Original Article

Inflammatory Markers in Peripheral Blood Cells Cannot Predict Intrauterine Insemination Outcome: A Retrospective Cohort Study

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Abstract

Background: Although infertility is known as a chronic inflammatory condition, the effect of the increased inflammatory response on IUI success is not clear. Systemic inflammation can be calculated by applying various hematological markers. **Aims:** We aimed to evaluate the ability of hematologic parameters of inflammation in predicting intrauterine insemination (IUI) outcome. **Study Setting and Design:** A total of 334 infertile couples included in this retrospective cohort study. The study population comprised of all couples who were candidates for ovulation induction and IUI due to polycystic ovary syndrome (PCOS) (n = 147) or unexplained infertility (UI) (n = 187). **Materials and Methods:** The inflammatory parameters in the complete blood count parameters, such as neutrophil-lymphocyte ratio, platelet lymphocyte ratio, platelet distribution width, plateletcrit were obtained on IUI day and compared between the two groups. The predictive values of these markers for IUI outcome were calculated. **Results:** There were 44 pregnancies (13.2%) in the whole study cohort. There were no significant differences between the pregnant and nonpregnant groups regarding the evaluated parameters (all $P > 0.05$). Also, no significant difference was observed between the patients with PCOS and UI in terms of those parameters. The area under receiver operating characteristic (ROC) curve analysis revealed that none of the inflammatory markers can predict pregnancy in intrauterine insemination cycles. Further prospective studies are needed to verify our findings. **Conclusion:** We found no relationship between the hematologic inflammatory markers and IUI outcome. Therefore these markers cannot be used for prediction of pregnancy.

Keywords: *Complete blood count, inflammatory markers, intrauterine insemination, polycystic ovary syndrome, unexplained infertility*

Introduction

Infertility is defined as a failure to conceive after 12 months of intercourse without contraception.[1] Intrauterine insemination (IUI) is a cheaper and noninvasive method compared to assisted reproductive techniques.[2-4] Successful implantation requires a quality embryo and coordinated communication with the endometrium.[5] Chemokines, hormones, growth factors, and cytokines are important factors in fetal-maternal interaction.[6] The mutual effects among these results induce a local inflammatory response and systemic inflammation.[7] Pregnancy success depends on good embryo implantation.[8] Hundreds of molecules were investigated and a lot of mechanisms were detailed to justify the impacts of maternal immune response on pregnancy.[9-11] Although infertility is known as a chronic inflammatory condition, the effect of the increased inflammatory response on IUI success is not clear. Systemic inflammation can be calculated by applying various hematological markers.[12] It was shown that

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measurement of neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), and platelet to lymphocyte ratios (PLR) may provide clues to prognosis and diagnosis for diseases associated with chronic low-grade inflammation.[13-15] They can be measured easily and cheaply.[16] As far as we know, no research has been conducted regarding the inflammatory markers of complete blood count (CBC) in patients undergoing IUI treatment.

In the present study, we analyzed the effects of hematological inflammatory markers on treatment success in patients undergoing ovulation induction (OI) and IUI.

Materials and Methods

The current retrospective cohort study was conducted at the University of Health Sciences Zekai Tahir Burak Women's Health Care Training and Research Hospital between May 2014 and December 2016. The institutional review board of the hospital approved the study (# 23/2014) and the principles of the Declaration of Helsinki were applied.[17] A written consent form was obtained from all participants.

A total of 334 infertile couples aged between 18 and 43 were included in this retrospective cohort study. Baseline hormone levels and hysterosalpingography were performed. Exclusion criteria included women with endometriosis, pelvic adhesions, pelvic inflammatory disease. The couples with anatomical abnormalities, hydrosalpinx, systemic disease, endocrinopathy, autoimmune disease, hematologic disease, glucocorticoid, and anti-inflammatory drug use were excluded from participation.

