Jinekoloji - Obstetrik ve Neonatoloji Tıp Dergisi
The Journal of Gynecology-Obstetrics and Neonatology

ÖZGÜN ARAŞTIRMA / ORIGINAL ARTICLE

DOI: 10.38136/jgon.1787533

Use of Inflammatory Markers in the Diagnosis of Ectopic Pregnancy

Enflamatuvar Belirteçlerin Ektopik Gebelik Tanısında Kullanımı

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ABSTRACT

Aim: This study aimed to evaluate the utility of inflammatory markers, including the systemic immune-inflammation index, systemic inflammatory response index, and pan-immune inflammatory value, in diagnosing ectopic pregnancy.

Material and Methods: A retrospective analysis was conducted on 105 patients diagnosed with ectopic pregnancy and 105 patients with intrauterine pregnancies treated at our hospital between November 2022 and September 2023. Ectopic pregnancy diagnoses were confirmed through ultrasonography. Data collected included age, gravida, parity, white blood cell count, neutrophil count, monocyte count, lymphocyte count, and platelet count. The inflammatory markers were calculated and compared between the groups using statistical software.

Results: Significant differences were observed in white blood cell, neutrophil, monocyte, and lymphocyte counts. The systemic immune-inflammation index values were significantly higher in the ectopic pregnancy group (890.70±545.30) compared to the intrauterine pregnancy group (1121.50±537.59, p=0.004). Similarly, the systemic inflammatory response index and pan-immune inflammatory value were significantly elevated in the ectopic pregnancy group (systemic inflammatory response index: 70.31±29.70 vs. 89.07±32.49, p=0.000; pan-immune inflammatory value: 477.56±270.08 vs. 679.77±319.18, p=0.000). Receiver operating characteristic curve analysis showed moderate diagnostic accuracy for these markers with area under the curve values of 0.670, 0.670, and 0.710, respectively.

Conclusions: Inflammatory markers such as the systemic immune-inflammation index, systemic inflammatory response index, and pan-immune inflammatory value show potential as diagnostic tools for ectopic pregnancy. Incorporating these markers into clinical practice could improve diagnostic accuracy, complementing traditional methods. Further research with larger sample sizes is required to validate these findings and enhance their clinical application.

Keywords: Ectopic pregnancy, biomarkers, inflammation, immune system

ÖZ

Amaç: Bu çalışmada, sistemik immün inflamasyon indeksi, sistemik inflamatuar yanıt indeksi ve pan-immün inflamatuar değer gibi inflamatuar belirteçlerin ektopik gebelik tanısında kullanılabilirliğinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Kasım 2022 ve Eylül 2023 tarihleri arasında hastanemizde tedavi edilen 105 ektopik gebelik tanılı hasta ve 105 intrauterin gebelik tanılı hasta üzerinde retrospektif bir analiz yapıldı. Ektopik gebelik tanıları ultrasonografi ile doğrulandı. Toplanan veriler arasında yaş, gravida, parite, lökosit sayısı, nötrofil sayısı, monosit sayısı, lenfosit sayısı ve trombosit sayısı yer aldı. İnflamatuvar belirteçler hesaplandı ve istatistiksel yazılım kullanılarak gruplar arasında karşılaştırıldı.

Bulgular: Lökosit, nötrofil, monosit ve lenfosit sayılarında anlamlı farklılıklar gözlendi. Sistemik immün-inflamasyon indeksi değeri ektopik gebelik grubunda (890,70±545,30) intrauterin gebelik grubuna (1121,50±537,59, p=0,004) kıyasla anlamlı olarak daha yüksekti. Benzer şekilde, sistemik inflamatuvar yanıt indeksi ve pan-immün inflamatuvar değer ektopik gebelik grubunda anlamlı olarak yükselmiştir (sistemik inflamatuvar yanıt indeksi: 70,31±29,70 vs. 89,07±32,49, p=0,000; pan-immün inflamatuvar değer: 477,56±270,08 vs. 679,77±319,18, p=0,000). Alıcı işletim karakteristik eğrisi analizi, sırasıyla 0,670, 0,670 ve 0,710 eğri altında kalan alan değerleri ile bu belirteçler için orta düzeyde tanısal doğruluk göstermiştir.

