Case report
Prikaz bolesnice

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Vaginal bleeding in the second trimester of pregnancy leading to preterm birth - a possible case of NETosis

Vaginalno krvarenje u drugom tromjesečju trudnoće kao uvod u prijevremeni porod – mogući slučaj NET-oze

Andrijana Muller[1,2], Martina Vulin[1,2], Darjan Kardum[2,3], Mladen Ugljarević[4], Mihaela Vranješ[5], Domagoj Vidosavljević[2]

[1]Clinic of Obstetric and Gynecology, University Hospital Center Osijek, Osijek, Croatia.
[2]Faculty of Medicine, J.J. Strossmayer University, Osijek, Croatia.
[3]Department of Pediatrics, Zadar General Hospital, Zadar, Croatia.
[4]Department of Pathology, National Memorial Hospital Vukovar, Croatia.
[5]Faculty of Veterinary Medicine, University of Zagreb, Croatia.

Summary

Introduction: Preterm birth is a matter of significant concern due to its association with increased perinatal morbidity and mortality. While various causes of preterm birth have been identified, there are still unknown factors that pose challenges in terms of diagnostics and therapy.

Case report: Presents COVID positive tertiparous women in second trimester, with an uneventful course of pregnancy, admitted at the Clinic with symptoms of abdominal pain, vaginal bleeding, fever, accompanied by vaginal discharge containing liquid blood and coagula. Notably, the patient expelled a cloth of necrotic tissue from the uterus, exhibiting visible accumulations of fibrin and abundant inflammatory infiltrates of neutrophils, suggesting NETosis as potential cause. These manifestations gradually led to the development of symptoms consistent with chorioamnionitis, ultimately resulting in a preterm birth at 27 weeks of gestation.

Conclusion: In similar cases involving complicated pregnancies leading to preterm birth, it is important to consider NETosis as a potential differential diagnosis.

Keywords: chorioamnionitis; preterm delivery; NETosis

Sažetak

Uvod: Prijevremeni porod predstavlja značajan uzrok povećanog perinatalnog morbiditeta i mortaliteta. Iako su identificirani različiti uzroci prijevremenoga poroda, još uvijek postoje nepoznati čimbenici koji predstavljaju dijagnostičke i terapijske izazove.

Prikaz bolesnice: opisuje COVID pozitivnu trećerotkinju s, do tada urednom trudnoćom, primljenom na Kliniku u drugom tromjesečju s kliničkom slikom abdominalne boli, vaginalnog krvarenja i vrućice, popraćene vaginalnim iscjetkom koji je sadržavao tekuću krv i koagule. Tijekom hospitalizacije došlo je do ekspulzije djelomično nekrotične tkivne formacije iz maternice, sastavljene od nakupina fibrina i obilnih upalnih infiltrata neutrofila, što sugerira netozu kao potencijalni uzrok. Te manifestacije postupno su dovele do razvoja simptoma koji su u skladu s korioamnionitisom, što je u konačnici rezultiralo prijevremenim porodom u 27. tjednu trudnoće.

Author for correspondence/*Autor za dopisivanje:* Assoc.prof. Domagoj Vidosavljević, MD, PhD, Faculty of medicine, Cathedra for gynecology and obstetrics, J.J. Strossmayer university Osijek, J. Huttlera 4, 31000 Osijek E-mail: domagoj.vidosavljevic@gmail.com

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Zaključak: U sličnim slučajevima trudnoća kompliciranih znacima prijevremenoga poroda treba uzeti u razmatranje i mehanizme netoze kao etiološkog čimbenika.

