealed that NLR showed no significant correlation with EMT diagnosis ($r = 0.215$, $P = 0.126$). FS and CA-125 presented better correlation than NLR ($r = 0.815$, $P < 0.001$; $r = 0.674$, $P < 0.001$). The correlation of three markers combined detection ($r = 0.782$, $P < 0.001$) was higher than CA-125 but lower than FS. Combining sensitivity and specificity analysis showed FS was an important parameter for EMT diagnosis (**Table 4**).

Discussion

Serum markers assisting EMT diagnosis are the research trend at present. Of which FS, NLR, and CA-125 received more attention as indicators of hormone, inflammation, and tumor [4-6, 8, 9]. EMT is a process of inflammatory response associated with immune cell dysregulation. As an indicator of the inflammatory response [10], a number of studies have shown that NLR level was elevated in EMT patients [11, 12], and significantly higher than that in benign tumor group and healthy controls. It is suggested that NLR was a marked feature of abnormal inflammatory response and immune suppression in EMT. However, NLR also can be used as a prognostic indicator for patients with systemic inflammatory response and other diseases, such as coronary heart disease, myocardial infarction, lung cancer, ovarian cancer, and colorectal cancer [13-17]. CA-125 is a type of tumor associated antigen which belongs to non-specific antigen and is mainly used for the diagnosis of gynecological tumors [18]. Some reports have confirmed that serum CA-125 level was correlated with EMT severity. Thus, CA-125 has certain advantages as an EMT screening marker [8]. FS is a member of the

family of transforming growth factor - β that is antagonistic with activin. Torres and Rombaut reported that FS is mainly located in the glandular epithelium cytoplasm of ectopic endometrial, and its level was increased significantly in EMT patients [19, 20]. Consistent with these observations mentioned above, our study demonstrated that FS, NLR and CA-125 levels in EMT patients were significantly higher than that in benign tumor group and healthy control ($P < 0.05$).

Furthermore, our study has also compared the sensitivity, specificity and correlation of FS, NLR, and CA-125 single or combined detection in EMT diagnosis. At present, serum CA-125 has been used in assisting EMT diagnosis, but our results showed that its specificity and sensitivity were lower than NLR and FS, suggesting the limitation of single CA-125 detection and importance of FS for assisting. Correlation analysis showed that there was no obvious correlation between NLR and EMT diagnosis ($r = 0.215$, $P = 0.215$), whereas FS and CA-125 had better correlation with EMT than NLR. Therefore, NLR cannot be treated as a single diagnosis indicator for EMT, and this is consistent with the clinical practice. NLR is associated with a variety of diseases such as tumor and metabolic syndrome and its elevation alone is difficult for EMT diagnosis. However, most of the EMT patients showed increased NLR value. A Meta-analysis revealed that the sensitivity and specificity of serum CA-125 were significant in ovarian cancer, but its sensitivity and accuracy were lower in EMT [9], which further displayed its limitation in EMT diagnosis. More researches considered that CA-125 can be used as a monitoring indicator for EMT progression and prognosis [21]. FS presented higher sensitivity in EMT diagnosis than serum CA-125 (86.7% vs. 60.9%), and higher specificity than NLR (89.9% vs. 60.7%), which is consistent with Florio P's report [22]. However, FS level is also increased in inflammation and ovarian benign tumor. It was reported that most of EMT belongs to the ovaries type, and FS has high sensitivity and specificity for ovarian EMT. For non-ovarian EMT, its independent diagnosis may have certain risk. Our study did not focus on diagnosis accuracy, EMT classification, and the severity of disease. Therefore, though "necessary" combined detection for early EMT or non-ovarian EMT may slightly elevate sensitivity but decrease specificity. The overall accuracy may increase, but it needs further clinical trials to confirm.

So far, a number of biomarkers combined diagnosis has become a key in numerous disease diagnosis and treatment. S. Cho. H et al. [23] found that NLR and CA-125 combined detection can significantly increase diagnostic sensitivity (69.3%), which was better than that NLR (59.7%) or CA-125 (55.8%) single diagnosis. Furthermore, two indicators combined detection also presented good specificity. In this study, we found that FS is an important indicator for EMT diagnosis with high sensitivity and specificity. Three indicators combined diagnosis showed highest sensitivity that was better than S. Cho. H's results. Though the specificity was slightly decreased because of the indicators' increase, it was still higher than NLR or CA-125 single or combined detection but not FS. Three indicators combined detection showed significant correlation with EMT diagnosis ($r = 0.782$, $P < 0.001$), but lower than that FS. Therefore, we could preliminarily choose the three indicators in clinic to reach high sensitivity and specificity which was helpful for EMT early diagnosis and treatment.

In conclusion, FS, NLR, and CA-125 all can be used as diagnostic indicator for EMT. FS single detection showed high specificity, sensitivity, and correlation with EMT diagnosis when considering the cost and effectiveness. Its combination with CA0125 can provide more sufficient basis for diagnosis. The sensitivity, specificity and correlation of NLR are not significant in EMT diagnosis.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ke Li, Department of Medical Oncology, Third Affiliated Hospital of Kunming Medical University, Tumor Hospital of Yunnan Province, Kunming 650118, Yunnan Province, China. Tel: +86-0871-68185656; Fax: +86-0871-68185656; E-mail: likelivek@sina.com

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