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Correlation of clinical and biological evidence – a dominant therapeutic element of succeeding in ectopic pregnancy

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Abstract

Tubal pathology, smoking, pelvic inflammatory disease, miscarriage, medical or surgical abortion, usage of intrauterine devices (IUDs) for women with salpingitis latent injuries, older than 40 years, are risk factors for ectopic pregnancy. The objective of this study concerns the correlation of the clinical and biological evidence for the early diagnosis of the ectopic pregnancy and, as soon as possible, for the estimation for eventual risk of complications that may appear. The transvaginal ultrasound test, minimal increases in serum beta-human chorionic gonadotropin (β-hCG) dynamics and blood counts are investigations of choice in achieving our objective. Overcoming β-hCG critical level (>1198 IU/mL), the decrease of platelets and changes in platelet constants announce the imminent risk of ectopic pregnancy rupture and the need to take a quick decision on the course of treatment.

Keywords: ectopic pregnancy, hemoperitoneum, β-hCG, ultrasound.

Introduction

The ectopic pregnancy has as a feature the blastocyst implantation outside the uterine endometrium cavity in the trunk, abdomen, ovary, intramural or in the cervical canal, being favored by sexually transmitted diseases (STDs), tubal pathology, reproductive technologies, Fallopian tube sterilization and usage of intrauterine devices (IUDs). The ectopic pregnancy seriously compromises a woman's obstetrical future, 1/3 of ectopic implantation occurring in nulliparous, when 50% of women with this disease will remain with infertility. The pregnancy etiology is not fully understood [1].

The frequency of ectopic pregnancy, according to the scientific literature, ranges from 0.58 to 1.3% of all births due to the high incidence of the acute salpingitis, increased usage of estroprogestative contraceptives, IUDs and growing number of tubal surgical interventions. The frequency of ectopic pregnancy represents approx. 1–2% of all reported pregnancies [2].

It is considered that the incidence of ectopic pregnancy is higher in developed countries, where the absolute number of ectopic pregnancies is not known, in comparison to the undeveloped world [3].

Generally, the ectopic pregnancy is identified before rupture, by serial determinations of beta-human chorionic gonadotropin (β-hCG) and transvaginal ultrasonography (TVU) [4].

Presently, TVU is considered as the standard method that provides a high quality examination and a certain and early preoperatory diagnosis [5, 6].

There were described three major options for the ectopic pregnancy: the expectative management, surgical treatment and medical treatment [7].

When the diagnosis of non-ruptured ectopic pregnancy is established early, there is usually preferred a drug treatment (Methotrexate). The drug treatment in the above-presented conditions has a success rate of 52–94% [8].

The risk of an ectopic pregnancy is three to four times higher in women who smoke above one pack of cigarettes per day. Fallopian tube is affected by cigarette smoke, which impairs ciliary function and causes smooth

muscle contraction, being compromised oocyte-cumulus complex and transport of the embryo in the uterus. Tubal infertility and hydrosalpinx represents the main risk factors for ectopic pregnancy in *in vitro* fertilization (IVF) techniques. The hormonal changes of age (35–45 years) alter the tubal function, increasing the risk of an ectopic pregnancy [9].

Birth control pills based on progesterone only, due to diminish the tubal motility, increase the rate of ectopic pregnancy.

The antibiotics prophylaxis in the event of a pharmacological or surgical induced abortion has a protective effect against inflammatory damage to the Fallopian tubes.

Because tubal ectopic pregnancy is the most frequent form of ectopic pregnancy localization, we will refer to tubal ectopic pregnancy as ectopic pregnancy, specifying another type of ectopic pregnancy, when necessary.

Patients, Materials and Methods

The study includes 49 pregnant women, with ectopic pregnancies, which are in evidence at the "Filantropia" Municipal Hospital, Craiova, Romania. To whom there were made clinical examinations and laboratory investigations: ultrasonography, hormonal and biochemical determinations [β-hCG, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, blood counts (blood group ABO and Rh, counting of reticulocytes)]; 19 of the pregnant women were clinically supervised by the following analyses: β-hCG's dynamic, blood tests [complete blood count (CBC)], coagulation tests [Quick time, international normalized ratio (INR), activated partial thromboplastin time (APTT), fibrin monomer test (FMT), fibrin degradation products (FDPs), D-dimers] and ultrasound examination.

Results

From all the clinically supervised pregnant women, we evidenced three representative cases.

Case No. 1

Left tubal ectopic pregnancy, gravida 1, para 0, with signs of a ruptured ectopic pregnancy on the 5th week of pregnancy; the case risk data indicate: amenorrhea, vaginal bleeding in small amounts and mild abdominal pain.

The laboratory investigations indicate that the patient was hemodynamically stable with a minor increase in the dynamics of the β-hCG serum. There were reported no significant changes in platelet counts and platelet indices [mean platelet volume (MPV), platelet distribution width (PDW)] due to prompt surgery, knowing that ruptured ectopic pregnancy involves the consumption of platelets and inflammatory phenomena (Table 1).

