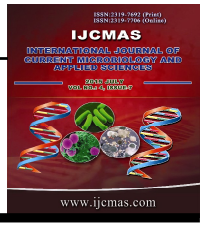
Int.J.Curr.Microbiol.App.Sci (2015) 4(7): 441-450

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 441-450**
http://www.ijcmas.com



Original Research Article

Potential of the Blood Neutrophil to Lymphocyte Ratio, the Serum Interleukin-6, and the Fibroblast Growth Factor-2 in the Diagnosis of Endometriosis

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Keywords

Fibroblast growth factor-2, Interleukin-6, Markers for endometriosis, Neutrophil/ lymphocyte ratio

A B S T R A C T

The main aim of this study to test the diagnostic potential of NLR, IL-6 and FGF-2, both individually and in a combined score, as markers for endometriosis. These markers were measured in eighty patients undergoing laparoscopy at Ain Shams University Women's Hospital. Group A included 40 sub-fertile women with endometriosis and Group B included 40 sub-fertile women without endometriosis. Group A had statistically highly significant higher level of NLR, IL-6 and FGF-2 compared to group B. The Receiver operating characteristic (ROC) Curve of IL-6 for predicting endometriosis had the highest area under the curve (AUC), of 0.85 with a cutoff of 2.8 pg/ml. It showed a sensitivity and specificity of 80% and 77.5%, respectively. It was followed by NLR with an AUC of 0.74 at a cutoff of 1.11 and a sensitivity and specificity of 92.5% and 50%, respectively. FGF-2 had an AUC of 0.77 at a cutoff of 7.7 with a sensitivity and specificity of 67.5% and 82.5%, respectively. The combined marker, at a cutoff of 3.1, had the best sensitivity (97%) and a high AUC of 0.85. Logistic regression analysis showed that an increase in the NLR by one, in the serum level of IL-6 or in serum level of FGF-2 by one unit (pg/ml) was associated with increasing the likelihood of endometriosis by 8.13, 21.44 or 6.99, respectively. The suggested blood tests are useful, practical, easily available and reliable tests in the diagnostic workup of

Introduction

Endometriosis is a polygenic disease which has a multifactorial etiology. It is diagnosed in 10%-15% of women during the reproductive age.[1, 2] Today, the implantation theory of Sampson[3] is the most widely accepted etiology of this disease.[4] Recent technical progress in transvaginal ultra-sonography[5] and magnetic resonance imaging[6] improved diagnostic accuracy. These tools, together with symptoms and pelvic examination, appear to detect ovarian endometriomas with a high sensitivity and specificity; however, current non-invasive techniques are still limited in detecting

extraovarian lesions.[7] Given such a situation, a serological test that can reliably detect endometriosis would be very useful.

Interest has recently focused on a possible role of the immune system in the development of endometriosis. Activated peritoneal macrophages and their secreted growth factors and cytokines induce proliferation on the endometrial implants which suggests an inflammatory pathogenic cascade.[8, 9]

Those factors play a critical role in decreased immunologic surveillance, recognition and destruction of ectopic endometrial cells and possible facilitation of the implantation of ectopic endometrial tissues.

Many of these immunologic changes can be demonstrated both locally within the peritoneal cavity and systemically in the circulation. This has been taken as evidence that endometriosis is in many ways a systemic disease.[4]

Although peritoneal fluid is a good material to study the pathophysiology of endometriosis, it is not only difficult and invasive to obtain from patients but also easily contaminated with blood. Therefore, development of a serum marker for endometriosis would be valuable for clinical purposes as it reduces the costs and possible morbidity of the diagnostic procedures currently used.[10]

Cytokines, particularly IL-6,[11, 12, 13, 14, 15] and growth factors such as fibroblast growth factor[16](FGF-2) have been shown to differ between women with endometriosis compared with controls. Decreased proliferation of peripheral blood lymphocytes in response to recognition of endometrial cells and antigens has also been reported.[17, 18, 19]

Neutrophilia, accompanied by a relative lymphocytopenia, yielded an increased NLR in patients with endometriosis.[19]In this study, we evaluated NLR, serum IL-6 and FGF-2 in patients with endometriosis and investigated whether these markers can be used reliably as diagnostic markers for endometriosis.