Sonuç: Sistemik immün-inflamasyon indeksi, sistemik inflamatuar yanıt indeksi ve pan-immün inflamatuar değer gibi inflamatuar belirteçler ektopik gebelik için tanısal araçlar olarak potansiyel göstermektedir. Bu belirteçlerin klinik uygulamaya dahil edilmesi, geleneksel yöntemleri tamamlayarak tanısal doğruluğu artırabilir. Bu bulguları doğrulamak ve klinik uygulamalarını geliştirmek için daha büyük örneklem boyutlarıyla daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Ektopik gebelik, biyobelirteçler, enflamasyon, bağışıklık sistemi

Cite as: Polat M, Sapmaz MA, Erbey S, Akın F. Use of inflammatory markers in the diagnosis of ectopic pregnancy. Jinekoloji-Obstetrik ve Neonatoloji Tıp Dergisi 2025;22(4):493–498.

Geliş/Received: 19.09.2025 • **Kabul/Accepted:** 10.12.2025

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Çevrimiçi Erişim/Available online at: https://dergipark.org.tr/tr/pub/jgon

INTRODUCTION

Ectopic pregnancy (EP) is a pathological pregnancy condition that occurs when a fertilised ovum implants outside the uterus, typically in the fallopian tubes, but can also occur in the ovary, cervix or abdominal cavity (1). The incidence of EP is estimated to be between 1 and 2 percent of all pregnancies. In recent years, the frequency of diagnosis has increased due to improved early diagnosis methods and the development of more sensitive ultrasonography technologies (1). EP is considered an acute obstetric emergency with the potential to result in significant maternal morbidity and mortality if not diagnosed and treated in a timely manner and may result in significant complications such as tubal rupture, haemorrhagic shock and maternal death. Tubal rupture is clinically manifested by symptoms such as severe abdominal pain, vaginal bleeding and hypotension (1). Furthermore, failure to treat EP early may have adverse effects on future fertility and increase the risk of recurrent EP. Therefore, it is very important to diagnose EP early to prevent these complications (1).

Diagnostic methods currently used to diagnose EP include measurement of human coryonic gonadotropin (HCG) levels, transvaginal ultrasonography (TVUSG) and evaluation of serum progesterone levels. Transvaginal ultrasonography is one of the most effective methods to determine the localisation of EP. While HCG levels increase at a regular rate in normal intrauterine pregnancy, this increase is usually slow or irregular in EP. The combination of these two methods provides high sensitivity and specificity in the early diagnosis of EP (2-4).

The role of inflammatory response in EP has been an important area of interest for researchers in recent years. The inflammatory response is a defence mechanism initiated by the body in response to infection or tissue damage. In EP, abnormal localisation of the implanted embryo and associated tissue damage may play a role as a trigger for the inflammatory response (5,6).

The systemic inflammation index (SII) is a biomarker calculated using platelet (PLT), neutrophil (NEU) and lymphocyte (LYM) counts, and its formula is PLTxNEU/LYM. A high SII value is indicative of increased inflammatory activity in the body. In one study, it was shown that SII was elevated in patients with EP treated with surgery compared to patients treated with methotrexate (7). In another study, SII was identified as a valuable marker for predicting the risk of tubal EP rupture (8). A recent study showed that patients with low SII values responded better to methotrexate in the treatment of tubal EP (9).

Systemic immune response index (SIRI) is calculated using neutrophil (NEU), monocyte (MON) and lymphocyte (LYM) counts and is used to assess the severity of the inflammatory response. Although there is little literature examining the role of SIRI in EP, one study has shown that SIRI is elevated in caesarean scar pregnancies, potentially providing a diagnostic benefit in such cases (10).

Pan-immune inflammatory value (PIV) is used as a combined indicator of inflammatory processes and immune response. PIV is calculated using platelet (PLT), neutrophil (NEU), monocyte (MON) and lymphocyte (LYM) counts. At the time of our study, there were no publications in the literature on the relationship between PIV and EP.

Evaluation of the inflammatory response in EP is of great importance for early diagnosis and determination of appropriate treatment strategies. The use of inflammatory markers such as SII, SIRI and PIV may be valuable in clinical practice to determine the severity and treatment requirements of EP. The aim of our study was to examine the association of SII, SIRI and PIV values with EP and to determine whether they can be used as diagnostic tools.