Ključne riječi: koriamnionitis, prijevremeni porod, NET-oza

Introduction

Bleeding in the second trimester of pregnancy can be caused by various causes, as outlined in perinatal literature. These include abnormal placentation, placental abruption, vasa previa, and coagulopathies. Furthermore, infection emerges as a noteworthy contributor to bleeding, ultimately leading to preterm birth. Several ascending infectious agents contribute to local inflammation, which may escalate into intraamniotic infection or inflammation. In some cases, it can manifest as a septic condition in the pregnant woman.[1,2]

Sepsis is a multiorgan condition, signifying the host's dysregulated response to infection, ultimately leading to multiorgan failure and death, with a highly complex pathogenetic background. Pregnancy further complicates the maternal response to infection due to immunologic and cardiovascular changes designed to support fetal development. As a result, addressing sepsis necessitates a precision-based approach.[2]

While intraamniotic infection primarily targets the placental membrane, chorion, amnion, amniotic fluid, placenta, and uterine wall, in severe cases, it can progress to septicemia in the pregnant woman. The mechanisms and pathways of intraamniotic infection have been extensively studied.[3,4]

The mother's capacity to respond to various viruses and bacteria can be influenced by the overall degree of immune modulation through the control of the immune response.[2] Research exploring the pathophysiological mechanisms of immune diseases, including the impact of SARS-CoV-2 infection, has the potential to provide a fresh perspective on the possible sequence of events during pregnancy.

This case of a pregnant woman experiencing bleeding in the second trimester serves as the study foundation and offers a potential new perspective on a different inflammatory response.[5] NETosis represents a specific form of cell death, distinct from both necrosis and apoptosis in which neutrophils release neutrophil extracellular traps (NETs) into the extracellular space.NETs are web-like structures composed of decondensed chromatin (DNA and histones) coated with antimicrobial proteins (e.g., neutrophil elastase, myeloperoxidase).[6] The formation of mesh structures induced by neutrophils represents a mechanism of inflammatory reaction observed in SARS-CoV-2 infection, infections caused by other agents, and autoimmune inflammatory responses, as observed in this patient.[5] During pregnancy, such mesh formation occurs in conditions like preeclampsia, pathological placentation, and endothelial injury, serving as a vital defense mechanism for the host.[7,8]

NETs are expansive structures released extracellularly from activated neutrophils in response to infection. These structures play a pivotal role in preventing the dissemination of microorganisms in the blood by mechanically trapping them and leveraging their coagulant function to confine them within the circulation.[8]

Case report

A 37-year-old woman in her third pregnancy admitted to our Clinic at 24 weeks of gestationadmitted due to significant vaginal bleeding. A vaginal clinical examination showed liquid blood and coagula.The patient exhibited mild respiratory infection symptoms and tested positive for SARS-CoV-2 (PCR testing). During the time of admittance, a delta subtype of coronavirus was dominant in the population. Laboratory results showed leukocytosis (L) at 13.2×10^9 /L, CRP at 4.1 mg/L, erythrocytes (E) at 3.78×10^{12}/L, hemoglobin (Hg) at 125 g/L, hematocrit (Htc) at 0.36 L/L, platelets (Trc) at 367×10^9/L, packed volume (PV) at 1.3, fibrinogen at 3.5, APTV at 0.80. Fetal biometrics and amniotic fluid levels were physiological. An isoechoic area of 31 × 13 mm (Figure 1. and 2.), without detectable vascularization, was observed above the cervical orifice. Urine culture and cervical swabs were sterile. We administered antibiotic therapy with azithromycin, cephalosporin, and penicillin with microionized progesterone vaginally. Tranexamic acid (TXA) has been introduced into the therapy, 1 g intravenously for five days. Since the patient's condition improved, she was discharged home with antibiotic therapy. After one week the laboratory findings were: L 16.0×10^9/L; CRP 12.2 mg/L. The pregnant woman subjectively felt better, and the bleeding stopped. The control ultrasound finding was unchanged. We prescribed a peroral cephalosporin antibiotic for another five days.

The patient was readmitted to our Clinic at the 26th week of pregnancy due to heavy vaginal bleeding. Transabdominal ultrasound and colour Doppler described normal fetal biometrics. The resistance index (RI) of the umbilical artery was 0.50.