The ultrasound examination confirmed the free fluid (representing blood) in the pouch of Douglas (hemoperitoneum), a heterogeneous mass adjacent to the ovary (blob sign) but separated from this; the pregnancy was evacuated by surgical treatment – laparotomy with salpingostomy.

Table 1 – ***The laboratory investigations in Case No. 1***

Parameter	Result	Biological reference values
Leukocytes	6.57×10^3/mm^3	$4–9\times10^3$/mm^3
Erythrocytes	4.08×10^6/mm^3	$4–5\times10^6$/mm^3
Hemoglobin	13.5 g/dL	12–15 g/dL
Hematocrit	37.2%	36–45%
MCV	91.2 fL	88–95 fL
MCH	33.1 pg	28–32 pg
MCHC	36.3 g/dL	32–36 g/dL
Platelets	290	150–400
Lymphocytes	36.4%	20–40%
Monocytes	8.1%	0–8%
Neutrophils	51.1%	50–75%
Eosinophils	3%	0–3%
PDW	12.3 fL	11–16 fL
MPV	10.4 fL	7.4–10.4 fL
ALT	13 U/L	2–40 U/L
Glycemia	77.9 mg/dL	70–110 mg/dL
INR	1.02	0.9–1.1
Serum β-hCG:		
Sample 1	1125 µIU/mL	>5 µIU/mL in pregnant women
Sample 2 – after 48 h	1198 µIU/mL	

MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PDW: Platelet distribution width; MPV: Mean platelet volume; ALT: Alanine aminotransferase; INR: International normalized ratio; β-hCG: Beta-human chorionic gonadotropin.

The surgical piece was macroscopically examined, subsequently processed by histopathological methods, the sections being stained histochemically and immunohistochemically. The examination of the histochemically-stained pieces with the Hematoxylin–Eosin (HE) highlighted the presence of secondary and mesenchymal villosities, with angiogenesis foci (Figure 1, A and B). Also, there is observed the presence of the extravillous trophoblast in the salpingian wall of the giant-cellular and diffuse interstitial type. The salpingian wall presents a decidual transformation, with dilated, thrombosed, diffuse hematic infiltrated and perivascular vessels (Figure 1C). The cross-sections examined using Goldner–Szekely (GS) trichrome staining highlight the decidual change of the salpingian wall into involution (Figure 2A), thrombosed vessels with perivascular inflammatory infiltrates (Figure 2B). The immunohistochemical examination by using the cytokeratins (CKs) 7 and 8, as well as pan-cytokeratin antibody (MNF-116) highlighted an intensely positive trophoblast immunostaining (Figure 3, A, B and E). The immunostaining with CK19 was intensely positive in the salpingian mucosa (Figure 3C). The cytoplasm of the syncytial buds was intensely positive to the immunostaining with MNF-116 (Figure 3E). Instead, the immunostaining with CK20 was negative in the trophoblast cytoplasm of the mesenchymal villosities with stromal degenerescence (Figure 3D). The salpingian fibromuscular tunica was intensely positive to the CD34 staining (Figure 4A), as well as in the angiogenesis foci in the mesenchymal villosities (Figure 4B). The immunostaining to estrogen is positive in the villositary trophoblast cytoplasm (Figure 5A), but negative to progesterone (Figure 5B). Still, the progesterone is positive intra-nuclearly in the salpingian mucosa (Figure 5C).

Case No. 2

Right tubal ectopic pregnancy, eight weeks' gestation, uncomplicated; patient requested specialist advice for vaginal bleeding in small amount for approx. 3–4 weeks.

The patient was hemodynamically stable. Increased levels of serum β-hCG, well above the predictive value cited in the literature (>1750 IU/mL), which announces the imminent risk of uterine tube rupture; minimum value of the PDW were useful in therapeutic decision making, case completed with laparoscopic intervention (Table 2; Figures 6 and 7).