MATERIALS AND METHODS

The study was initiated after the approval numbered AESH-EK1-2023-773 was obtained from the Ethics Committee of Ankara Etlik City Hospital and the study was conducted in accordance with the Declaration of Helsinki. The files of patients who were treated with the diagnosis of EP between November 2022 and September 2023 in our hospital and patients diagnosed with intrauterine pregnancy (IUP) presenting to the outpatient clinic were extracted. EP patients were classified as group-1 and IUP patients as group-2. Inclusion criteria for EP group;

1) Patients with tubal EP confirmed by ultrasonography,
2) Patients over 18 years of age
3) Patients with hemogram results at the time of diagnosis in the medical record

Our exclusion criteria for the EP group

1) Diagnosed with ruptured EP
2) Leukaemia, lymphoma and bone marrow diseases that may affect blood counts
3) Those with active infection
4) Patients with diseases that increase systemic inflammation (systemic lupus erythematosus, diabetes mellitus, hypertension, vasculitis, rheumatoid arthritis, renal failure, liver failure)
5) Heterotopic pregnancy and other ectopic pregnancies located outside the tuba uterina

Our inclusion criteria for the IUP group;

1) Pregnant women with intrauterine fetal cardiac activity detected by ultrasonography
2) Patients whose haemogram is checked at the first visit
3) Those over 18 years of age

Our exclusion criteria for the IUP group;

1) Those with leukaemia, lymphoma and bone marrow diseases that may affect blood counts
2) Those with active infection
3) Patients with diseases that increase systemic inflammation (systemic lupus erythematosus, diabetes mellitus, hypertension, vasculitis, rheumatoid arthritis, renal failure, liver failure)

As a result of the file search, 211 patients diagnosed with EP were found and when the inclusion and exclusion criteria were applied, it was seen that 153 patients could be included in the study. Among 153 EP patients, permutation randomisation was applied and 105 patients were included in group-1. In the cohort of 2455 patients diagnosed with IUP, 2104 patients were eligible for inclusion when inclusion and exclusion criteria were applied. A randomisation procedure based on permutations was applied to 2104 patients, resulting in the inclusion of 105 patients in Group 2.

After identifying patients who could be included in both groups, relevant data, i.e. age, gravida, parity, white blood cell (WBC), neutrophil (NEU), monocyte (MON), lymphocyte (LYM) and platelet (PLT) values were obtained from the medical records. These were used to calculate SII, SIRI and PIV values. The formula used to calculate SIRI is PLTxMON/LYM, formula is PLTxNEU/LYM for SII, the formula is expressed as PLTxMONxNEU/LEU for PIV.

In our hospital, blood samples of the patients are collected in a 3 mL BD Vacutainer™ Blood Collection K2EDTA tube (lot 4141891; Becton Dickinson, Plymouth, England) for complete blood count and measured by fluorescence flow cytometry method on a Sysmex XN-1000 (Sysmex Europe GmbH, Bornbarch 1, 22848 Norderstedt, Germany).

Statistical Analysis

In the power analysis performed pre-study, alpha-error: 0.05, beta-error: 0.95 and effect size: 0.5 (medium) were taken as alpha-error: 0.05, beta-error: 0.95 and effect size: 0.5 (medium) for analysis with t-test and it was decided to include a total of 210 patients with 105 patients in each group.

All data were entered into the statistical software SPSS version 25.0 (Armonk, NY, IBM Corp., USA) and analysed. Mann–Whitney U test was used to evaluate the gravida and parity values between IUP and EP groups. Independent t-test was used to evaluate age, neutrophil, monocyte, lymphocyte, platelet, SII, SIRI and PIV values between the groups. Logistic regression analysis was used for the diagnostic value, sensitivity and specificity analyses of SII, SIRI and PIV values for EP and area under the curve (AUC) values were determined by calculating the receiver operating characteristic (ROC) curve. Results were considered significant if the p-value was less than 0.05.

