Diagnostic value of a urine test in pregnancy of unknown location

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BACKGRO: Pregnancy of unknown location (PUL) is a term used when there is a positive pregnancy test but no sonographic evidence for an intrauterine pregnancy (IUP) or ectopic pregnancy (EP). This term is a classification and not a final diagnosis.
OBJECTIVE: This study aimed to evaluate the diagnostic value of the Inexscreen test on the outcome of patients with pregnancies of unknown location.
STUDY DESIGN: In this prospective study, a total of 251 patients with a diagnosis of pregnancy of unknown location at the gynecologic emergency department of the La Conception Hospital, Marseille, France, between June 2015 and February 2019 were included. The Inexscreen (semiquantitative determination of intact human urinary chorionic gonadotropin) test was performed on patients with a diagnosis of pregnancy of unknown location. They participated in the study after information and consent collection. The main outcome measures (sensitivity, specificity, predictive values, and the Youden index) of Inexscreen were calculated for the diagnosis of abnormal pregnancy (nonprogressive pregnancy) and ectopic pregnancy.
RESULTS: The sensitivity and specificity of Inexscreen for the diagnosis of abnormal pregnancy in patients with pregnancy of unknown location were 56.3% (95% confidence interval, 47.0%−65.1%) and 62.8% (95% confidence interval, 53.1%−71.5%), respectively. The sensitivity and specificity of Inexscreen for the diagnosis of ectopic pregnancy in patients with pregnancy of unknown location were 81.3% (95% confidence interval, 57.0%−93.4%) and 55.6% (95% confidence interval, 48.6%−62.3%), respectively. The positive predictive value and negative predictive value of Inexscreen for ectopic pregnancy were 12.9% (95% confidence interval, 7.7%−20.8%) and 97.4% (95% confidence interval, 92.5%−99.1%), respectively.
CONCLUSION: Inexscreen is a rapid, non−operator-dependent, noninvasive, and inexpensive test that allows the selection of patients at high risk of ectopic pregnancy in case of pregnancy of unknown location. This test allows an adapted follow-up according to the technical platform available in a gynecologic emergency service.

Key words: abnormal pregnancy, ectopic pregnancy, pregnancy of unknown location

Introduction

Pregnancy of unknown location (PUL) is a term used when there is a positive pregnancy test but no sonographic evidence for an intrauterine pregnancy (IUP) or ectopic pregnancy (EP). This term is a classification and not a final diagnosis.[1] PULs account for 8% to 31% of early pregnancy diagnoses.[2−7] Between 6% and 20% of PULs are EPs.[4] Thus, a follow-up of PUL is necessary to determine the outcome of the pregnancy. The diagnosis of PUL often leads to a follow-up with multiple laboratory and ultrasound evaluations.[1,2,8]

The poor growth kinetics of human chorionic gonadotropin (hCG) levels in PUL is suggestive of a miscarriage or EP.[9] However, the kinetics of the hCG level is not specific because a 66% increase in hCG every 48 hours is reported in 85% of progressive intrauterine pregnancies and 13% of EPs.[10,11] Thus, the diagnostic value of hCG kinetics in the follow-up of PUL is limited. Ectopic pregnancies are important to diagnose early because they can be life-threatening if they progress. Early diagnosis allows for drug treatment instead of surgery.

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Patient consent is not required because no personal information or details are included.

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Why was this study conducted?

Monitoring pregnancy of unknown location (PUL) is a source of anxiety for patients with multiple consultations to obtain a diagnosis. This study aimed to evaluate the diagnostic value of the Inexscreen test on the outcome of patients with PUL.

Key findings

The sensitivity and specificity of Inexscreen for the diagnosis of abnormal pregnancy in patients with PUL were 56.3% (95% confidence interval [CI], 47.0%−65.1%) and 62.8% (95% CI, 53.1%−71.5%), respectively. The sensitivity and specificity of Inexscreen for the diagnosis of ectopic pregnancy (EP) in patients with PUL were 81.3% (95% CI, 57.0%−93.4%) and 55.6% (95% CI, 48.6%−62.3%), respectively.

What does this add to what is known?

Inexscreen is a test that can specify the risk of EP in case of PUL and would allow to set up an adapted follow-up according to the technical platform available in a gynecologic emergency service.

