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Pregnancy of unknown location — still diagnostics and therapeutic problem

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Abstract: Pregnancy of unknown location is still a significant diagnostic and therapeutic problem in the population of women of reproductive age. The management of women with PUL can vary widely, as there are still no standardized guidelines for the diagnosis and treatment of such a clinical situation. The diagnostic effort in this initial diagnosis should be directed both to the exclusion of ectopic pregnancy, which carries a significant risk to the patient, as well as a properly implanted pregnancy, since the implementation of treatment may be associated with adverse effects on its subsequent fate. The authors reviewed the literature on this issue, taking into account their own experience in the management of pregnancies of unknown location. They conducted a critical analysis of the literature on PUL found in MEDLINE, Google Scholar, the Cochrane Central Register of Controlled Trials, and Clinical Trials.

Keywords: pregnancy of unknown location, ectopic pregnancy, ultrasound, human chorionic gonadotropin, biomarkers, review.

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Introduction

PUL is commonly defined as failure to see pregnancy on transvaginal ultrasound with a positive pregnancy test. PUL is therefore a diagnosis describing a clinical situation that requires further diagnostics [1]. It is not a definitive diagnosis, but in a number of women with this diagnosis, the actual location of the pregnancy cannot be determined, as both intrauterine pregnancy and ectopic pregnancy can terminate spontaneously without any external intervention [2]. The incidence of PUL among women who present to a physician after missed period and getting a positive

pregnancy test result, due to pain and/or abnormal bleeding, is estimated at 5–42% [1]. It is important to remember that PUL is not the same as ectopic pregnancy, but it is the exclusion of abnormal implantation of pregnancy that is the most important challenge facing the physician. If the pregnancy implants outside the uterine cavity, late diagnosis can have serious consequences.

Another major problem is the unnecessary implementation of therapy if it turns out that a viable intrauterine pregnancy is revealed in the natural course of PUL.

Any pregnancy implanted outside the endometrial cavity is defined as an ectopic pregnancy (EP) [3]. The etiology remains poorly defined but is likely to be a combination of impaired embryo–tubal transport and alterations in the tubal environment allowing early implantation. Ectopic pregnancy continues to be one of the most common causes of mortality in women in the first trimester of pregnancy and accounts for 5–10% of all pregnancy-related deaths [4].

Diagnosis of ectopic pregnancy can be difficult and delayed due to sometimes uncharacteristic symptoms. Considered typical, the triad of symptoms in the form of delayed or absent period, abnormal bleeding and abdominal pain rarely occur simultaneously, especially in the early stages of ectopic pregnancy. Because of the accompanying pain, it should be differentiated from urological, surgical or gastroenterological conditions as well adnexitis or complications of ovarian tumors [5, 6]. In women with such symptoms, who present to emergency departments, the incidence of ectopic pregnancies is as high as 18%. It is worth noting that about 9% of women with ectopic pregnancies report no pain at all and only 64% report tenderness in the adnexa [7]. Knowledge of the risk factors for EP allows a quicker correct diagnosis, and thus, in some cases, avoiding surgical treatment.