Of 334 patients, 147 were diagnosed with polycystic ovary syndrome (PCOS), and 187 women were diagnosed as unexplained infertility (UI). Sperm analysis was performed in the andrology laboratory and it was evaluated according to guidelines.[18]

All patients were asked to respect the 3-day abstinence period. Semen samples from the patients were collected by masturbation in a special room nearby the laboratory. After being liquefied for 30 min at room temperature, the collected semen samples (prewashed) were evaluated by a computer-assisted semen analyzer for conventional semen parameters, including sperm concentration and sperm motility. The rest of the sperm was processed using the standard swimming method with a sperm preparation medium (SpermRinse Solution, Vitrolife, Gothenburg, Sweden). Postwash analysis was again performed by the computer-assisted semen analyzer. Sperm analysis was performed by the same andrology laboratory technician in the andrology laboratory of the current hospital according to the quality control program.

The blood sample for hematologic markers of inflammation was collected on the same day of intrauterine insemination. All analyses were conducted using the impedance method in the hematology laboratory with Beckman coulter LH-780 fully automated hematology analyzer (Beckman coulter Inc., Brea, CA, USA) (intra-assay variation coefficient 1.6%, interassay variation coefficient 1.65%). The CBC parameters of the patients, were recorded and evaluated. NLR, MLR, and PLR ratios were evaluated by dividing the absolute neutrophil, monocyte, and platelet count, respectively, by the absolute lymphocyte counts. The predictive values of inflammatory markers for insemination outcomes were estimated.

All IUI cycles were performed with ovarian stimulation and included either clomiphene citrate (orally administered 50–150 mg/day), starting from days 3 to 5 of the menstrual cycle or gonadotropin at days 2–3 of the cycle. The follicular development was confirmed when at least one follicle of ≥18 mm was seen on transvaginal ultrasonography and then human chorionic gonadotropin (hCG) 5,000 IU intramuscular or 250 mcg recombinant hCG subcutaneous was administered. All patients underwent IUI 36 h after hCG administration. Following the insemination, the patients received 200 mg/day vaginal progesterone supplementation for luteal support until 12 weeks of gestational age. Transvaginal sonography was performed 6 weeks after insemination. Clinical pregnancy was explained as the presence of a fetal heartbeat at 6–7 weeks of pregnancy.

Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). The assumption of normality was tested via the Shapiro–Wilk test. Data are shown as means ± standard deviation for continuous variables. The nonparametric variables and data without normal distribution were tested using the Mann–Whitney *U*-test. The receiver operator characteristics curve (ROC) analysis was performed to reveal the hematological parameters. A $P < 0.05$ was considered statistically significant.

The sample size calculation for the entire study population, a two-tailed comparison with 0.5 effect size, a 5% level of significance (alpha) and a power of 0.95 with an allocation ratio of 1:1, gave a study population of 105 versus 105 women in each group. Sample size calculations were performed using the G*Power version 3.1.5 (Heinrich Heine Universität, Düsseldorf, Germany) general power analysis program.[19]

Results

A total of 334 patients were included in the study (147 patients with PCOS and 187 patients with UI). The median ages of patients in the PCOS group and the unexplained groups were 24 (18–38) years and 28 (18–43) years, respectively ($P < 0.001$). There were 25 (13.4%) pregnant patients in the PCOS group and 19 (12.9%) pregnant patients in the UI group ($P = 0.905$).

The median inflammatory values were for the PCOS and unexplained group is defined in Table 1. The median inflammatory marker values were for the pregnant and nonpregnant groups are defined in Table 2. The difference in inflammatory marker values among defined groups were not found to be statistically significant ($P > 0.05$). The area under ROC curve analysis revealed that none of the inflammatory markers can predict pregnancy in IUI cycles [Figure 1].