RESULTS

When the statistical analyses of the groups were performed, the mean age of group-1 and group-2 was 28.99±5.20 and 28.99±5.20 years, respectively, and there was no significant difference between them (p=0.745). Gravida and parity values were compared between the two groups using the Mann–Whitney U test, and no significant difference was observed (U=4387.0, p=0.949 and U=4364.0, p=0.895, respectively). The distribution of both variables was similar across the groups. The mean WBC values were 12.86±4.97 in group-1 and 10.91±1.96 in group-2 and there was a statistically significant difference between them (p=0.000). The mean PLT values of the groups were 231.24±59.71 and 251.32±53.50, respectively, and the difference between them was statistically significant (p=0.017). The mean NEU values were 6.58±1.68 in group-1 and 7.53±1.81 in group-2 and the difference between them was statistically significant (p=0.000). The mean value of MON was 0.55±0.15 in group-1 and 0.62±0.18 in group-2 and there was a significant difference between them (p=0.003). The mean LYM values of the groups were 2.34±0.65 and 1.80±0.43, respectively, and the difference between them was statistically significant (p=0.000). Mean SII values were 890.70±545.30 in group-1 and 1121.50±537.59 in group-2 and the difference between them was significant (p=0.004). Mean SIRI values were 70.31±29.70 in group-1 and 89.07±32.49 in group-2 and the difference between them was significant (p=0.000). The mean PIV values of the groups were 477.56±270.08 and 679.77±319.18, respectively, and there was a statistically significant difference between them (p=0.000) (Table 1).

ROC curve analysis for the use of SII value as a parameter in the diagnosis of ectopic pregnancy showed AUC 0.670, sensitivity 0.83 and specificity 0.50, which were found to be significant for this variable (p=0.007). Positive predictive value (PPV) was 0.57 and negative predictive value (NPV) was 0.79 (Table 2 and Figure 1). When ROC curve analysis was performed for the use of SIRI value as a parameter in the diagnosis of ectopic pregnancy, AUC 0.670, sensitivity 0.56 and specificity 0.72 were found to be significant

Table 1. Comparison of Variables According to Groups

Variable	Group-1 (EP) (n=105) Mean±Standard Deviation	Group-2 (IUP) (n=105) Mean±Standard Deviation	t- statistic	p- value
Age	28.99±5.20	28.73±5.41	0.325	0.745
White Blood Cell (*10^3/µl)	12.86±4.97	10.91±1.96	4.818	0.000*
Platelet (*10^3/µl)	231.24±59.71	251.32±53.50	-2.404	0.017*
Neutrophil (*10^3/µl)	6.58±1.68	7.53±1.81	-3.730	0.000*
Monocyte (*10^3/µl)	0.55±0.15	0.62±0.18	-2.989	0.003*
Lymphocyte (*10^3/µl)	2.34±0.65	1.80±0.43	6.550	0.000*
SII †	890.70±545.30	1121.50±537.59	-2.910	0.004*
SIRI ‡	70.31±29.70	89.07±32.49	-4.136	0.000*
PIV §	477.56±270.08	679.77±319.18	-4.716	0.000*
Variable	Group-1 (EP) (n=105) Median (IQR) **	Group-2 (IUP) (n=105) Median (IQR)	U value	p- value
Gravida	2 (1–2)	2 (1–2)	4387.0	0.949
Parita	1 (0–1)	1 (0–1)	4364.0	0.895

†: systemic immune-inflammation index. **‡:** systemic inflammatory response index**. §:** pan-immune inflammatory value *: statistically significant **: Interquartile Range

Table 2. Logistic Regression and ROC Results for SII Value

Parameter	Value	P Value	Threshold
ROC Area Under Curve	0.670	0.007*	764.80
Sensitivity	0.83		
Specificity	0.50		
Positive Predictive Value	0.57		
Negatif Predictive Value	0.79		

*: statistically significant

Table 3. Logistic Regression Results for SIRI Value

Parameter	Value	P Value	Threshold
ROC Area Under Curve	0.670	0.001*	82.67
Sensitivity	0.56		
Specificity	0.72		
Positive Predictive Value	0.62		
Negatif Predictive Value	0.67		