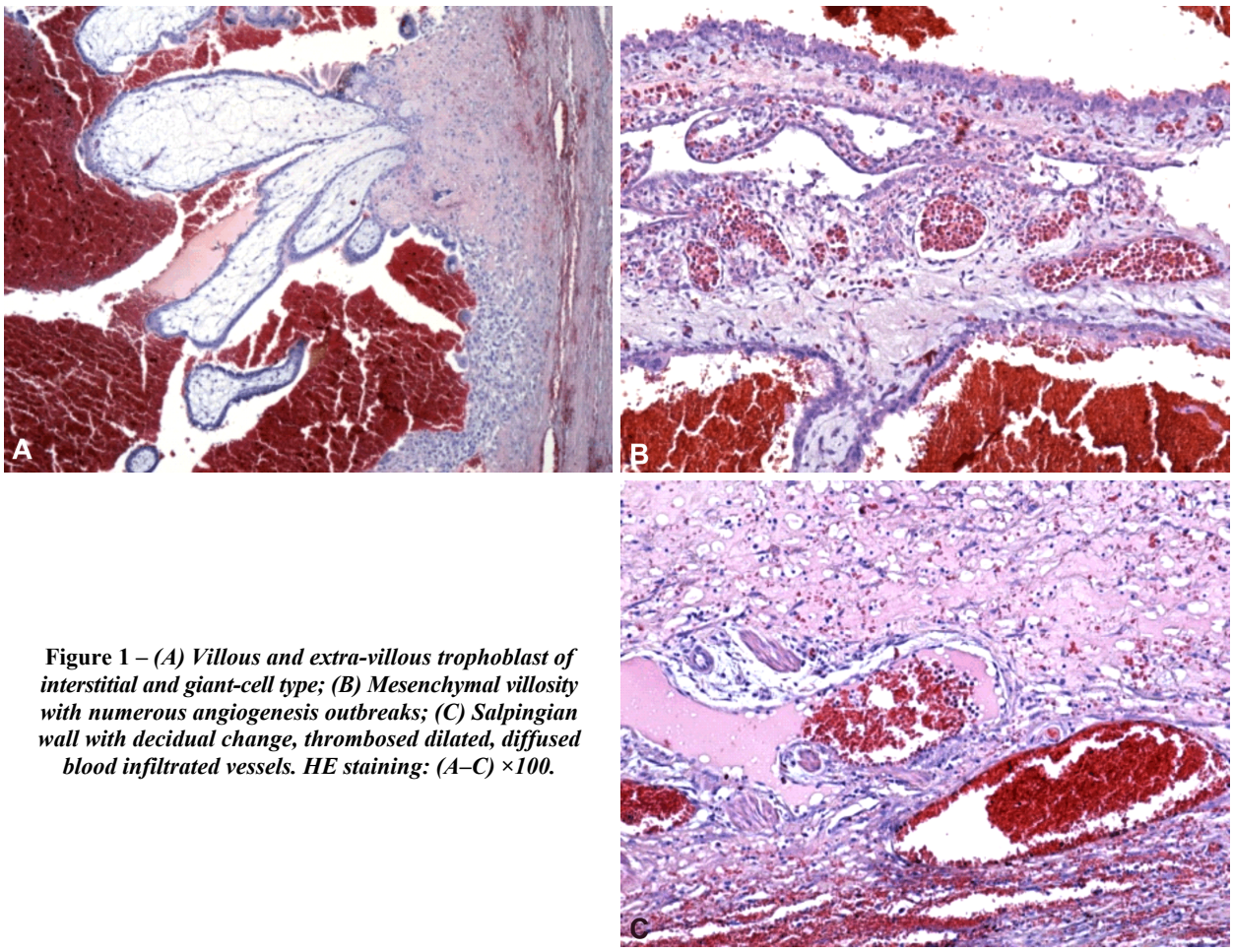

Figure 1 – *(A) Villous and extra-villous trophoblast of interstitial and giant-cell type; (B) Mesenchymal villosity with numerous angiogenesis outbreaks; (C) Salpingian wall with decidual change, thrombosed dilated, diffused blood infiltrated vessels. HE staining: (A–C) ×100.*

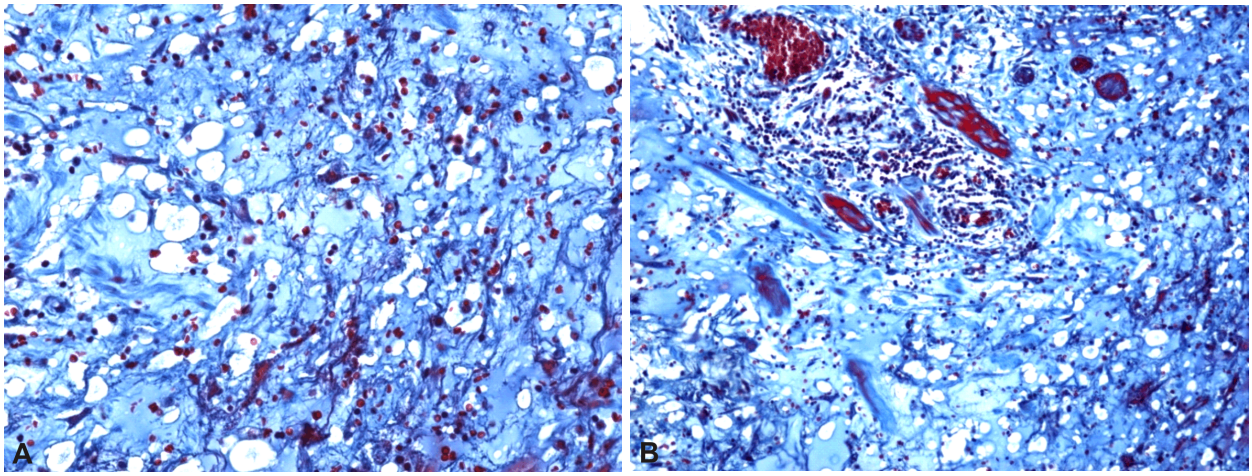

Figure 2 – *(A) Salpingian wall with necrobiosis decidua with diffuse blood infiltrates; (B) Salpingian wall with thrombosed vessels and perivascular inflammatory infiltrates. GS trichrome staining: (A and B) ×100.*