Table 1: The distribution of inflammatory markers for the polycystic ovary syndrome and unexplained group

	PCOS (*n*=147)	Unexplained infertility (*n*=187)	*P*
WBC	7.8 (4.2-16.4)	8.3 (4-16.4)	0.32
PDW	16.5 (11.1-18.4)	16.5 (11.9-17.9)	0.684
PCT	0.24 (0.14-0.41)	0.24 (0.15-0.4)	0.859
MPV	8.8 (6.7-13.1)	8.9 (7-12)	0.645
NLR	2.17 (1.09-9.42)	2.07 (0.5-11.11)	0.470
MLR	0.23 (0.09-1)	0.22 (0.1-1)	0.273
PLR	119.38 (1-298.33)	113.75 (47.11-283.08)	0.125

The assumption of normality was tested via the Shapiro-Wilk test. The nonparametric variables and data without normal distribution were tested using the Mann-Whitney *U*-test. PCOS=Polycystic ovary syndrome, WBC=White blood cell, PDW=Platelet distribution width, PCT=Plateletcrit, MPV=Mean platelet volume, NLR=Neutrophil to lymphocyte ratio, MLR=Monocyte to lymphocyte ratio, PLR=Platelet to lymphocyte ratio

Table 2: The distribution of inflammatory markers for the pregnant and nonpregnant group

	Pregnant (*n*=44)	Nonpregnant (*n*=290)	*P*
WBC	8.3 (5.3-14.1)	8.1 (4-16.4)	0.337
PDW	16.5 (11.9-18.3)	16.5 (11.1-18.4)	0.764
PCT	0.25 (0.14-0.36)	0.24 (0.15-0.41)	0.861
MPV	9 (7.5-13.1)	8.85 (6.7-12.5)	0.375
NLR	2.17 (0.5-6)	2.1 (0.93-11.11)	0.129
MLR	0.23 (0.14-0.73)	0.23 (0.09-1)	0.254
PLR	118.99 (47.11-231.67)	116.4 (1-298.33)	0.798

The assumption of normality was tested via the Shapiro-Wilk test. The nonparametric variables and data without normal distribution were tested using the Mann-Whitney *U*-test. WBC: White blood cell, PDW: Platelet distribution width, PCT: Plateletcrit, MPV: Mean platelet volume, NLR: Neutrophil to lymphocyte ratio, MLR: Monocyte to lymphocyte ratio, PLR: Platelet to lymphocyte ratio

Discussion

The mother's immune system plays an important role in the implantation phase; exaggerated inflammatory responses may also reduce the likelihood of implantation and pregnancy. In this study, we aimed to determine simple inflammatory markers of CBC in patients undergoing OI and IUI cycles and to find out whether any of them has a predictive value for pregnancy. In our study, there were no significant differences between pregnant and nonpregnant patients in terms of CBC parameters. Also, no significant difference was observed between the patients with PCOS and UI. The small number of patients might have caused the CBC parameters to be insignificant.

The accumulating evidence indicates that one of the most important mechanisms of PCOS pathogenesis is insulin resistance. Therefore, the use of insulin sensitizers such as inositol isoforms has received increasing attention due to their safety profile and efficacy.[20,21]

In one study it was suggested that high C-reactive protein (CRP) which is produced by main macrophages in response to a wide range of acute and chronic inflammatory conditions levels were associated with poor cycle results.[22] But, others found no relationship between CRP levels and *in vitro* fertilization (IVF) results.[23,24] The OI and IUI is the standard first-line therapy before the IVF. Success in fertilization and implantation may affect treatment outcomes. But, it has not been clearly shown which parameter is responsible for the failed cycles. To date, many efforts have been made to identify an accurate algorithm that sees the woman's age and ovarian reserve markers as a means to optimize the initial dose of recombinant follicle stimulating hormone in the IVF procedure. However, the available evidence on PCOS women, particularly those with high AMH, does not appear to be sufficient.[25,26]

The insemination may lead to neutrophil migration to the uterus and inflammation.[27-29] A striking infiltration of neutrophils into the uterus of mice was observed but the specific process has not yet been clarified.[28] Song *et al*. showed that the insemination increased the number of uterine neutrophils and some entered the uterine cavity and they could interact with the sperm cells or increase the inflammation.[30] This rapid and intense process in the uterus can be important in ensuring the optimal environment for successful implantation.[31] There are also difficulties with ovarian stimulation in patients affected by certain conditions such as endometriosis.[32,33]

