

# Alteration of peripheral blood cells in tubal ectopic pregnancy

Zmiany w komórkach krwi obwodowej w ciąży ektopowej jajowodowej

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## Abstract

**Objectives:** To investigate whether mean platelet volumes and leukocyte counts are altered significantly in patients with tubal ectopic pregnancy (TEP).

**Materials and Methods:** Retrospective analysis of mean platelet volumes and leukocyte counts of 138 TEP patients, diagnosed between 2005 and 2012, and the control group consisting of 72 pregnant was performed. Patients with TEP were further subdivided into 2 subgroups composed of 72 ruptured and 66 non-ruptured cases. Statistical analysis was performed using the Kruskal-Wallis and the Mann-Whitney U tests.

**Results:** Mean platelet volume was found to be larger in patients with TEP (whether ruptured or non-ruptured) when compared to controls ( $p=0.007$ ). However, no significant difference could be observed between the ruptured or non-ruptured cases ( $p=0.89$ ). With respect to leukocytosis, the TEP group with tubal rupture had significantly higher white blood cell numbers when compared to the non-ruptured TEP and the control groups ( $p=0.022$  and  $p<0.001$ , respectively).

**Conclusions:** Mean platelet volume seems to be higher in ectopic pregnancy and this finding evokes a possible role of increased platelet activity in the pathophysiology. Leukocytosis may occur more apparently in EP cases with tubal rupture. However, further prospective, controlled and with a larger sample size studies must be conducted to find clues on the correlation between the clinical entities and laboratory findings.

Key words: **ectopic pregnancy / tubal pregnancy/ mean platelet volume / leukocyte count /**

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## Streszczenie

**Cel pracy:** Celem badania było sprawdzenie czy średnia objętość płytek krwi oraz liczba leukocytów zmieniają się istotnie u pacjentek z ciążą ektopową jajowodową (TEP).

**Materiał i metoda:** wykonano retrospektywną analizę średniej objętości płytek krwi oraz liczby leukocytów u 138 pacjentek z TEP, zdiagnozowanych w latach 2005 do 2012 oraz w grupie kontrolnej 72 pacjentek ciężarnych. Pacjentki z TEP podzielono na dwie podgrupy składające się z 72 przypadków pękniętej ciąży ektopowej i 66 niepękniętej. Analizę statystyczną wykonano w oparciu o test Kruskala-Wallisa i U Manna-Whitneya.

**Wyniki:** Średnia objętość płytek krwi była większa u pacjentek z TEP (zarówno w pękniętej jak i niepękniętej ciąży ektopowej) w porównaniu do grupy kontrolnej ( $p=0,007$ ). Jednak nie zaobserwowano istotnych różnic pomiędzy przypadkami z pękniętą i niepękniętą ciążą ektopową ( $p=0,89$ ). W odniesieniu do leukocytozy, pacjentki z TEP i pękniętym jajowodem miały istotnie wyższą liczbę białych krwinek w porównaniu do pacjentek z TEP i niepękniętym jajowodem i do pacjentek z grupy kontrolnej ( $p=0,022$  i  $p<0,001$ , odpowiednio).

**Wnioski:** Średnia objętość płytek krwi wydaje się być wyższa w ciąży ektopowej i ta cecha powoduje zwiększoną aktywność płytek krwi w patofizjologii. Leukocytoza może pojawiać się częściej w pękniętej jajowodowej ciąży ektopowej. Konieczne są dalsze prospektywne, kontrolowane badania na większej grupie badanej aby znaleźć związek pomiędzy jednostkami klinicznymi a wynikami laboratoryjnymi.

Słowa kluczowe: **cięża ektopowa / ciąża jajowodowa / średnia objętość płytek krwi / leukocyty /**

## Introduction

Ectopic pregnancy (EP) is an implantation of a fertilized ovum outside the intrauterine cavity. In vast majority of EP cases, the fertilized ovum is implanted in the fallopian tubes. Actually, the fallopian tube is the site for oocyte pickup, fertilization, early proliferation of embryonic stem cell and transport of embryo. The etiology of the ectopic implantation of the fertilized ovum remains obscure [1].