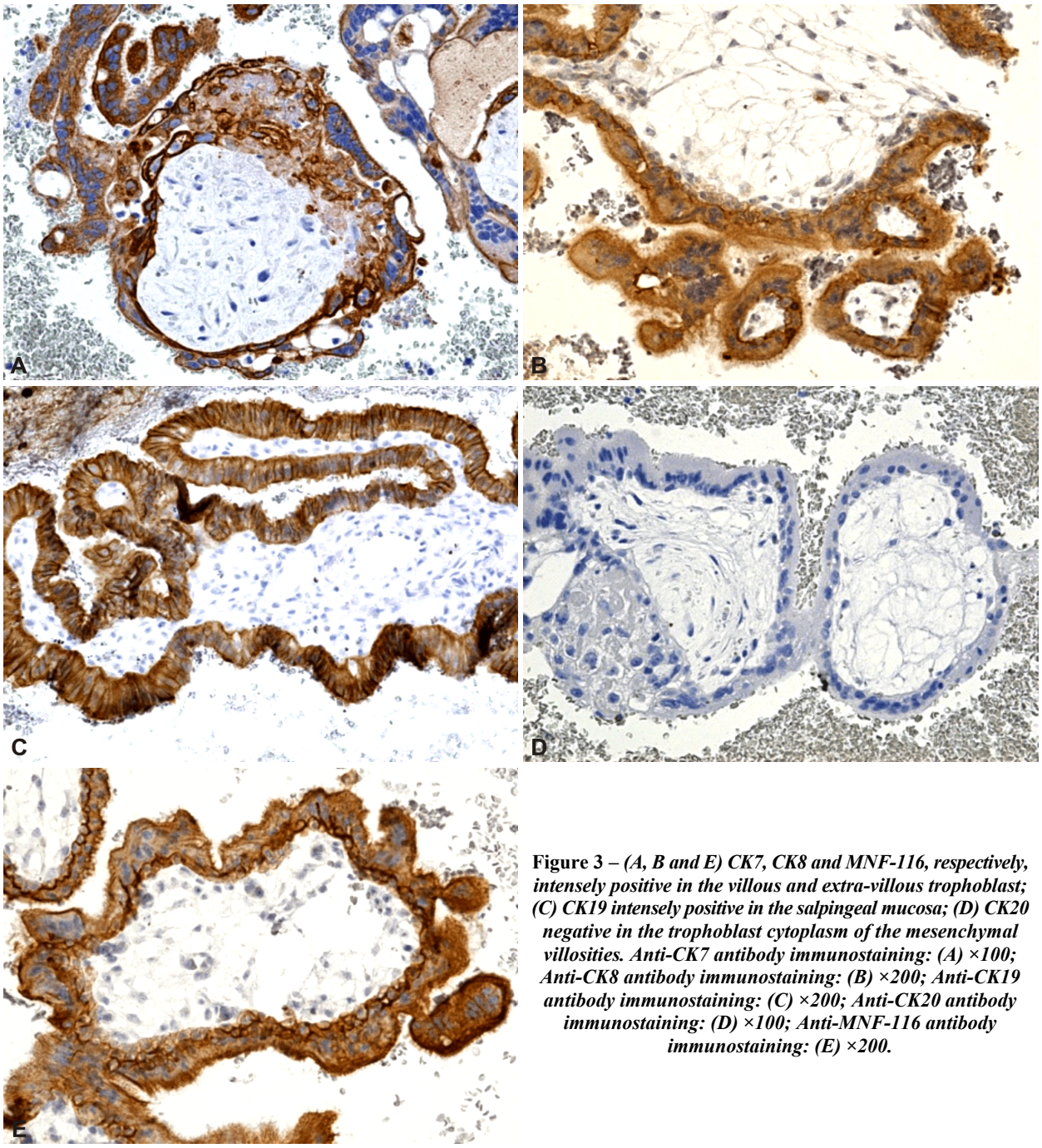

Figure 3 – ***(A, B and E) CK7, CK8 and MNF-116, respectively, intensely positive in the villous and extra-villous trophoblast; (C) CK19 intensely positive in the salpingeal mucosa; (D) CK20 negative in the trophoblast cytoplasm of the mesenchymal villosities. Anti-CK7 antibody immunostaining: (A) ×100; Anti-CK8 antibody immunostaining: (B) ×200; Anti-CK19 antibody immunostaining: (C) ×200; Anti-CK20 antibody immunostaining: (D) ×100; Anti-MNF-116 antibody immunostaining: (E) ×200.***