*: statistically significant

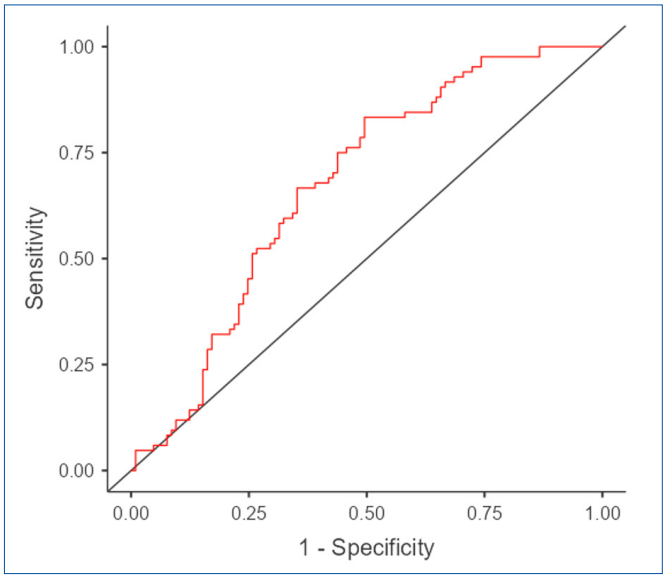

Figure 1. Receiver Operating Characteristic (ROC) Curve for SII

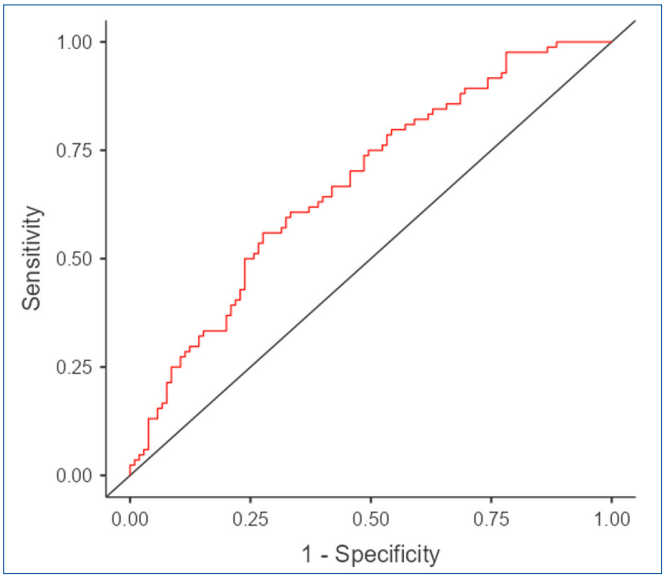

Figure 2. Receiver Operating Characteristic (ROC) Curve for SIRI

for this variable (p=0.001). PPV and NPV were 0.62 and 0.67, respectively (Table 3 and Figure 2). When ROC curve analysis was performed for the use of PIV value as a parameter in the diagnosis of ectopic pregnancy, AUC 0.710, sensitivity 0.56 and specificity 0.83 were found and found to be significant for this variable (p=0.000). PPV was 0.72 and NPV was 0.70 (Table 4 and Figure 3).

Table 4. Logistic Regression Results for PIV Value

Parametre	Value	P Value	Threshold
ROC Area Under Curve	0.710	0.000*	617.29
Sensitivity	0.56		
Specificity	0.83		
Positive Predictive Value	0.72		
Negatif Predictive Value	0.70		

*: statistically significant

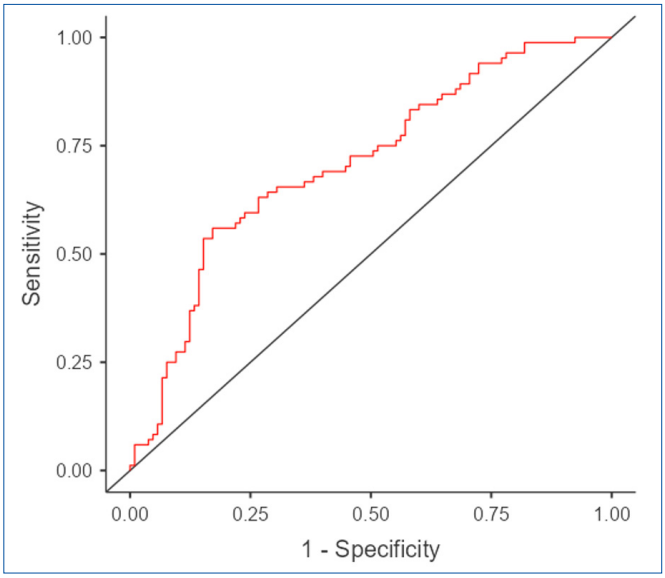

Figure 3. Receiver Operating Characteristic (ROC) Curve for PIV

DISCUSSION

Review of the literature shows that the studies examining the relationship between SII and EP are primarily concerned with the correlation between these factors and methotrexate response and the risk of rupture of EP (7-9). In contrast, SIRI has only been investigated in caesarean scar pregnancies and no study has examined the relationship between PIV and EP. The aim of this study was to analyse the diagnostic and prognostic value of inflammatory markers such as SII, SIRI and PIV in patients diagnosed with EP. The findings suggest that these inflammatory markers may be effective in the diagnosis and treatment of EP. In addition, a noteworthy aspect of this study is that it is one of the first to analyse the relationship between EP and SIRI and PIV.