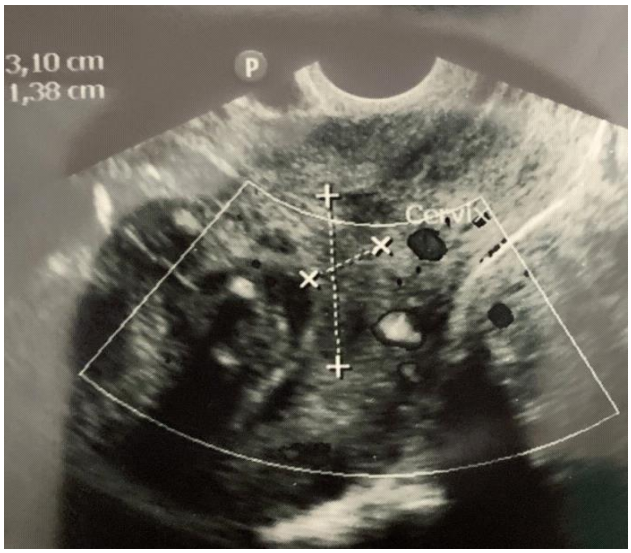

Figure 1 Ultrasonic picture presenting isoechoic area measuring 31 × 13 mm above the cervical orifice.
Slika 1. *Ultrazvučni prikaz izoehogenog područja dimenzija 31×13 mm, smještenog iznad cervikalnog ušća*

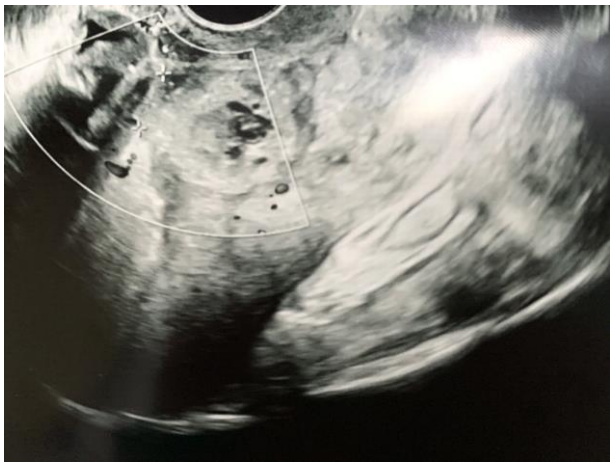

Figure 2 Previously described isoechoic area without detectable vascularization.
Slika 2*. Prethodno opisano izoehogeno područje bez doplerski detektibilnih protoka*

The transvaginal ultrasonic length of the cervix was 35 mm with the persistent isoechoic area above the cervical orifice.Upon admission, the patient was afebrile with a body temperature of 36.5 °C. The laboratory findings were as follows: leukocytes (L) 20.0 × 10^9 /L, erythrocytes (E) 4.08 × 10^{12} /L, hemoglobin (Hg) 131 g/L, hematocrit (Hct) 0.379 L/L, mean corpuscular volume (MCV) 92.9 fL, mean corpuscular hemoglobin (MCH) 32.1 pg, mean corpuscular hemoglobin concentration (MCHC) 346 g/L, platelets (Trc) 349 × 10^9 /L, mean platelet volume (MPV) 9.2 fL, packed volume (PV) 1.26 L, prothrombin time 0.88 INR, fibrinogen 5.2 g/L, and C-reactive protein (CRP) 16.0 mg/L.

During hospitalization, the patient received triple antibiotic therapy as *per Romero* and a two-day course of dexamethasone (2×6 mg/day).[9] Antibiotics were initiated after thorough consideration, as a last-resort therapy, bearing in mind all potential side effects on mother and fetus (including necrotizing enterocolitis) to the patient's nonspecific clinical symptoms. The patient experienced pain during urination and discharged vaginal content (Figure 3.) after three days.