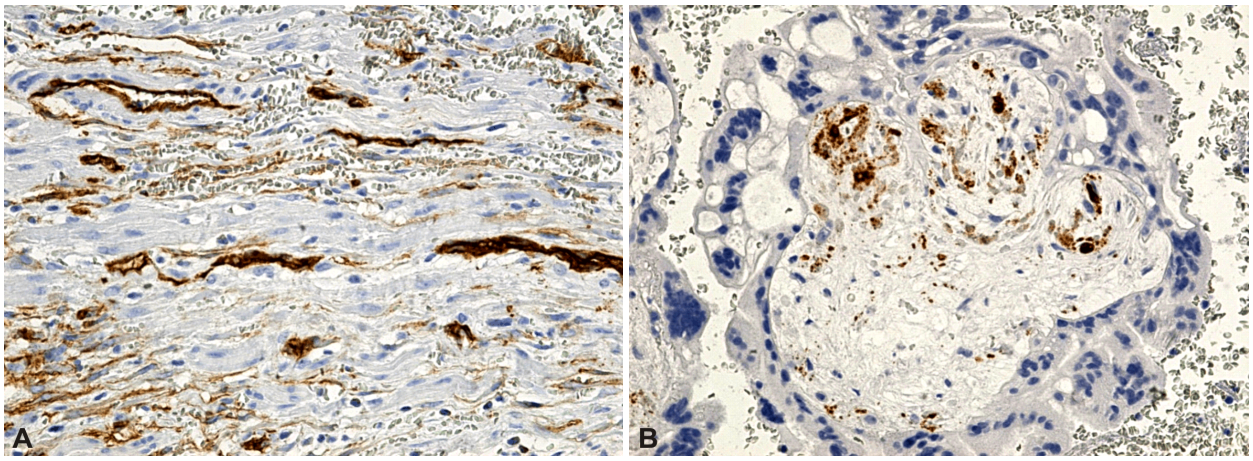

Figure 4 – ***(A and B) Vessels of the salpingian fibromuscular tunica and of CD34 intensely positive mesenchymal villosity. Anti-CD34 antibody immunostaining: (A and B) ×100.***

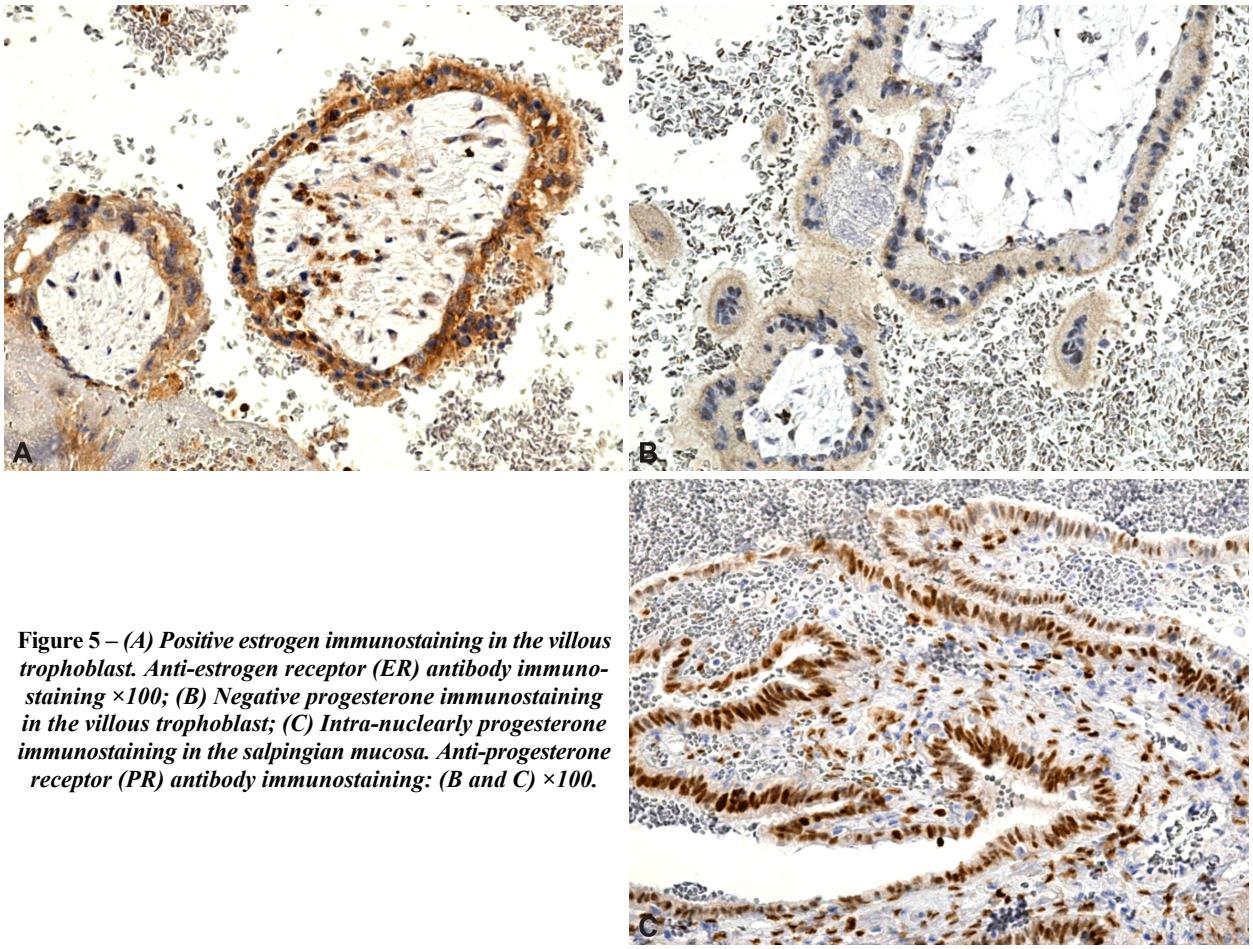

Figure 5 – *(A) Positive estrogen immunostaining in the villous trophoblast. Anti-estrogen receptor (ER) antibody immunostaining ×100; (B) Negative progesterone immunostaining in the villous trophoblast; (C) Intra-nuclearly progesterone immunostaining in the salpingian mucosa. Anti-progesterone receptor (PR) antibody immunostaining: (B and C) ×100.*