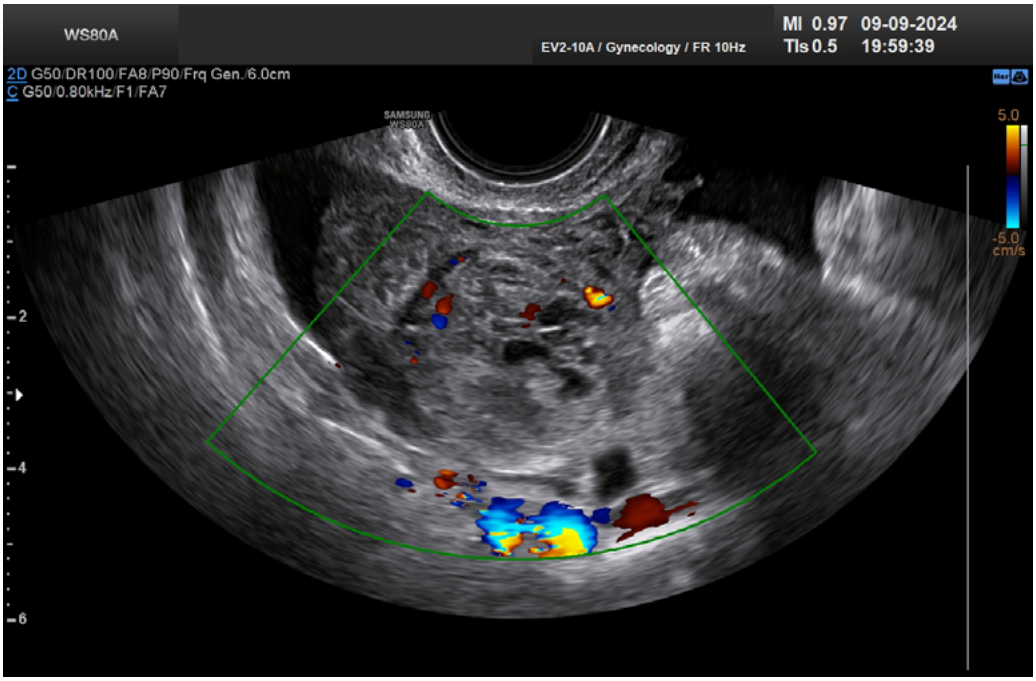

Fig. 1. Visualized TVUS ectopic tubal pregnancy.

Consequently, it leads to a lower risk of death and, which is also worth noting, reduces the cost of treatment. However, it should be remembered that half of patients diagnosed with an EP have no known risk factors [8–10].

Ectopic pregnancy should be sought primarily in the fallopian tube (Fig. 1), much less frequently in the rudimentary uterine horn, cesarean section scar, cervix, ovary or abdominal cavity.

Materials and Methods

We present a review of diagnosis and management of pregnancy of unknown location. We performed a literature search in MEDLINE, Google Scholar, the Cochrane Central Register of Controlled Trials, and Clinical Trials. The database search focused on selected topics related to factors to consider in pregnancy of unknown location. The authors also contributed through individual, independent literature searches.

Defining PUL

PUL is a diagnostic problem in a population of women with above-normal serum b-hCG levels and no confirmation of intrauterine or ectopic pregnancy location on transvaginal ultrasound. PUL is not a diagnosis. It is a term used to classify a pregnancy until the final clinical outcome is known [1].

One of the main problems of PUL is that delaying its diagnosis can consequently lead to failure to implement treatment in a duly manner. The goal of management according to the International Society of Ultrasound in Obstetrics and Gynecology is to reduce the rate of PUL diagnoses to less than 15% [1, 11].

Units specializing in the management of early pregnancy should strive to maintain this suggested rate.

Due to advances in medicine regarding both equipment and staff training, the percentage of PUL diagnoses made is projected to steadily decrease over time. Ultrasonography is by far the best method for determining the location of early pregnancy. A lower rate of PUL is thought to correlate largely with higher quality of images obtained by transvaginal ultrasound (TVUS) [1, 12].

Currently, the primary diagnostic method for pregnancy of unknown location is a combination of transvaginal ultrasound and serial determinations of human chorionic gonadotropin (hCG) β-subunit concentrations [13].

The purpose of diagnostics is to differentiate between a normal developing intrauterine pregnancy (IUP), spontaneous miscarriage (failed PUL) and ectopic pregnancy (EP) [13].

It should be clearly emphasized that despite the progress made in recent years, unambiguous determination of the location of pregnancy in its early stages is still a major challenge. On TVUS examination, the characteristic features of intrauterine pregnancy are: the presence of a gestational sac, yolk sac and confirmation of embryonic cardiac activity. These parameters can be visualized typically between 4.5 and 6 weeks of gestation.