Materials and Methods

This was an observational study including 80 patients who underwent laparoscopy over a period of nine months at Ain Shams University Women's Hospital for evaluation of their infertility. The protocol of this study was approved by the ethics committee of the faculty of medicine in Ain Shams University. Patients were allocated into one of two groups on the basis of their postsurgical diagnosis. Statistical consultation advised that forty patients with endometriosis (group A) and forty women without the disease (group B; patients having other causes of infertility) need to be included in the study to detect a significance level (α) of 0.05 and power of 0.9 ($\beta = 0.1$). They were randomly selected from the patients presenting for laparoscopy during the nine months of the study. Inclusion criteria consisted of patients in the reproductive age (20-44 years) who underwent laparoscopy for evaluation of infertility.

All participants had laparoscopy during the proliferative phase (determined as the first 12 days of the menstrual cycle) of a regular menstrual cycle.

Exclusion criteria included 1) administration of any hormonal treatment over the previous 3 months, as it could influence the progression of endometriosis as gonadotrophin releasing hormone analogues, danazol and combined oral contraceptive

pills, 2) Processes that could increase the levels of IL−6 in blood, independently of endometriosis (ovarian cysts of any kind, pelvic inflammatory disease or neoplasms). 3) Suspected or ascertained diagnosis of malignancy, 4) Two or more concomitant causes of infertility diagnosed at laparoscopy, 5) Presence of any general autoimmune disease, active inflammatory disease or elevated erythrocyte sedimentation rate (ESR) on admission or 6) Refusal to participate in the study. Written informed consent for the collection of clinical information and peripheral blood was obtained before surgery from all patients.

On laparoscopy, any endometriosis was graded using the American Society of Reproductive Medicine (ASRM) revised classification, [20]and the severity of the disease was classified as minimal/mild (stages I and II) or moderate/severe (stages III and IV). Biopsies of endometriosis were prepared for histopathologic examination. For measurement of the ESR, 0.8 ml blood was collected into a Seditainer tube with 0.2 ml buffered sodium citrate solution. Then, 4 ml of blood were drawn into Vacutainer tubes for biochemical analysis.

The quantitative detection of Interleukin (IL)-6 and FGF-2 levels in patients' serum was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit provided by (R&D Systems Inc., Minneapolis, USA) with a sensitivity of <0.7 and 3pg/ml, respectively. Differential white blood cell (WBC) counts obtained on a complete blood count as part of the preoperative workup were recorded. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count.

Threshold values, sensitivity and specificity of the proposed marker were calculated using receiver operating characteristic (ROC) curves. The area under the ROC curve (AUC) was calculated as a measure of the ability of each potential marker to discriminate between endometriosis cases (group A) and noncases (group B). An AUC of 0.5 indicated classifications assigned by chance. In order to determine which marker could best distinguish the patients with endometriosis from those without, a multivariate logistic regression was performed. Statistical significance was calculated using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Among the 40 endometriosis patients studied, 25 had minimal/mild disease and 15 had moderate/severe disease. Of the 40 patients in group B, 18 were diagnosed with normal laparoscopic findings (6 patients with male infertility and 12 with unexplained infertility), 19 with polycystic ovaries, 2 patients had a bicornuate uterus and one patient had subseptate uterus, diagnosed on concomitant hysteroscopy. ESR in all patients revealed similar values (statistically non-significant) between the two groups. There was no significant statistical difference between participants with respect to their age or duration of infertility. The mean age (+/-SD) for endometriosis cases was 27.95 years (+/- 6.18) while for other cases, it was 26.35 years (+/- 5.02) (*p*= 0.207). The mean (+/- SD) duration of infertility was 3.90 years (+/- 3.62) in the endometriosis group and 3.66 years (+/- 2.28) in the other group (*p*= 0.729).