Table 2 – *The laboratory investigations in Case No. 2*

Parameter	Result	Biological reference values
Leukocytes	9.54×10^3/mm^3	$4–9\times10^3$/mm^3
Erythrocytes	3.43×10^6/mm^3	$4–5\times10^6$/mm^3
Hemoglobin	11.5 g/dL	12–15 g/dL
Hematocrit	32.8%	36–45%
MCV	95.6 fL	88–95 fL
MCH	33.5 pg	28–32 pg
MCHC	35.1 g/dL	32–36 g/dL
Platelets	337	150–400
Lymphocytes	18.1%	20–40%
Monocytes	5.5%	0–8%
Neutrophils	76%	50–75%
Eosinophils	0.2%	0–3%
Basophils	0.2%	0–1%
MPV	10 fL	7.4–10.4 fL
PDW	11 fL	11–16 fL
Glycemia	82.7 mg/dL	70–110 mg/dL
INR	1	0.9–1.1
Serum *β*-hCG	8604 µIU/mL	>5 µIU/mL in pregnant women
Serum *β*-hCG at 48 h from the first measurement	8921 µIU/mL	

MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MPV: Mean platelet volume; PDW: Platelet distribution width; INR: International normalized ratio; *β*-hCG: Beta-human chorionic gonadotropin.

Case No. 3

Cervical ectopic pregnancy treated with Methotrexate, 35-year-old pregnant woman; one pregnancy (delivered by Cesarean section), one spontaneous miscarriage; gravida 3, para 2.

From the patient's medical history, we mention a 6-week amenorrhea, mild abdominal pain and slight bleeding vaginal for about seven days, positive pregnancy test. The patient did not ask for medical advice until the moment of hospital presentation.

Due to the decrease in platelet count dynamic and to the subnormal value of the PDW (Table 3), the patient was given emergency Methotrexate 1 mg/kg/day – 50 mg/day, intramuscular injections for three consecutive days. The monitoring of seric *β*-hCG showed a progressive decrease and transvaginal ultrasound showed a collapsed gestational sac after seven days (Figure 8).

Table 3 – *The laboratory investigations in Case No. 3*

Parameter	Result	Biological reference values
Platelets	700	150–400
PDW	10.5	11–16 fL
Platelets at 72 h	681	150–400
PDW at 72 h	10.8	11–16 fL
Platelets at 96 h	438	150–400
PDW at 96 h	10.7	11–16 fL

PDW: Platelet distribution width.

Follow-up scan revealed complete resolution that

occurred three months after the medical therapy. Early diagnosis and conservative treatment prevented installing bleeding through the erosion of the blood vessels of the cervix and lead to avoid hysterectomy.

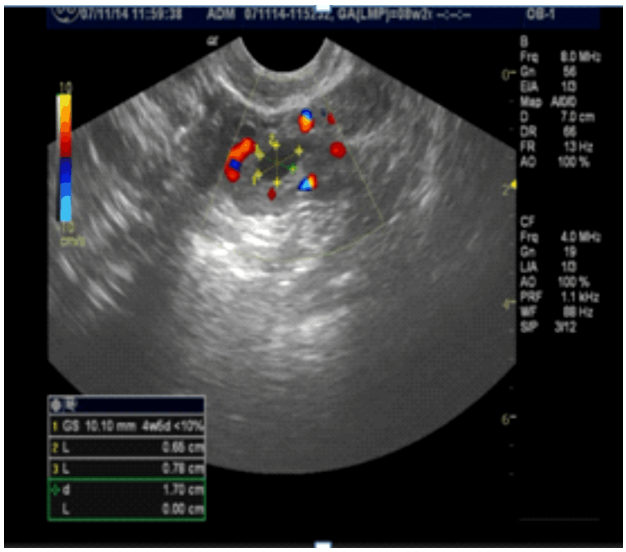

Figure 6 – ***Transvaginal ultrasound image of an ectopic tubal pregnancy: a mass with a hyperechoic ring around the gestational sac (10 mm – four weeks and five days); empty adnexal gestation sac.***

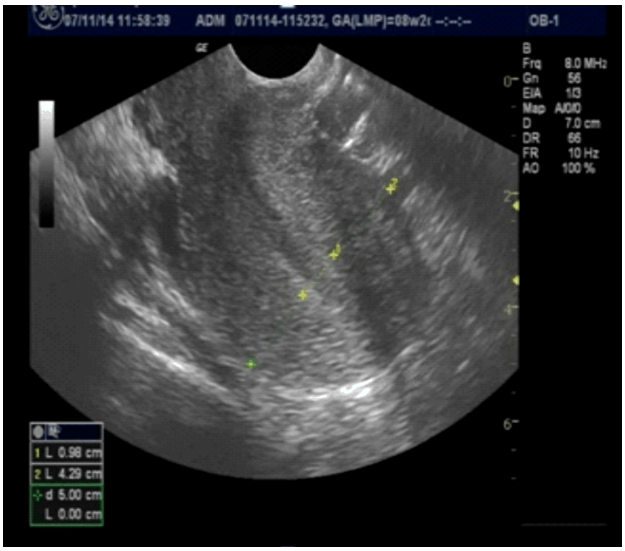

Figure 7 – ***Absence of intrauterine pregnancy (empty uterus) at eight weeks of amenorrhea.***