On the other hand, the images suggestive of ectopic pregnancy are the presence of a heterogeneous structure of mixed echogenicity in the projection of the adnexa, (sensitivity 60%), the finding of a gestational sac within the adnexa with or without fetal cardiac activity, the visualization of a pseudo sac in the uterine cavity (it occurs in about 20% of cases of ectopic pregnancy). Ruptured ectopic pregnancy is accompanied by free fluid in the peritoneal cavity (in about 56%

of cases) (Fig. 2). TVUS can identify EP with a sensitivity of 87% to 94% and specificity of 94% to 99% when serial testing is performed. For a single test, TVUS identifies EP with a sensitivity of 73.9% and specificity of 98.3% [1, 12].

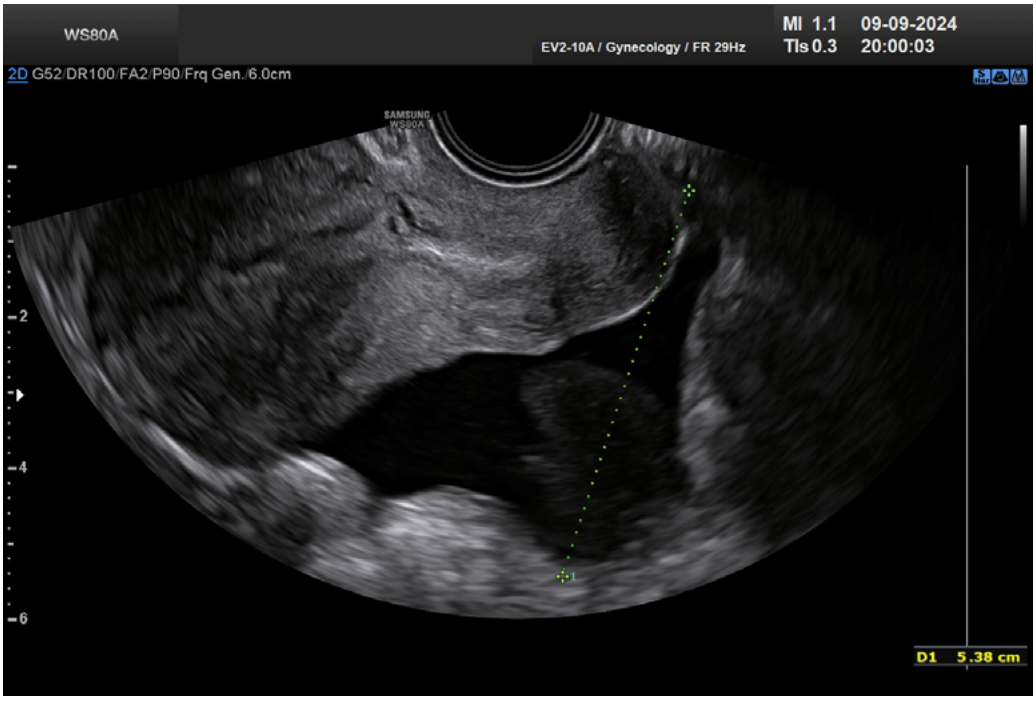

Fig. 2. Free fluid with cloths after rupture of ectopic pregnancy in TVUS.

In cases of PUL, TVUS alone is often performed, which significantly narrows the field of observation. The adnexa may be beyond the reach of the transvaginal probe and only TAUS examination allows their visualization and identification of ectopic pregnancy located outside the lesser pelvis, in the abdominal cavity.

According to Mullany, TVUS has been shown to be more accurate and sensitive compared to TAUS in the diagnosis of early EP [13]. Specifically, three-dimensional TVUS combined with color Doppler US was shown to be more effective than conventional 3D-US for the diagnosis of early caesarean scar pregnancy [14].

Many centers are striving to standardize ultrasound criteria for the diagnosis of PUL. According to experts from the United Kingdom, the United States, Belgium, the Netherlands and Australia, by standardizing ultrasound criteria for IUP and EP, in the case of a positive pregnancy test, a clinical cases can, based on the ultrasound result, be classified into one of five categories [1, 15]:

1. Defined EP: presence of a gestational sac with yolk sac and/or embryo with or without cardiac activity outside the uterine cavity.
2. Probable EP: heterogeneous lesion in the projection of the adnexa or a structure resembling a gestational sac in their projection.
3. PUL: absence of IUP or EP images.