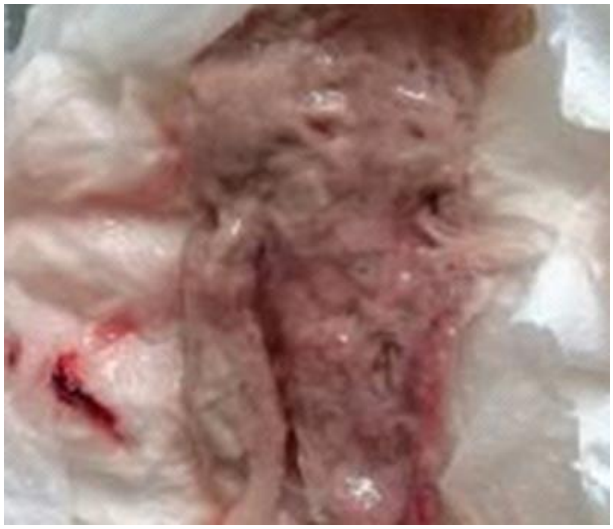

Figure 3 Spontaneous expulsion of previously ultrasonically described formation- a white-gray flat piece of tissue, measuring 3 cm in diameter.
Slika 3. *Spontano izbačeni prethodno ultrazvučno prikazani – blijedosivi komad tkiva promjera 3 cm.*

The expelled mass, sent for pathohistological examination, was described as a white-gray flat piece, 3 cm in size, showing necrotic tissue with visible accumulations of fibrin and abundant inflammatory infiltrates of neutrophils suggesting NETosis (Figure 4-5). Notably, there was no identifiable placental tissue in the discharged material. Despite antibiotic therapy, symptoms re-emerged after two days, with renewed vaginal bleeding. Laboratory findings at this point were leukocytosis (L) at 19.1 × 10^9/L, CRP at 17.2 mg/L, erythrocytes (E) at 3.14 × 10^{12}/L, hemoglobin (Hg) at 100 g/L, and platelets (Trc) at 422 × 10^9/L. Obstetric examination revealed an 80% shortened cervix with cervical orifice dilated to 2 cm. The patient reported overall weakness and rapidly deteriorating general condition despite being afebrile.

Due to acute deterioration of the patient's general condition, the consillium decided to administer neuroprophylaxis involving 4 grams of intravenous magnesium sulfate ($MgSO_4$), followed by an emergency C-section.

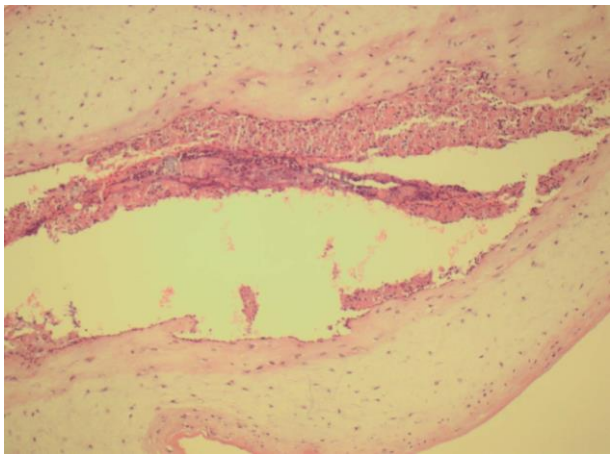

Figure 4. Subchorionic fibrin deposits with neutrophils (HE staining, 10×).
Slika 4.*Subkorijalni fibrinski depoziti s neutrofilima (bojanje HE, 10×)*

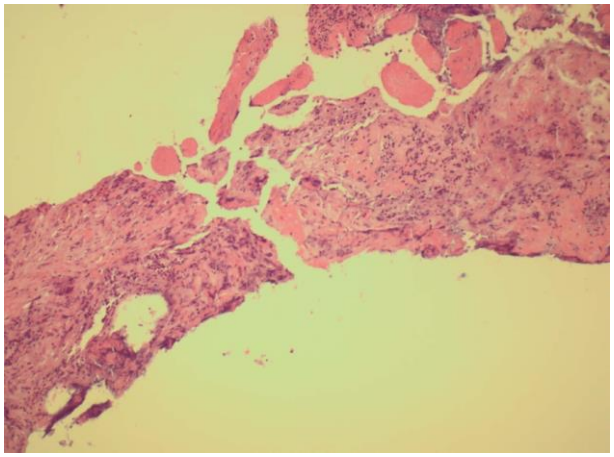

Figure 5 Fibrin deposits with neutrophils (HE staining, 10×).
Slika 5*. Fibrinski depoziti s neutrofilima (bojanje HE, 10×)*