Embryo implantation is a complex and critical process in which the embryo attaches to the endometrium and initiates pregnancy. Successful implantation of the embryo into the endometrium requires the coordination of multiple biochemical and cellular events. A considerable amount of evidence suggests that inflammation is a crucial factor in this process and has the potential to influence the success of implantation (11). The role of inflammation in processes such as decidualisation of stromal cells and enhancement of endometrial receptivity during embryo implantation is critical. These processes are associated with high levels of pro-inflammatory cytokines and prostaglandins (11). An inflammatory environment is necessary for the attachment and invasion stages of the blastocyst. This is achieved by appropriate guidance of the innate immune system by the trophoblast (12). The role of inflammation in the process of embryo implantation is critical for successful implantation of the embryo into the endometrium. Molecules such as pro-inflammatory cytokines, prostaglandins and immunomodulators play a very important role in this process and ensure the success of implantation by increasing endometrial receptivity (11-13). In our study, SII, SIRI and PIV values were higher in the group with live pregnancy compared to the EP group. We consider that inflammation should increase during embryo implantation in IUP and the observed low inflammatory values may be attributed to abnormal implantation formation in EP.

In the literature, there are studies showing that inflammatory markers other than SII, SIRI and PIV are increased in EP. In the study by Ülkümen et al., lower platelet count was observed in the EP group compared to the IUP group, and this finding was similar in our study (6). Mean platelet volume value (MPV), which is an inflammatory marker, was found to be significantly higher in Karaman et al. study, whereas no significant difference was observed in the other study (5). Although MPV is an inflammatory marker, it is less effective than SII, SIRI and PIV in showing inflammation. Although the literature examining the role of SIRI in EP is scarce, one study has shown that SIRI is elevated in caesarean scar pregnancies and potentially provides diagnostic utility (10). However, the low SIRI values observed in tubal EPs in our study suggest that this may be specific to caesarean scar pregnancies.

The results of the ROC analysis showed that SII is an important diagnostic indicator for EP with an AUC of 0.670, sensitivity of 83% and specificity of 50%. In the light of these findings, it can be said that SII has a moderate accuracy rate in the diagnosis of EP and can serve as a valid marker in clinical practice. In the ROC analysis, SIRI was found to have an AUC of 0.670, a sensitivity of 56% and a specificity of 72% in the diagnosis of EP. In the light of these findings, it seems reasonable to suggest that SIRI can be used as a diagnostic tool for EP with a moderate accuracy rate. The results of the ROC analysis of the PIV revealed an AUC of 0.710, a sensitivity of 56% and a specificity of 83% for the diagnosis of EP. We believe that PIV has a middle degree of accuracy in the diagnosis of EP and can be used as a marker in clinical practice.

Currently, serum HCG and progesterone levels have an important value as biochemical parameters in the diagnosis of EP. It has been reported that the sensitivity of HCG in the diagnosis of EP ranges

between 96% and 100% and the specificity between 79% and 100% (2,14). Similarly, it was reported that the sensitivity of serum progesterone value in the diagnosis of EP ranged between 63% and 100%, and the specificity ranged between 35% and 100% (3,15,16). The findings of our study suggest that SII, SIRI and PIV values can be used as complementary parameters in the diagnosis of EP together with serum progesterone levels, especially in cases where β-HCG elevation is minimal.

CONCLUSION

This study shows that inflammatory markers such as SII, SIRI and PIV may play an important role in the diagnosis and management of EP. These markers may be valuable in clinical practice to determine the severity and treatment requirements of EP. Further studies are needed to confirm these findings and to finalise the status of the use of inflammatory markers in clinical practice. Furthermore, additional studies in different patient groups and with larger sample sizes will further clarify the diagnostic and prognostic value of these markers.

Conflict of Interest: There is no conflict of interest in our study.

Funding: There is no financial support in our study.

Author Contributions: xxxxxxx

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