Endothelial damage, angiogenesis, inflammation, increased vascular permeability, and tissue hypoxia are histopathological processes involved in the pathogenesis of EP [1-4]. Hypoxia stimulates placentation and implantation mainly through the vascular endothelial growth factor (VEGF), a potent angiogenic factor produced by platelets [2, 3]. Levels of this cytokine are increased not only at the site of implantation, but also in the systemic circulation [2-4]. In the literature, a correlation has been established between the platelet count and mean platelet volume (MPV) [5-7]. In normal pregnancy, dilutional thrombocytopenia is accompanied by a compensatory increase in the MPV and platelet distribution width (PDW). Mean platelet volume may reflect the platelet function, and increased MPV can be interpreted as 'increased platelet function' [8].

## Objectives

To the best of our knowledge, changes in MPV and leukocyte counts (WBC) in TEP have not been investigated in the literature. The purpose of this unique study was to investigate if MPV and WBC were altered in TEP.

## Materials and Methods

**Study Design:** This retrospective study was conducted at the obstetrics and gynecology department of a tertiary center after the approval of Institutional Review Board. Medical records of 138 TEP patients (72 ruptured, 66 non-ruptured) diagnosed between 2005 and 2012 and 72 pregnant constituting the control group were assessed in terms of maternal demographics

and mean platelet volumes. Patients with chronic anemia, hemoglobinopathies, chronic inflammatory diseases, renal or hepatic failure, congenital heart disease, diabetes mellitus, coagulopathies, history of smoking as well as patients on drugs that may interfere with the coagulation cascade (non-steroidal anti-inflammatory drugs, oral contraceptives, anticoagulants, etc.) or hemodynamically unstable patients were excluded from the study.

The diagnosis of TEP was confirmed upon admission to our clinic. Blood samples were obtained as soon as the diagnosis was made. As stated above, only hemodynamically stable patients were included in this series.

**Outcome Parameters:** Laboratory results of the blood samples obtained only within the first hour of admission were taken into account. Complete blood count samples which were drawn into vacutainer tubes containing 0.04 mL of 7.5% K3 salt of EDTA were analyzed within 1 hour after sampling with a commercially available analyzer (CELL-DYN 3700; Abbott Diagnostics, Abbott Park, Illinois, USA).

**Statistical analysis:** Data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 13.0 for Windows). All differences associated with a p value  $<0.05$  were considered to be statistically significant. The Kruskal-Wallis and the Mann-Whitney U tests were used to compare groups in terms of MPV values.

## Results

Demographics such as age, gestational age, obstetric history (parity, previous deliveries and abortions, etc.) are demonstrated in Table II.

No statistically significant difference was observed between the groups with regard to these parameters.

Mean platelet volumes in the EP (ruptured and non-ruptured) and the control groups are presented in Table II.

**Table I.** Demographics in ectopic pregnancy and control groups.

	Ruptured EP	Non-ruptured EP	Control	p Value
Age	29.9±6.0	30.6±5.8	29.6±5.7	0.57
Gestational age (weeks)	6.2±2.6	5.9±1.4	6.4±2.5	0.14
Gravida	3.7±2.6	3.2±1.9	3.2±1.8	0.51
Parity	2.0±1.7	1.6±1.6	1.7±1.6	0.35
Previous abortion(s)	0.8±1.4	0.8±1.0	0.5±1.0	0.8
Previous live birth(s)	1.9±1.7	1.4±1.6	1.6±1.6	0.8

Abbreviation: EP = Ectopic pregnancy

**Table II.** Mean platelet volumes in EP and control groups.

Group	n	MPV (fL)	WBC (/mm <sup>3</sup> )
Ruptured EP	66	9.45±1.85	14,088.0±6109.4
Non-ruptured EP	72	9.50±1.70	9779.1±3490.9
Control	72	7.84±1.00	9234.7±2108.5