4. Probable IUP: presence of a gestational sac in the uterine cavity.
5. Defined IUP: presence of a gestational sac in the uterine cavity with a yolk sac and/or embryo with or without cardiac activity.

Categories 1 and 5 are considered final diagnoses.
Patients in categories 2, 3, 4 may be classified into categories 1 or 5 during subsequent examinations. A patient with PUL should be monitored until an accurate diagnosis can be established.
PUL classification at the initial scan:

1. True PUL (no evidence of a pregnancy seen inside or outside the uterus);
2. PUL likely EP (possible adnexal mass seen);
3. PUL likely IUP (possible intrauterine gestation sac but no yolk sac or fetal pole seen);
4. PUL likely miscarriage (possible products of conception seen in the endometrial cavity).

The final outcome in a woman initially classified as a PUL is as follows:

1. Ectopic pregnancy (EP) is confirmed/ Identified/by TVS or at the time of surgery [16].
2. Intrauterine pregnancy (IUP) is confirmed/ identified/ by TVS, regardless of the viability. This category should be further subdivided based on viability:
   - Viable IUP (normal ultrasound milestones for gestational age)
   - IUP of uncertain viability (definitive ultrasonic evidence of an IUP but milestones are insufficient to state if the gestation is viable) or
   - Nonviable intrauterine gestation (definitive ultrasonic evidence of empty sac, embryonic demise, or retained trophoblastic tissue).
3. A spontaneously resolved PUL should be used for women who start as having a PUL but have a spontaneous resolution of serum hCG to undetectable levels without surgical or medical intervention.
4. Persistent PUL (PULP). In these cases, hCG does not decline spontaneously, an abnormal increase or plateau of hCG occurs (a variation of less than 15% in hCG titration over three consecutive 48-hour interval measurements), and TVUS does not show intrauterine or ectopic gestation. Approximately 2% of patients with PUL are classified as PULP [9]. These similar to the term PUL, the term persisting PUL is a classification and not a final diagnosis [16].

The final outcome of PULP include:

   a. A non-visualized EP is defined as an increasing serum hCG level after uterine evacuation.
   b. A treated persistent PUL is defined as those women who are treated medically without confirmation of the location of the gestation by TVS, laparoscopy, or uterine evacuation.
   c. A resolved persistent PUL is defined as resolution of serum hCG levels after expectant management or after uterine evacuation (without medical therapy) without evidence of chorionic villi on pathology.
   d. A histologic IUP is defined as identification of chorionic villi in the contents of the uterine evacuation.

5. Failed PUL (PULF): In this case, the spontaneous outcome of gestation occurs with negative human chorionic gonadotropin (hCG), but the exact location of gestation (i.e. whether intrauterine or ectopic) is never identified. Between 50% and 70% of PULs are classified as PULF. A PULF is defined as a woman who has a negative pregnancy test 2 weeks after her initial follow-up. In women with suspected PUL, serum beta hCG is repeatedly determined and TVUS is performed to eliminate the risk of not recognizing an ectopic pregnancy. Consequently, however, the majority of women with initially non-localized pregnancy will be diagnosed with IUP (17%–41%) or PULF (47%–70%) rather than EP (8%–16%). Two groups

of women were identified: those with a low risk of complications, in which the final outcome is IUP or FPUL, or those with a high risk of complications, in which the final outcome is EP or PPUL. The inclusion of women in one of the groups allows for concentrating observations on women with high risk of complications, while limiting observations on women with low risk [1, 15].

An important area of clinical research is to establish criteria for expectant management in women with PUL. Using more precise and consistent language in the descriptions of patients, their risk factors and diagnoses, there should be a strategy to identify women who require increased monitoring, as opposed to those whose pregnancies are likely to resolve spontaneously without intervention.