A female baby, born very prematurely at 27 + 1/7 weeks of gestation, weighed 1100 grams, length of 36 cm, and received an Apgar score of 10/10. Due to extreme prematurity, as per our hospital protocol, the newborn was promptly transferred to the Neonatal Intensive Care Unit for specialized care. The placenta was sent for pathohistological examination. The pathohistological examination resulted in a diagnosis of chorioamnionitis acuta incipiens. Notably, there was no inflammation observed in the villi or the umbilical cord. The fetal membranes appeared macroscopically blurred and were covered with siderophages, along with sparse inflammatory infiltrates of neutrophils. The postoperative course was uneventful, involving standard postoperative infusion, analgesic therapy, and low-molecular-weight heparin prophylaxis.

The newborn experienced mild respiratory distress and received treatment with Continuous Positive Airway Pressure (CPAP) and Humidified High-flow nasal cannulae (HHFNC) for a duration of 42 days. Notably, surfactant was not administered. Antibiotic therapy with ampicillin and gentamicin was discontinued after two days as there were no signs of infection. Parenteral nutrition and trophic enteral feeds were initiated upon admission, and full enteral feedings were successfully achieved on the 28th day of life. Apnea of prematurity was addressed through treatment with parenteral doxapram and both parenteral and oral caffeine. Remarkably, the apnea subsided after two weeks. The newborn was discharged at 36 weeks of gestation. Subsequent retinopathy screening and postnatal brain ultrasound yielded normal results, with no indications of periventricular lesions or brain hemorrhage. At 18 months of corrected age, there are no signs of developmental delays.

Discussion

The precise identification of the causes of vaginal bleeding during pregnancy can be challenging from a clinical standpoint. While abnormal placentation, placental abruption, vasa praevia, coagulopathy, and infections are recognized as potential contributors to bleeding, in numerous cases, the specific cause of pregnancy-related bleeding remains uknown.[10]

Acute chorioamnionitis stands out as the most common diagnosis in placental pathology. It signifies the presence of the "amniotic fluid infection syndrome" (AFI) and manifests in the context of sterile intraamniotic inflammation, where no detectable microorganisms are present[10] Vaginal bleeding may be one of the clinical signs associated with chorioamnionitis.[11]

Microorganisms and their products, including endotoxin, peptidoglycans, and glycans, have the potential to induce an intra-amniotic inflammatory response.[1,12,13] Interestingly, numerous patients with chorioamninitis have had no microorganisms detected, indicating cases of sterile inflammation or NETosis. NETosis is triggered by danger signals or alarmins that activate the inflammasome.[14]

Several guidelines recommend the use of antibiotics to treat intraamniotic infection or inflammation. Commonly suggested antibiotics include ampicillin, cefazolin, and gentamicin. In cases of penicillin allergy, an alternative regimen may involve clindamycin and vancomycin, or erythromycin as an alternative option.[15,16] The choice of antibiotics may vary based on the specific circumstances, and healthcare professionals typically

consider factors such as the patient's medical history and local resistance patterns when making treatment decisions.