Also, there was no significant statistical difference detected (p= 0.254) between both groups as regards the type of infertility (either primary or secondary). Primary infertility formed 67.5% of the endometriosis group and 52.5% of the other group.

Endometriosis group (group A) had a significantly higher mean and median values of NLR and IL-6 compared to group B (Table 1). While the mean value of FGF-2 was not significantly different than that of group B, because some of its serum values were very elevated (off scale), skewing the mean value of FGF-2, the median value compensated for this drawback and showed highly significant difference between the two groups.

For the differential diagnosis between patients with and without endometriosis, table 2 shows the AUCs and figure 1 shows the ROC curves for each of the three markers together with that of the combined marker, obtained by multiplying IL-6 levels by NLR (the two tests whose best cut-offs had the highest sensitivity). The combined marker had a very high sensitivity (97%) and a reasonable specificity (75%). So whenever the test is negative, a laparoscopy would be of little benefit to the patient, but if the test is positive, there could be a significant rate of overdiagnosis which could impact on treatment, and a laparoscopy needs to be done. The endometriosis group was also evaluated according to the severity of the disease. Table 3 showed no significant differences in the levels of NLR, IL-6 and FGF-2 between patients with minimal-to-moderate disease (stages I and II) and moderate-to-severe disease (stages III and IV).

To determine whether serum markers could identify the patients with or without endometriosis, a stepwise logistic regression analysis was performed. Then the variables were taken in the logistic regression model as measured, it could distinguish between endometriosis patients and patients with other causes of infertility with a sensitivity of 67.5% (0.58 – 0.75) and specificity of 82.5% (0.72 – 0.90). The equation calculated out of that logistic regression curve was as follows:

$$p = \frac{1}{1+e^{-Y}}$$

$$(Y = -5.544 + \text{NLR} \times 1.032 + \text{IL-6} \times 1.301)$$

Where p is the probability that a particular subject would have endometriosis, e is a mathematical constant, called Euler's number (equal to 2.7 approximately).

As shown in table 4, an increase in the NLR by one or in serum level of IL-6 by one unit (pg/ml) was associated with increasing the likelihood of endometriosis by 2.81% or 3.68%, respectively.

Categorization of the different markers according to the best cutoff values obtained by the ROC curves was also done, in case the assumption that linearity between the risk of diagnosing endometriosis and level of markers may be violated. When the variables are categorized in the logistic regression model by the best cutoff values obtained by the ROC curves, it could distinguish between endometriosis patients and patients with other causes of infertility with a sensitivity of 92.5% (0.84 – 0.97) and specificity of 77.5% (0.66 – 0.82). The equation calculated out of that logistic regression curve is as follows:

$$p = \frac{1}{1+e^{-Y}}$$

$$(Y = -3.987 + \text{NLR} \times 2.095 + \text{IL-6} \times 3.065 + \text{FGF-2} \times 1.944)$$

As shown in table 5, an increase in the best cutoff level of NLR by one, of serum IL-6 or FGF-2 by one unit (pg/ ml) was associated with increasing the likelihood of endometriosis by 8.13%, 21.44% or 6.99%, respectively.

Currently, the gold standard for diagnosis of endometriosis is laparoscopic inspection of the abdominal cavity and histological demonstration of lesions.[21, 22] Current evidence suggests that endometriosis induces local and systemic inflammatory processes. Numerous studies have focused on markers of inflammation in an effort to find less invasive methods for diagnosing endometriosis.[23, 24, 25, 26]

It was postulated that activated macrophages can promote the proliferation, adhesion, and vascularization of endometriotic lesions by the secretion of several molecules.[27]Cytokines, particularly IL-6,[11, 12, 13, 15] and growth factors such as FGF−2[16, 28]have been shown to differ between women with endometriosis compared with controls. Decreased proliferation of peripheral blood lymphocytes in response to recognition of endometrial cells and antigens has also been reported.[17, 18, 19]The current study reached a similar observation as that of Cho et al.[19] which is that there is an increased NLR in patients with endometriosis. Yet, the latter study was a retrospective one and their entire control group (n = 384) did not undergo laparoscopy to rule out any possible pathology that may affect the NLR. But the current study is a prospective one and misclassification bias was limited by confirmation with laparoscopy the presence or absence of endometriosis in all study subjects. Discrepancy between the current study results and those of Somigliana et al.[29] who found no value for serum IL-6 in the diagnosis of endometriosis, may be due to differences in the strictness of exclusion criteria. The presence of patients with non-endometriotic ovarian cysts and/or with pelvic inflammatory disease may have reduced the sensitivity of IL-6 in Somigliana series as these pathologies have been reported to be associated with increased serum levels of this molecule[30]and should thus have been ruled out from their datasheet (or analysed in a separate subgroup).