Biochemical diagnostics of PUL

Biomarkers

The only clinically relevant markers for the localization of EP, so far, are the hCG beta subunit and serum progesterone. A single measurement of β hCG in serum is applicable only to determine whether the obtained value of hCG concentration is above the level at which the gestational sac should be visible on ultrasound. The cut-off value of the level of chorionic gonadotropin, at which pregnancy should be visible in the uterine cavity on TVUS, is not clearly defined. Currently, most authors take the beta HCG value between 1,500 and 2,000/2,500 mIU/ml as the cut-off point [17, 18].

The probability of visualizing a pregnancy sac is then 80.4% and 91.2% for beta HCG values of 2,000 and 2,500 mIU/ml, respectively. When the hCG value is above the discriminatory zone and no intrauterine pregnancy is seen on TVUS examination, ectopic pregnancy should be suspected. However, several studies have documented the appearance of embryos with cardiac activity in pregnancies in which the gestational sac was not visible on TVUS at hCG values above 2,000 mIU/ml [1, 19].

To achieve a 99% probability of visualizing an intrauterine gestational sac on TVUS, the discriminative value of hCG should reach 3,510 mIU/ml [20].

Van Mello's meta-analysis has shown that a single determination of hCG levels in PUL has no prognostic value [2].

Chorionic gonadotropin levels should be determined every 48 hours and changes in their levels from the previous determination should be analysed.

Monitoring serum progesterone levels in the diagnosis of PUL is supposed to help determine whether a pregnancy is viable. Progesterone levels below 5 ng/ml are associated with an unfavourable prognosis for the subsequent fate of the pregnancy, while levels above 20 ng/ml are correlated with a normally developing viable IUP. However, in a significant proportion of ectopic pregnancies, progesterone levels range between 5 and 20 ng/ml, thus limiting its use in daily clinical practice [1, 17].

In view of the fact that diagnostics, including serial transvaginal ultrasound and serial measurement of serum hCG levels and taking into account progesterone levels, is not sufficient in PUL, in order to minimize the risk of PUL rupture it is of utmost importance to detect additional non-invasive markers that enable PUL diagnosis in early pregnancy. Particularly promising are

studies of exosomal biomarkers, which are being conducted in early pregnancy and its complications, as well as in hypertension and pre-eclampsia and in many other diseases, including cancer. Exosomes are small membrane-bound vesicles (size 30–100 nm) of endocytic origin, containing a variety of bio-molecules such as RNA (including microRNA), certain sets of lipids and proteins. In physiological early pregnancy, trophoblast-derived exosomes can be detected in the maternal circulation from the beginning of pregnancy, and their number is steadily increasing. Placental dysfunction leads to their increased excretion into the maternal circulation, followed by the release of various substances from them, including anti-angiogenic factors, pro-inflammatory mediators and microRNAs [21].

Serum miR-21 and miR-141 have been proposed as potential biomarkers in the diagnostics of early stage pregnancies and non-invasive diagnostics of pre-eclampsia (PE), intrauterine stunting and early pregnancy loss. Serum miRNAs are relatively stable, with a clear differential pattern of expression in embryonic tissues of normal and ectopic pregnancies.

In 2013, Fu *et al.* showed the possibility of using miR-376c as a predictive biomarker of EP [22]. Zhao *et al.* confirmed significantly elevated serum levels of miR-323-3p in EP determined by real-time PCR relative to those of miR-517a, miR-519d and miR-525-3p, with a sensitivity rate of 37% (with a consistent specificity of 90%) when used as a single marker. They also found that the combination of hCG and progesterone and miR-323-3p showed diagnostic accuracy with a sensitivity of 96.3% and specificity of 72.6% [23].

From the aspect of implantation, a great role seems to be played by mir-212 and the regulation on the Olfactomedin 1 (OLFM1) and C-terminal-binding protein (CTBP). Recent studies show that downregulation of OLFM1 in the endometrium and the fallopian tubes is associated with receptive endometrium and ectopic pregnancy.

Dominguez *et al.* revealed a markedly different miRNA expression pattern in embryonic tissues from normal and ectopic pregnancies by studying a sample of 23 patients with EP and 29 normal pregnancies [24].