Recent studies indicate that WBC subtypes and NLR are critical markers of systemic inflammatory and are prognostic factors in a variety of diseases.[34-38] The

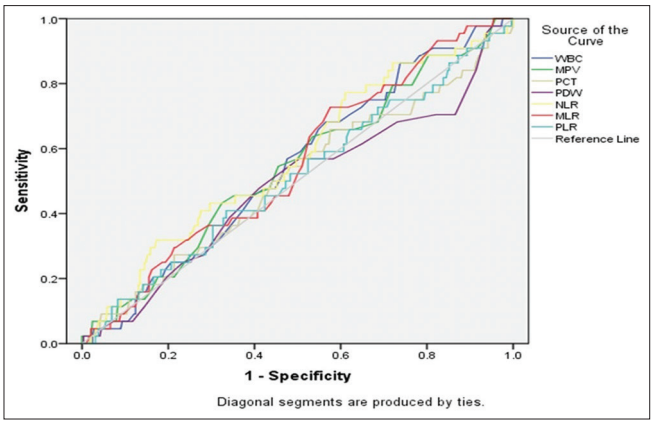

Figure 1: Area under ROC curve analysis of inflammatory markers for the prediction of pregnancy

systemic inflammatory response can be measured with a CBC. NLR and PLR, which are recommended as new markers for an immune response can be measured easily and inexpensively.[16]

Chronic inflammation may occur due to autoimmune illness, benign and malignant neoplasia. The neutrophil count increase and the lymphocyte count may relatively decrease because of chronic inflammation. Cho *et al*. showed that relative changes in spreading WBCs were connected with a systemic inflammatory reaction.[27] In their study neutrophil counts were increased in patients with endometriosis; lymphocytes, eosinophils, and basophil counts were decreased.[27] Neutrophilia-associated lymphocytopenia causes increased NLR in endometriosis patients. Our study is the first to evaluate the NLR and PLR in OI cycles in Turkey. We couldn't find any relationship between the hematologic inflammatory markers and IUI outcome therefore these markers cannot be used for prediction of pregnancy. Also, we investigated serum hemoglobin, hematocrit, monocyte, WBC levels but there is no significant relationship between each group.

High and low mean platelet volume (MPV) values are known to be associated with the inflammation process.[34] However, we found no significant difference in mean MPV values among all the groups. Several studies have reported no relation, while some have a positive association between diseases and CBC parameters. The main limitations of our study were the fact that our delivery results were not complete and involves a single institution and may not represent the general population.

Conclusion

This is the first study investigating the inflammatory markers of CBC in patients undergoing IUI treatment. Simple inflammatory markers assessed by CBC have no clinical significance for the prediction of pregnancy in infertile patients undergoing OI and IUI. There is a need for large-scale prospective studies on this subject. If the pregnancy results of the patients can be followed, the relationship between different results and parameters can be found.

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Conflicts of interest

There are no conflicts of interest.