In the current study, elevated serum IL-6 in women with endometriosis was noted, but did not distinguish the various stages of the disease. This finding confirms findings of another report[23]that IL-6 levels should be viewed as a qualitative diagnostic test rather than a quantitative test of severity.

A previous study has observed significantly higher levels of IL-6 in minimal–mild endometriosis,[13]while another study showed significantly higher levels of IL-6 in moderate–severe endometriosis.[15]The latter employed the assay during the secretory phase of the menstrual cycle and used different commercially available kits than the ones used in the current study which uses serum, rather than plasma assays. Both factors can play a role into the different results obtained.[13, 15]Thus, the effect of the stage of endometriosis on the predictive value of the test is still a debatable subject which still needs further studies to be clarified.

FGF-2 has been found in the serum[16, 28]and peritoneal fluid[31]from women with endometriosis. In the current study, the mean serum level of FGF-2 showed a non-significant difference between group A and B as some of its serum values were out of scale. But the median value compensated for this drawback and showed highly significant difference in the serum level of FGF-2 between both groups.

Table.2 Cutoff values of the NLR, IL-6, FGF-2 and combined marker for screening of endometriosis

	Cutoff value	AUC (95%CI)	Sensitivity (%)	Specificity (%)
NLR	1.11	0.74 (0.62-0.85)	92.5	50.0
IL-6	2.8 pg/ml	0.85 (0.77-0.93)	80.0	77.5
FGF-2	7.7 pg/ml	0.77 (0.66-0.88)	67.5	82.5
Combined	3.1	0.85 (0.78 -0.90)	97	75

Table.3 Relation between disease severity and levels of the studied markers

	Stage 1& 2 Median (IQR)	Stage 3 & 4 Median (IQR)	p value	95% CI
NLR	1.5 (0.96-3.5)	1.6 (1.1-3.6)	>0.05	0.56-0.35
IL-6 (pg/ml)	3.7 (2.2-440)	3.1 (2.2-450)	>0.05	108-65
FGF-2 (pg/ml)	9 (4-40)	9.5 (3-150)	>0.05	32-3.8
p value >0.05 = non-significant				

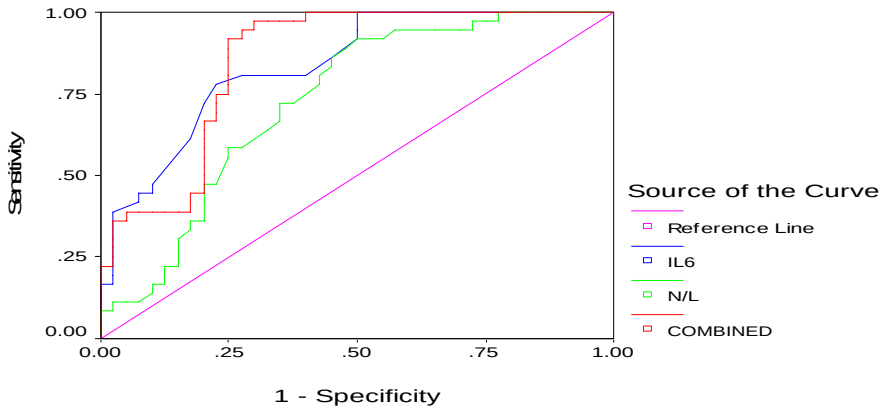
Table.4 Variables in the equation of the logistic regression model according to the level of different variables

		p value	Exp(B)	95.0% C.I. for Exp(B)
Step 1 (a)	NLR	0.027	2.871	1.131-7.287
	IL-6	0.002	3.699	1.609-8.504
	FGF-2	0.867	0.999	0.987-1.011
	Constant	0.000	0.004	
Step 2 (a)	NLR	0.023	2.81	1.150-6.846
	IL-6	0.002	3.65	1.608-8.393
	Constant	<0.001	0.004	
Exp(B)= the increase in the odds of finding endometriosis for every unit increase of the designated variable.				