Strategies for monitoring PUL have been suggested. Bobdiwala et al[12] suggested classifying PUL into 2 groups: low risk and high risk of EP with different follow-ups for the 2 groups, including progesterone and hCG tests. This strategy requires at least 2 visits.

Several biologic markers have been studied to determine the final diagnosis in the case of PUL: progesterone, CPK, inhibin A, activin A, and placental messenger RNA.[11−15] The use of these markers requires a specific technical platform, and their diagnostic threshold values are not yet well established for use in clinical practice. In addition, the use of these markers is expensive, and the results are often not available for emergencies.

Cole et al[13,14] reported that urinary hCG metabolite assay would be more effective than blood hCG for the diagnosis of EP, but these were small populations, and ultrasound is still needed to detect EP. hCG is composed of an alpha subunit and a beta subunit, which are noncovalently linked. A proportion of hCG molecules in pregnancy serum and urine samples have breaks or a missing peptide bond between beta subunit residues 44 and 45 or beta subunit residues 47 and 48 (modified hCG). In normal pregnancies, the proportion (blood and urine) is 90% intact hCG to 10% modified hCG, whereas abnormal pregnancies (miscarriages or EPs) show almost only intact hCG.[16]

The Inexscreen test (Fumouze Diagnostics, Levallois-Perret, France) is a urine test that detects intact hCG and modified hCG (human chorionic gonadotropin relative protein [hCGRP]) in pregnant women, allowing a distinction between normal and abnormal pregnancies, based on the ratio of intact hCG to hCGRP. The hCGRP concentration is decreased in case of abnormal pregnancies (miscarriages and EPs). In an abnormal pregnancy, intact hCG accounts for almost 100% of all hCGs, and there is very little hCGRP. The ratio of hCGRP to intact hCG is significantly decreased in abnormal pregnancies (EPs and miscarriages). In a normal pregnancy, intact hCG accounts for approximately 90% of all hCG isoforms present, and hCGRP accounts for approximately 10%.[17]

The diagnostic value of the Inexscreen test was evaluated in patients with an established diagnosis of IUP or EP. The sensitivity and specificity of the test for the diagnosis of EP were 97% and 83%, respectively. To the best of our knowledge, there is no prospective study evaluating Inexscreen in the follow-up of PUL. Therefore, there is an interest in evaluating Inexscreen in PUL. This study aimed to evaluate the performance of the Inexscreen test in predicting the outcome of patients with PUL.

Materials and Methods

This was a prospective monocentric study conducted in the gynecologic emergency department of the La Conception Hospital, Marseille, France, between June 2015 and February 2019. This was a pilot study. There is no similar study in the literature that has allowed us to calculate several subjects to include.

The inclusion criteria were patients with a diagnosis of PUL. The patients participated in the study after information and collection of consent. The exclusion criteria were patients whose pregnancy outcome was not obtained, patients wishing to withdraw from the study, and patients with an invalid or false negative test for pregnancy diagnosis. The diagnosis of PUL was defined by the presence of positive blood hCG in patients consulting for pelvic pain, metrorrhagia, and/or amenorrhea and the absence of sonographic diagnosis of pregnancy (absence of IUP or EP).

IUP was defined as the presence of an intrauterine gestational sac containing a yolk bladder or an embryo.[1,12] EP was defined by the presence of a uterine vacuity and a laterouterine collection and an extra uterine gestational sac with or without an embryo, without an intrauterine image associated with positive plasma hCG.[1,18] The diagnosis of nonprogressive pregnancy (NPP) was defined by a decrease in plasma hCG to negativity with no sonographic evidence of IUP or EP found during follow-up of PUL. Therefore, NPP includes IUP and EP that are not diagnosed by ultrasound and not suspected clinically and are not progressive until a negative hCG. Pelvic sonography was performed by transabdominal and transvaginal routes (Hitachi Alpha, EUB-6500F, Hitachi Medical Systems Europe Holding, Steinhausen, Switzerland) by a gynecologist department physician.