References

1. Schmidt L, Christensen U, Holstein BE. The social epidemiology of coping with infertility. Hum Reprod 2005;20:1044-52.
2. Devkota UN. The role of incubation during sperm preparation for intrauterine insemination in the treatment of male factor infertility. J Nepal Med Assoc 2003;35:248-54.
3. Cakmak H, Taylor HS. Implantation failure: Molecular mechanisms and clinical treatment. Hum Reprod Update 2011;17:242-53.
4. Solakhan M, Demir M. Effects of Sperm Parameters on Pregnancy Rate in Patients Undergoing Intrauterine Insemination. Gynecol Obstet Reprod Med 2020;26:199-202. [doi: 10.21613/gorm. 2020.1040].
5. Johnson AR, Milner JJ, Makowski L. The inflammation highway: Metabolism accelerates inflammatory traffic in obesity. Immunol Rev 2012;249:218-38.
6. Su RW, Fazleabas AT. Implantation and establishment of pregnancy in human and nonhuman primates. Adv Anat Embryol Cell Biol 2015;216:189-213.
7. Fettke F, Schumacher A, Canellada A, Toledo N, Bekeredjian-Ding I, Bondt A, *et al*. Maternal and fetal mechanisms of B cell regulation during pregnancy: Human chorionic gonadotropin stimulates B cells to produce IL-10 while alpha-fetoprotein drives them into apoptosis. Front Immunol 2016;7:495.
8. Markanday A. Acute phase reactants in ınfections: Evidence-based review and a guide for clinicians. Open Forum Infect Dis 2015;2:1-7.
9. Yuksel OH, Verit A, Sahin A, Urkmez A, Uruc F. White blood cell counts and neutrophil to lymphocyte ratio in the diagnosis of testicular cancer: A simple secondary serum tumor marker. Int Braz J Urol 2016;42:53-9.
10. Xiang F, Chen R, Cao X, Shen B, Liu Z, Tan X, *et al*. Monocyte/lymphocyte ratio as a better predictor of cardiovascular and all-cause mortality in hemodialysis patients: A prospective cohort study. Hemodial Int 2018;22:82-92.
11. Bhat T, Teli S, Rijal J, Bhat H, Raza M, Khoueiry G, *et al*. Neutrophil to lymphocyte ratio and cardiovascular diseases: A review. Expert Rev Cardiovasc Ther 2013;11:55-9.
12. Levin I, Gamzu R, Mashiach R, Lessing JB, Amit A, Almog B. Higher C-reactive protein levels during IVF stimulation are associated with ART failure. J Reprod Immunol 2007;75:141-4.
13. Almagor M, Hazav A, Yaffe H. The levels of C-reactive protein in women treated by IVF. Hum Reprod 2004;19:104-6.
14. Orvieto R, Chen R, Ashkenazi J, Ben-Haroush A, Bar J, Fisch B. C-reactive protein levels in patients undergoing controlled ovarian hyperstimulation for IVF cycle. Hum Reprod 2004;19:357-9.
15. Kotilainen T, Huhtinen M, Katila T. Sperm-induced leukocytosis in the equine uterus. Theriogenology 1994;41:629-36.