Abbreviations: MPV: mean platelet volume; fL: femtoliter, EP: ectopic pregnancy; WBC: white blood cell count

MPV values were significantly higher in EP patients than controls ( $p=0.007$ ). When EP patients were further subclassified into the ruptured or the non-ruptured groups, MPV values were higher than controls ( $p<0.001$ ). However, there was no difference between the ruptured or non-ruptured EP patients as for MPV values ( $p=0.89$ ). White blood cell count was significantly higher in the ruptured EP group when compared to the non-ruptured EP ( $p=0.022$ ) and the control ( $p<0.001$ ) groups.

## Discussion

Tubal ectopic pregnancy is an important cause of maternal morbidity and mortality, particularly due to the risk of tubal rupture and hemorrhage, especially in cases of misdiagnosis. Annual rates of tubal ectopic pregnancies and mortality due to ruptured tubal ectopic pregnancies are 11.5/1000 and 0.4/1000, respectively. An increased incidence of tubal ectopic pregnancy worldwide may be attributed to the rising incidence of pelvic inflammatory disease caused by the Chlamydia trachomatis infection and a popularized use of assisted reproductive techniques [9].

Ultrasonography needs to be performed for patients suffering from pain or bleeding in early pregnancy in order to confirm the viability of the fetus and localization of the gestational sac. In case of an unsatisfactory ultrasonographic examination, measurement of the serum  $\beta$ -human chorionic gonadotropin ( $\beta$ -hCG) level may be helpful. However, sometimes it may be difficult to distinguish between an ectopic pregnancy, spontaneous abortion and early ongoing intrauterine pregnancy by means of a single  $\beta$ -hCG measurement. In such circumstances, repeated  $\beta$ -hCG measurements should be obtained. Doubling of serum  $\beta$ -hCG

concentrations over 48 hours suggests the presence of a viable intrauterine pregnancy. If the rise in  $\beta$  hCG is abnormal or  $\beta$ -hCG concentrations are static, then a possibility of an ectopic pregnancy must be taken into consideration. In addition to a repeated  $\beta$ -hCG measurements, endometrial curettage or laparoscopy can be used to confirm a tubal ectopic pregnancy [3, 4, 9].

Therefore, establishment of a diagnosis of tubal ectopic pregnancy is often time-consuming and costly. Indeed, it causes psychological stress in patients, and incurs financial burden to health care services. Sometimes the diagnosis of a tubal EP can be difficult at an early stage since not only one third of the cases do not exhibit any clinical signs, but also because some patients display almost no symptoms prior to tubal rupture. When a woman presents with a suspicion of an ectopic pregnancy, the diagnosis is frequently based on the initial consultation and measurement of the  $\beta$ -hCG levels, despite a considerable improvement in the resolution of ultrasonographic images [1,3].

Platelets participate in some bodily processes like endothelial damage, angiogenesis and hypoxia that contribute to the pathogenesis of EP. Mean platelet volume, a reflector of the platelet function, is a hematologic parameter that is routinely investigated in all pregnant women [10, 11]. Markers reflecting the changes in platelet volumes and leukocyte counts may be useful and easily affordable indicators of the tubal EP. High-density platelets have an elevated volume and it is reasonable to assume that platelet activation, and subsequent granula release, disturb the platelet density distributions [5, 7, 8]. Changes in platelet volumes may be more sensitive as a measure of altered function in normal pregnancy than the platelet counts, and MPV may be correlated with the severity of hypertensive disorders

of pregnancy [5,7]. Previous retrospective and longitudinal studies have investigated the role of MPV in the prediction of pre-eclampsia. Pregnant women with higher MPV in the second trimester are supposed to be at a risk of pre-eclampsia [5,7,8]. A relatively large cross-sectional study provided evidence that reduced platelet counts and elevated MPV have a 90% sensitivity and 83.3% specificity in predicting pre-eclampsia [5-7]. Rather than a single measurement, monitoring of MPV from the start of the mid-trimester might aid the clinicians in evaluating the at-risk patients [6-8].