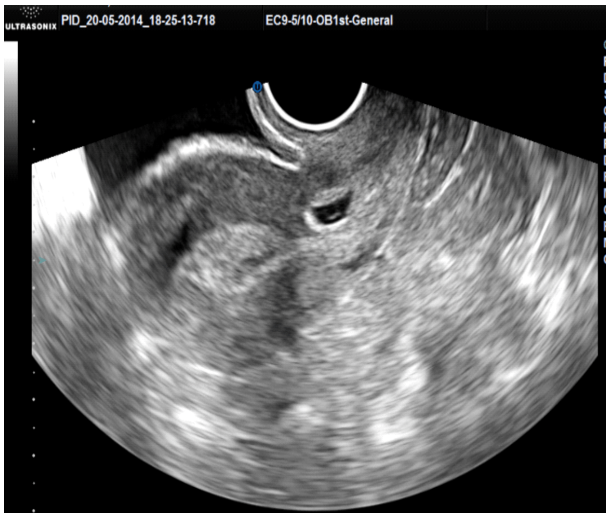

Figure 8 – ***Transvaginal ultrasound images of an ectopic cervical pregnancy: empty uterine cavity, gestational sac within the cervical canal, below the level of the cervical internal os, with trophoblastic ring and embryonic echo without cardiac activity.***

Discussion

The lack of the Fallopian tube submucosal layer allows the early penetration of the trophoblast and ensures the easy access through the epithelium and the implantation of the fertilized egg in the muscular wall [10].

Acute and chronic salpingitis may either stop embryo downhill and implantation in its trunk. Infections, especially with *Chlamydia trachomatis*, lead to the absence of the spontaneous activity of the interstitial cells of Cajal in the oviduct, pacemaker cells responsible for the oviduct motility and egg transport [9, 11]. The antibiotherapy makes the inflammatory process affect less the tubal permeability than the epithelium alteration that remains irreversible.

External fertilization occurs in 1/3 of the tube, where from, as a result of the kinetic activity of the tube, the egg migrates to the uterus. The change of the tubal physiology (mechanics, dynamics, biochemistry), the development process of the egg, the late egg capture, when fertilization occurs outside the tube, can create the premises of an ectopic implantation [12].

The anatomical causes of the tubal ectopic implantation, which can disrupt the tubal peristalsis are represented by the inflammatory stenosis (sequelae of trivial salpingitis or unapparent or treated tuberculosis, which may affect the tubal epithelium) tumors of the uterus (myoma of the cervix or included in the broad ligament by compression exerted on the tube path), tubal malformations (hypoplastic tubes, tubal diverticula), endometriosis, often interstitial located, tubal plastic surgery sequelae (ectopic pregnancy, tubal stump remaining relapses). Functional factors, spasmodic factors can alter the ovarian peristalsis through the steroid secretions; estroprogestative by the estrogens in their composition can cause a muscle spasm in the tubal isthmus; continuous intake of progestative microdoses increases the risk of ectopic pregnancy by their inhibiting action on the tube muscle contractions; the intrauterine devices increase the risk of ectopic pregnancy, but only in women with latent injured salpingitis; induction of ovulation by human gonadotropins can cause ectopic pregnancy – during multiple ovulation, the first oocyte has a normal transport, but hormonal changes linked to the first yellow body disrupts the transport of additional ova. Chronic exposure to nicotine can lead to dysfunction of the Fallopian tubes by affecting the level of the CB1 cannabinoid receptor with a role in the embryonic transport along the oviduct [13, 14].

If ectopic pregnancies occurred in IVF techniques there has been observed a high concentration of E-cadherin – an intercellular adhesion molecule, in the implantation tubal zone only in women who had IVF, suggesting such

a factor rather biological than mechanical, of association with the IVF techniques.

The most often found risk factors in cervical ectopic pregnancy are the factors that may cause changes in the cervical area: dilation and curettage, IVF techniques, but taking into consideration the scarcity of this ectopic localization, the intents of associating these factors remains weak [15]. Still, we observed in our case of cervical pregnancy, the association of two factors, Cesarean (C)-section and previous curettage.

The transvaginal ultrasound test, the dosages in the dynamics of β-hCG serum and the blood counts are investigations of choice in the diagnosis of the ectopic pregnancy. Increased levels of serum β-hCG over the predictive value cited in the literature (>1750 IU/mL), which announces the imminent risk of uterine rupture tube, and the minimum value of PDW are useful in therapeutic decision making in ectopic pregnancies [16]. In normal pregnancy, the serum β-hCG increases after a *log*-linear model up to 80 days from the last period, with 60–100% every 48 hours.