16. Robertson SA, Mau VJ, Tremellen KP, Seamark RF. Role of high molecular weight seminal vesicle proteins in eliciting the uterine inflammatory response to semen in mice. J Reprod Fertil 1996;107:265-77.
17. World Medical Association. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA 2013;310:2191-4.
18. Geneva S, Press W. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Cambringe: Behalf of the Wolrd Health Organization; 2010.
19. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175-19.
20. Facchinetti F, Unfer V, Dewailly D, Kamenov ZA, Diamanti-Kandarakis E, Laganà AS, *et al*. Inositols in polycystic ovary syndrome: An overview on the advances. Trends Endocrinol Metab 2020;31:435-47.
21. Facchinetti F, Appetecchia M, Aragona C, Bevilacqua A, Bezerra Espinola MS, Bizzarri M, *et al*. Experts' opinion on inositols in treating polycystic ovary syndrome and non-insulin dependent diabetes mellitus: A further help for human reproduction and beyond. Expert Opin Drug Metab Toxicol 2020;16:255-74.
22. Robertson SA. Seminal plasma and male factor signalling in the female reproductive tract. Cell Tissue Res 2005;322:43-52.
23. Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. J Surg Oncol 2005;91:181-4.
24. Bishara S, Griffin M, Cargill A, Bali A, Gore ME, Kaye SB, *et al*. Pre-treatment white blood cell subtypes as prognostic indicators in ovarian cancer. Eur J Obstet Gynecol Reprod Biol 2008;138:71-5.
25. Burnik Papler T, Vrtačnik Bokal E, Prosenc Zmrzljak U, Stimpfel M, Laganà AS, Ghezzi F, *et al*. PGR and PTX3 gene expression in cumulus cells from obese and normal weighting women after administration of long-acting recombinant follicle-stimulating hormone for controlled ovarian stimulation. Arch Gynecol Obstet 2019;299:863-71.
26. Peluso C, de Oliveira R, Laporta GZ, Christofolini DM, Affonso Fonseca FL, Laganà AS, *et al*. Are ovarian reserve tests reliable in predicting ovarian response? Results from a prospective, cross-sectional, single-center analysis. Gynecol Endocrinol 2020. p.1-9. doi: 10.1080/09513590.2020.1786509. Online ahead of print.
27. Cho S, Cho H, Nam A, Kim HY, Choi YS, Park KH, *et al*. Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. Fertil Steril 2008;90:2073-9.
28. Feng JF, Huang Y, Zhao Q, Chen QX. Clinical significance of preoperative neutrophil lymphocyte ratio versus platelet lymphocyte ratio in patients with small cell carcinoma of the esophagus. ScientificWorldJournal 2013;2013:504365. doi: 10.1155/2013/504365. eCollection 2013.
29. Wang D, Yang JX, Cao DY, Wan XR, Feng FZ, Huang HF, *et al*. Preoperative neutrophil-lymphocyte and platelet-lymphocyte ratios as independent predictors of cervical stromal involvement in surgically treated endometrioid adenocarcinoma. Onco Targets Ther 2013;6:211-6.
30. Song ZH, Li ZY, Li DD, Fang WN, Liu HY, Yang DD, *et al*. Seminal plasma induces inflammation in the uterus through the γδ T/IL-17 pathway. Sci Rep 2016;6:25118. doi: 10.1038/srep25118.
31. Liu J, Du J, Fan J, Liu K, Zhang B, Wang S, *et al*. The neutrophil-to-lymphocyte ratio correlates with age in patients with papillary thyroid carcinoma. ORL J Otorhinolaryngol Relat Spec 2015;77:109-16.
32. Šalamun V, Verdenik I, Laganà AS, Vrtačnik-Bokal E. Should we consider integrated approach for endometriosis-associated infertility as gold standard management? Rationale and results from a large cohort analysis. Arch Gynecol Obstet 2018;297:613-21.
33. Laganà AS, Vitagliano A, Noventa M, Ambrosini G, D'Anna R. Myo-inositol supplementation reduces the amount of gonadotropins and length of ovarian stimulation in women undergoing IVF: A systematic review and meta-analysis of randomized controlled trials. Arch Gynecol Obstet 2018;298:675-84.
34. Gasparyan AY, Ayvazyan L, Mikhailidis DP, Kitas GD. Mean platelet volume: A link between thrombosis and inflammation? Curr Pharm Des 2011;17:47-58.
35. Yılmaz ZV, Yılmaz E, İçer B, Küçüközkan T. Association of complete blood count parameters with gestational diabetes mellitus. Gynecol Obstet Reprod Med 2017;23:65-9.
36. Tayyar AT, Özkaya E, Yayla ÇA, Şentürk MB, Selçuk S, Polat M, *et al*. Evaluation of complete blood count parameters to predict ovarian torsion ın women with adnexal mass. Gynecol Obstet Reprod Med 2017;23:89-93.
37. Seçkin KD, Karslı MF, Yücel B, Özköse B, Yıldırım D, Aslan Çetin B, *et al*. Neutrophil lymphocyte ratio, platelet lymphocyte ratio and mean platelet volume; Which one is more predictive in the diagnosis of pelvic ınflammatory disease? Gynecol Obstet Reprod Med 2016;21:150-4.
38. Harrity C, Bereir MM, Walsh DJ, Marron KD. Moving from peripheral blood to local uterine immunophenotype analysis in patients with poor reproductive history: Pilot study of a novel technique. Ir J Med Sci 2019;188:893-901.