Determination process of the reference values for hematologic parameters in pregnant women revealed that hemoglobin levels are reduced, and the white blood cell count is elevated during pregnancy and platelet counts decrease during the third trimester. Platelet density is associated with platelet reactivity and high-density platelets have an elevated volume [5,7]. In different studies, conflicting results may be encountered for the MPV values, because it is measured by clinical hematology analyzers using sodium citrate as the anticoagulant [6-8]. In general, in medical practice EDTA is usually used as an anticoagulant for CBC measurements. However, since MPV increases significantly in a time-dependent manner, measurement in EDTA containing tubes may be unreliable [10,11]. Mean platelet volume varies with time in EDTA-anticoagulated samples and EDTA-induced changes in platelet morphology cause a progressive increase in MPV. It is possible to claim that the differences among all these study results are related to the use of different anticoagulants. Furthermore, different technologies for measuring MPV might yield different results.

Tubal EP is supposed to result from dysfunction of the fallopian tube, causing embryo arrest and changes in the tubal environment. Tubal inflammation due to smoking or infection may affect embryo-tubal transport by interfering with smooth muscle contractility and ciliary beat activity. This inflammatory micro-environment may provide pro-implantation signals, such as an increased IL-8 and decreased MUC1 expression, as a response to the arrested embryo. An arrested embryo may facilitate the expression of the uterine pro-implantation factors (uteroglobin, trophinin, VEGF) that subsequently establish a tubal environment suitable for implantation. A better understanding of the etiology of tubal EP is important for the development of improved preventative measures and novel treatments, and also evolvement of diagnostic screening methods [8].

In our study, we found that MPV was significantly higher in the EP patients when compared to controls. There was no significant difference between the EP cases, with or without uterine rupture, in terms of MPV values. Our data are insufficient to clearly state that thrombosis arising from an increased platelet activity is a pathogenetic factor responsible for EP. At least it can be suggested that as an hematologic parameter, MPV is routinely analyzed during the follow-up of every pregnant woman, and it can provide quite valuable clues alerting for the possibility of EP. In patients with high MPV values, further diagnostic or therapeutic strategies can be more rapidly developed to confirm EP or to foresee and prevent any possible complications. The most important limitation of our study is its retrospective design and lack of follow-up. Some parameters evaluated at the time of admission may sometimes be misleading. Although some patients have not been enrolled in the study due the presence

of previous and/or current conditions mentioned above as the excluding criteria, we cannot fully exclude the impact of previous, unreported treatments or other unpredictable factors. Rigorous longitudinal studies may be more reliable for the assessment of MPV as a risk factor for EP.

Observation of leukocytosis in EP patients with tubal rupture was another noteworthy finding in our series. To the best of our knowledge, there seems to be no publication in the literature (PubMed) regarding the leukocytosis in ruptured EP. Obviously, causes of leukocytosis are numerous and any co-existing infection could not be completely excluded in this retrospective study. However, we are of the opinion that leukocytosis in a patient with EP can be a sign of a possible tubal rupture and such a patient must be more cautiously evaluated. This laboratory finding can be quite useful and help doctors avoid the loss of precious time before making the correct diagnosis, since tubal rupture may not present very typically in clinical settings.

Even though we cannot state that MPV can serve as a serum biomarker that can accurately and precisely detect a tubal ectopic pregnancy, increased MPV values in patients with suspected EP must be further investigated and closely monitored to confirm the diagnosis and eliminate any hazardous complications like tubal rupture. Mean platelet volume is a practical tool with no additional expenses, because it is a hematologic parameter which is routinely studied in all pregnant women.

## Conclusion

As a result, we think that hemogram parameters such as leukocyte count and MPV may be elevated in TEP. Therefore, a high index of suspicion should arise in patients with higher MPV values. The actual diagnostic and predictive potentials of MPV and leukocytosis for thrombosis and rupture of TEP ought to be investigated in further studies.

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