In a study by *Romero et al*., the combination of antibiotics (ceftriaxone, clarithromycin, and metronidazole) has proven effective in cases involving cervical insufficiency and intraamniotic infection in 75% of cases, with an overall treatment effectiveness of approximately 60% of all cases.[9,15,16]

The efficiency of antibiotics in reducing decidual infection is a subject of debate. Studies involving microorganisms in the chorioamniotic membranes and decidua have indicated that microbial organisms primarily invade the amniotic cavity and are only secondarily present in the decidua. This challenges the initial perspective that organisms would be present in the decidua and, from there, invade the amniotic cavity. Empirical evidence utilizing morphologic and molecular microbiologic techniques contradicts this initial assumption.[17,18]

The pathological finding of chorioamnionitis is marked by the diffuse infiltration of neutrophils into the chorioamniotic membranes.[19]

Vital NETosis plays a crucial role in containing local infections, such as gram-positive cellulitis, as it enables polymorphonuclear neutrophils (PMNs) to swiftly release NETs while still maintaining the ability to chemotax and phagocytose live bacteria. Furthermore, PMNs undergoing vital NETosis can preserve their membrane integrity, effectively entrapping captured bacteria. Intravascular NET release enhances the capture of both bacteria and viruses within the bloodstream. Intravascular NETosis may also contribute to immunothrombosis.[8]

NET's contain several proteins that inhibit microbes, encompassing enzymes, antimicrobial peptides, calgranulin, and histones. The antimicrobial activity of NET's results from the synergistic actions of multiple components, and it is further enhanced by elevated local concentrations of mediators on the surfaces of the NET's.[20]

Considering both the clinical and pathohistological grading of intraamniotic infection, the described inflammatory response in NETosis could be regarded as an introduction to the clinical intraamniotic infection syndrome, particularly in relation to this patient.

Our patient underwent ultrasound examination and received treatment under the clinical diagnosis of intraamniotic infection. Clinical observation of tissue expansion, and the pathohistological analysis of discharged cloth revealed a cluster of fibrins infused with the infiltration of neutrophils. However, the pathohistological analysis of the fibrin substrate expelled from the birth canal did not provide a clear answer or explanation for the events that occurred during the pregnancy, other than neutrophillic infiltration. It is also important that COVID infection had played a role in the patient's general worsening condition and decision to terminate the pregnancy, however, it is hard to link acute COVID infection with expulsed tissue.

The origin of this fibrin is not entirely clear. Two fundamental mechanisms explain the accumulation of fibrin in the placenta: maternal floor infarction, where fibrin is mostly confined to the basal plate, and massive perivillous fibrin, where fibrin clusters are diffusely distributed in the intervillous spaces.[21] Morphologically and in terms of content, these mechanisms differ from the matrix type fibrinoid composed of connective matrix molecules, often without pure fibrin, and the fibrin type fibrinoid, which exhibits immunohistochemical properties of a blood clot and lacks a cellular trophoblastic component. The latter accumulates in conditions of hypoperfusion of the maternal vasculature associated with decidual vasculopathy and thew preeclampsia syndrome. The relatively abundant necrosis in the material complicates the determination of the location and type of the described fibrin accumulation according to the above division.

It is impossible to unequivocally determine the exact genesis of the material. Whether it is a separate fragment of subchorionic fibrin as part of chorioamnionitis, potentially explaining the neutrophilic infiltrate to some extent, or if it is a disorganized accumulation of perivillous deposited fibrin, where neutrophils are not typically present, morphologically fitting into the pathophysiological mechanism of NETosis, or if it is something else altogether, cannot be definitively declared by the pathologist. It may be necessary to await further studies addressing the same or at least a similar issue for more insights. These assumptions necessitate further laboratory and clinical research. The chronological arrangement of causal mechanisms in the presented patient is challenging to establish.

Conclusions

The causes of vaginal bleeding during pregnancy are numerous and can be very challenging from a clinical standpoint. This case presents a patient with vaginal bleeding and symptoms of intra-amniotic infection resulting in the formation of a fibrin substrate with neutrophilic depostion. One of the causes of preterm birth discussed in this report is NETosis. Despite prematurity, the overall perinatal outcome was favorable, pointing out to NET-osis as a possible cause. Additionally, since the pregnant

woman was SARS-COV-2 positive during her pregnancy, we attempted to illuminate the challenging pathophysiological events from the perspective of NETosis. Further extensive research is needed for confirmation, but it is important for physicians to be aware of this potential correlation.

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