A tubal ectopic pregnancy can lead to tubal rupture, tubal abortion or spontaneous resorption; tubal abortion consists in expelling the pregnancy by the fimbriae end being able to regress or being able to implant again into the abdominal cavity. Some ectopic pregnancies stop evolving and are resorbed without adverse effects on the patient; tubal rupture is associated with significant intra-abdominal hemorrhage [17].

Non-specific laboratory explorations announce the imminent risk of intra-peritoneal hemorrhage: decreased platelet count, decreased platelet indices (MPV, PDW) due to increased young, efficient platelet consumption. The early diagnosis of the ectopic pregnancy together with the application of the conservative treatment can prevent the onset of bleeding by eroding blood vessels and avoid surgery [18]. The optimal treatment protocol has been discussed repeatedly in the literature [19–21].

Therapy consists in a treatment with Methotrexate [50 mg/m^2 body surface area (BSA) in single dose or 1 mg/kg dose followed by 1, 3, 5, 7 days of Leucovorin additional dosages of 0.1 mg/kg days 2, 4, 6, 8), prostaglandins, Mifepristone (stops development of trophoblast), Chinese medicine plant-based potassium chloride or hyperosmolar glucose injected in the ectopic mass [22]. The surgical treatment includes laparotomy or laparoscopic approach. In the ectopic pregnancy, laparotomy has a potential advantage over the laparoscopic surgery when planning salpingostomy [23]. This necessity treatment was also applied in the first case of our study. The histopathological analysis of the received piece confirmed the presence of tubal pregnancy, being a direct correlation between the levels of serum β-hCG in the last 48 hours. The examination of the usual and immunohistochemical stained sections was conclusive for pregnancy, by the decidual change of the salpingian wall, the presence of thrombosed vessels and areas of blood necrosis being relevant for the salpingian wall rupture. The presence of the villous and extra-villous trophoblast in the salpingian cavity confirms ectopic pregnancy. From the study of specialized literature in the case of tubal ectopic pregnancies, we did not find an immunohistochemical study. The trophoblast cells express a high number of proteins. An immunohistochemical analysis of the normal pregnancy trophoblast in the first trimester highlighted an intensely positive reactivity of cytokeratins, both in the villous trophoblast and the extra-villous one [24]. In our case, there was highlighted the same aspect of CK7, CK8 and MNF-116 in the villous and extra-villous trophoblast. The immunostaining with CK19 was positive in the salpingian mucosa, suggesting a trophoblast response. Instead, the immunostaining with CK20 was negative in the uninvolved trophoblast of the mesenchymal villosity with stromal degenerescence. The same aspect was identified as a response reaction to the cytokeratins with high molecular weight (CK20) in the trophoblast cells of normal pregnancy. The immunostaining with hormonal antibodies like the estrogen, or progesterone, respectively, shows an inversely proportional reaction in the trophoblast cytoplasm. The immunohistochemical analysis of the villous and extra-villous trophoblast of the tubal pregnancy is in correlation to the ones mentioned in literature regarding normal pregnancy.

In the cervical pregnancy, the early diagnosis and drug treatment with Methotrexate is the treatment of choice, as the massive bleeding that may occur after curettage would determine an emergency surgical intervention, consisting in hysterectomy, the only way of saving the patient's life [25].

The termination of the pregnancy and the uterine preservation are achieved by the administration of Methotrexate for pregnancies less than 12 weeks [26, 27]. Methotrexate therapy should be followed by contraception because of teratogenic effects of this drug, which may persist in human tissues up to eight months after administration.

The surgical procedures include suction curettage or hysterectomy if the advanced pregnancies or uncontrolled bleeding by conservative methods [28].

Conclusions

There should be approached an individualized management for ectopic pregnancy. The physicians should suspect an ectopic pregnancy in a woman at reproductive age that presents abdominal or pelvine symptoms of ectopic pregnancy. Even though the ultrasounds examination remains a reliable early diagnosis technique, we should take into account the fact that the ultrasound diagnosis of ectopic pregnancy sometimes can be difficult. Estroprogestatives and ovulation inducers must be very wisely used and the insertion of the intrauterine devices shall be observing the contraindications, knowing that these factors are involved in the etiology of the ectopic pregnancy. Early detection and treatment of the uterine tumors, of the tubal malformation, inflammatory stenosis which can disrupt tubal peristalsis favoring ectopic implantation, aims to prevent ectopic pregnancies. Prenuptial screening for genital infections, especially those with *Chlamydia trachomatis* can prevent ectopic pregnancy, pelvic inflammatory disease playing a major role in the tubal adhesions and their obstruction. Transvaginal ultrasound, dynamic dosage of β-hCG in serum, blood counts and platelet-indices evaluation time are investigations of choice for early diagnosis of the ectopic pregnancy and for faster estimation and of the risk for complications. Morphologically and immunohistochemically, the villous

and extravillous trophoblast of the tubal pregnancy has the same reactivity to cytokeratins like in a normal pregnancy, so the localization of the respective implantation, and of the placental development, respectively, is an insignificant one.

Conflict of interests

The authors declare no conflict of interests.

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