The results of studies carried out by Sun *et al.* suggest that exosomal miR-378d, miR-100-5p and miR-215-5P in serum are promising biomarkers of early EP and can help distinguish EP/SA from VIP with high specificity. Their studies showed reduced levels of miR-146a-5p, miR-215-5p and miR-378d in serum exosome samples from women with EP compared to serum exosome samples from women with SA/VIP [21].

Other experimental markers being studied for their potential use in the diagnostics of PUL include inhibin A, activin, pregnancy-associated plasma protein (PAPP-A), A disintegrin and metalloprotease-12 (ADAM-12) and vascular endothelial growth factor (VEGF) [13]. Activin-AB was found to have a strong diagnostic correlation with EP. 15 ADAM-12 as a biomarker of PUL showed promise in the diagnostics of EP in a study by Rausch *et al.* but Horne *et al.* were unable to replicate these findings [13, 25, 26] (Table 1).

PAPP-A expression has been shown to be significantly lower in patients with EP than in women with an intrauterine pregnancy, suggesting its application in the diagnostics of PUL [27].

Creatine kinase, cancer antigen 125, inhibin pro-αC-related immunoreactivity and insulin-like growth factor-binding protein have all been evaluated but are also not used in clinical practice [13].

Inhibin A levels, on the other hand, can be used in clinical practice to predict the fate of PUL. Inhibin A may be useful for predicting spontaneous resolution of PUL, but is not as good as progesterone. Inhibin A levels are significantly lower in cases of PUL that resolve spontaneously

(i.e. PULF) compared to cases that lead to EP and IUP. Haematological assessment of complete blood count (CBC) samples has also been investigated as a diagnostic tool for EPs. Retrospective reviews have shown that white blood cell (WBC) levels, specifically monocyte counts, are higher in patients with tubal Eps.

Histopathological evaluation of an endometrial biopsy can be a diagnostic component in PUL. This makes it possible to differentiate abnormally developing pregnancies and fetal deaths from ectopic pregnancies. This is important for deciding on the use of methotrexate (MTX). The results obtained during endometrial biopsy confirming the presence of villi showed that up to 40% of patients were misdiagnosed as ectopic pregnancy. In addition, these women showed a 15%–20% decrease in β-hCG the day after the procedure. D&C is found to have higher sensitivity rates for EP diagnosis in comparison to endometrial biopsy pipelles; however, both procedures are limited in accuracy and further studies are needed to confirm diagnostic value.

Table 1. Sensitivity and specificity of promising biomarkers for ectopic pregnancy diagnosis.

Biomarker	Reference	Sample size	Sensitivity (%)	Specifity (%)	Positive predictive value (%)	Negative predictive value (%)
Activin-AB	Refaat and Bahathiq [28]	120	92.5	85	75.5	95.8
A disintegrin and metalloprotease-12 (ADAM-12)a	Rausch *et al.* [25]	199	70	84	—	—
β-human chorionic gonadotropin	Refaat and Bahathiq [28]	120	67.5	51.2	40.9	75.9
Micro-RNA miR-378d	Sun *et al.* [21]	36	89.1	64	—	—
Pregnancy-associated plasma protein	Zhang and Wang [27]	134	92.13	78.33	—	—
Progesterone	Refaat and Bahathiq [28]	120	27.5	50	21.5	58

Source: *Mullany K., Minneci M., Monjazeb R., Coiado O.C.*: Overview of ectopic pregnancy diagnosis, management, and innovation. Women's Health. 2023; 19: 1–13 [13].

Mathematical models

A number of mathematical models have been developed for the prediction of PUL outcome.

Mathematical models developed as decision support tools help determine the likely location and therefore risk of complications in women classified as a PUL [29].

These include logistic regression models and Bayesian networks, based on variables such as serum hCG and progesterone levels, endometrial thickness and the amount of vaginal bleeding. These models have been shown to have high sensitivities for the prediction of PUL outcome and can be used to rationalize follow-up. The most widely evaluated model developed is M4 [29].