Table.5 Variables in the equation of the logistic regression model according to the best cutoff level

	p value	Exp(B)	95.0% C.I. for Exp(B)
NLR	0.02	8.13	1.40-47.13
IL-6	<0.001	21.44	4.93-93.33
FGF-2	0.01	6.99	1.61-30.40
Exp(B)= the increase in the odds of finding endometriosis for every unit increase of the best cutoff level.			

Figure.1 Receiver operating characteristic curve of neutrophil-lymphocyte ratio (NLR), serum IL-6 and the combined marker for the differential diagnosis between patients with and without endometriosis



Bourlev et al.[16] came to a similar conclusion, but that study only recruited 25 patients with endometriosis and 14 patients without endometriosis (undergoing laparoscopic sterilization) and their samples were collected in any phase of the menstrual cycle.[16] Overall, our results do suggest that NLR, IL−6 and FGF-2 demonstrate much potential to work as clinical markers for endometriosis. Based on our data, IL-6 may be considered the best predictive marker for endometriosis, followed by NLR and, lastly, FGF-2. However, it should be also noted that the patient exclusion criteria used in our current study may not accurately recreate the infertility population commonly encountered in the clinic.

For example, patients with infertility related conditions (such as ovarian cysts, pelvic inflammatory disease, autoimmune disease or any active inflammation) were all excluded from our study. Follow-up investigation is needed to determine how well the NLR, IL-6 and FGF-2 markers work in a real world clinical population. Reexamination of the usefulness of the two most promising markers, NLR and serum levels of IL-6, as a combined marker is a more powerful test than examining any of the single markers. The overall sensitivity of the corresponding threshold value on the ROC curve increased significantly (from 92.5% and 80% with NLR and IL-6 alone, respectively) to 97%, without any major change in the specificity (from 77.5% with serum IL-6 alone to 75% with the combined marker). This encourages the use of this marker as a novel predictive tool for endometriosis and makes this approach clinically useful. So if the test is negative, a laparoscopy would be of little benefit to the patient, but if it is positive, there could be a significant rate of overdiagnosis which could impact on treatment. Then, laparoscopy becomes a very useful confirmatory test as well as it allows for operative treatment. Mihalyi et al.(15) reached a comparable result, concluding their odds of having endometriosis increased more than 78-fold for every unit increase of IL-6. The cost of the blood tests (including the costs of the kits used) per patient was about 166 Egyptian pounds, which is a very reasonable value, taking into consideration its high statistical power and the fact that it is a safe and non-invasive test, which makes the suggested blood tests a useful, practical, easily available and reliable test in the diagnostic workup of endometriosis.

The current study confirmed previous findings noting that when considered alone, each marker studied has limited diagnostic properties. However, we conclude that the combined marker (NLR, and IL-6) is better able to identify patients with endometriosis.

Confirmation in larger groups of patients should be obtained and, if confirmed, this simple measurement from NLR, in conjunction with serum IL-6 may lead to a much simpler and still accurate screening test for endometriosis. On the basis of the study findings, not all patients would need surgical diagnosis.

A possible relative limitation of current study is that it is designed to assess the ability of NLR, IL-6 and FGF-2 to detect endometriosis among a selected population of patients undergoing laparoscopic surgery for infertility. The strict exclusion criteria used limits the clinical value of the studied tests when applied in a real life daily routine. It warrants further studies on the groups of patients excluded in the current thesis.

Acknowledgments

We would like the staff of Ain Shams University Department of Clinical Pathology for their help in running the tests done during this research.

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