The M4 model [21] is a logistic regression model based on the initial serum hCG and the hCG ratio as variables. It was found to be able to classify 69.6% PUL as at low risk of complications with

a negative predictive value (NPV) of 97.5% (i.e. the model was correct in its low risk classification 97.5% of the time) [5]. It had a sensitivity of 88.0%, that is, it correctly classified 88.0% of PUL with a final outcome of EP/PPUL as at high risk of complications. This model was found to be superior in performance to a single progesterone cut-off of <10 nmol/L or the hCG ratio alone.

Recently, a new M6 two-steps polynomial logistic regression model is used in clinical practice [30]. This logistic regression model is based on the hCG ratio and the initial serum hCG and progesterone levels [1].

The M6 model required a minimum of two visits for all women. The M6 model is available for clinical use and is based on one of the largest cohorts of PUL reported to date [30].

Treatment

Having a PUL is a transient state with a large spectrum of outcomes: viable IUP (VIUP), non-viable IUP (NVIUP), EP, failed PUL (FPUL) and persistent PUL (PPUL).

There is no international consensus on the management of PUL, so patients are managed differently in different countries and centres. Guidelines for PUL management aim to determine the location and viability of the pregnancy, and usually recommend follow-up visits until a definitive diagnosis of viable or non-viable pregnancy is obtained. Medical intervention, commonly in the form of methotrexate (MTX), may be considered to treat asymptomatic PUL that is at risk of being an EP [31]. However, prediction of high-risk pregnancy is not error-free [32].

In most hemodynamically stable women with PUL with minimal or no clinical symptoms, the safest management is observation [13]. For persistent PUL, treatment with methotrexate (MTX) is preferred as less invasive. There are several MTX protocols for the treatment of EP: single-dose, double-dose and multi-dose protocols [33, 34]. No consensus has been reached as to which protocol is most effective [33, 34]. It has been found that, in women with lower initial serum β-hCG levels (<2,000 mIU/ml), single-dose and double-dose MTX protocols provide comparable cure success rates in women with PUL. However, women in the single-dose MTX group achieved a more rapid and adequate decrease in serum β-hCG levels compared to women in the double-dose MTX group. Single-dose and double-dose MTX protocols have comparable efficacy and safety and should be equally considered in women with serum β-hCG levels <2,000 mIU/mL, taking into account their clinical status and preferences [35].

The American College of Obstetricians and Gynecologists and the Royal College of Obstetricians and Gynecologists recommend expectant management in women with low initial serum β-hCG levels who are hemodynamically stable and accept the potential risk of fallopian tube rupture and haemorrhage [36, 37].

Surgery

Currently, laparoscopy is reserved for cases of PUL with signs of active bleeding into the peritoneal cavity. Abrasion of the uterine cavity by curettage (D&C) to distinguish EP from an abnormally developing or dead intrauterine pregnancy has many advocates who believe it is also important for preconception counselling. This is because a history of EP increases the risk of recurrence of this situation in a future pregnancy, while the confirmation of a failed intrauterine pregnancy may be associated with recurrent miscarriages requiring further diagnostics. Performing D&C also helps avoid unnecessary use of MTX [1].

Conclusion

Despite the progress that has been made in the diagnostics and treatment of PUL, intensive research is still underway to standardize diagnostic criteria and select appropriate management strategies for these women. Careful definition of the population of women at risk of PUL and the introduction of new, non-invasive and more specific methods of diagnosis and classification of results are aimed at objective interpretation of test results, optimization of management and will allow objective assessment of future reproductive prognosis. Consequently, this aims to improve the clinical care of women initially diagnosed with PUL.

As long as the patient is hemodynamically stable, PUL should be managed expectantly until the final outcome is determined. Women with a final outcome of an IUP or FPUL will rarely need any intervention. PPUL can be managed either expectantly, medically (Methotrexate) or surgically (laparoscopy/uterine curettage). There is no consensus on which of these management options is best.

Authors' contributions

M.K.T. searched and identified appropriate articles, and wrote the manuscript; M.B.N. and V.H. revised the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest

None declared.

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