Patients with PUL had regular follow-up through ultrasounds and hCG assays until a diagnosis of pregnancy localization (IUP or EP) and/or its

evolution (NPP). In case of PUL with an initial plasma hCG of >500 IU/L, the patient was evaluated every 48 hours with ultrasound monitoring and new plasma HCG assay until a final diagnosis (IUP, EP, or NPP). In case of a blood hCG of <500 IU/mL, the patient was evaluated 5 days later using ultrasound and a new plasma hCG assay. This follow-up was performed until the final diagnosis of IUP, EP, or NPP.

The Inexscreen box is a urine lateral flow test, an immunochromatographic test, and a solid-phase immunosorbent device with 2 reading windows. Line A detects intact hCG, and line B detects hCGRP.

In the case of progressive IUP, the intensity of line B is higher than or equal to that of line A. Subsequently, the test is considered positive 1 (P1). In case of an abnormal pregnancy (miscarriage or EP), the intensity of line A is higher than line B. Subsequently, the Inexscreen test is considered positive 2 (P2). If there is no pregnancy, both lines A and B are absent, but the control line is present. If the test is not valid, both lines A and B and the control line are absent.

The Inexscreen test was performed after the diagnosis of PUL, and the urine test was used only once per patient. Here, the Inexscreen tests were interpreted by 2 persons who were not aware of the clinical, biologic, and ultrasound data of the patients. In case of discrepancies in interpretation, a third opinion could be sought. The results of these tests were not known to the caretakers of the patient. Therefore, this test was only evaluated during the study and was not used in the management of the patients. We evaluated the diagnostic value of the Inexscreen test for the final diagnosis of abnormal pregnancy (EP and NPP) or EP in case of an initial diagnosis of PUL.

The sensitivity, specificity, and predictive values and the Youden index of Inexscreen were calculated in different situations. A Wilson score was employed to analyze data. The data were analyzed using SPSS (version 20.0; SPSS Inc, Chicago, IL). An information sheet was given to each patient included in the study. The study obtained the consent of the research ethics committee in obstetrics and gynecology on April 4, 2013 (CEROG 2013-GYN-03-02).

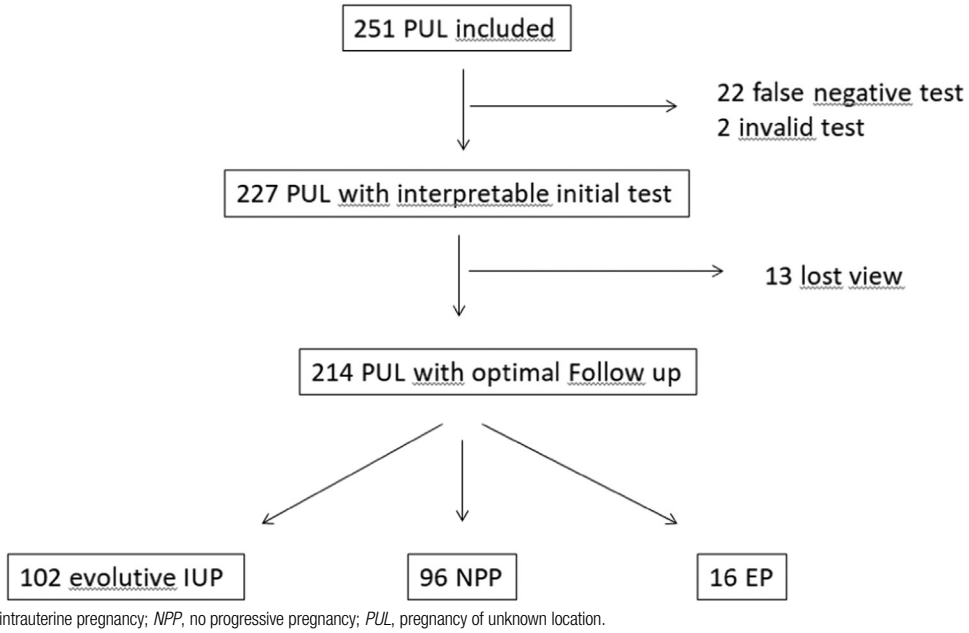
Results

During the study period, 721 patients were diagnosed with PUL between 5 weeks and 8 weeks and 5 days of amenorrhea. Of these patients, 470 were excluded because of their refusal to participate in the study or failure to perform the initial Inexscreen test.

The remaining 251 patients with a diagnosis of PUL were included. Of these patients, 24 were excluded: 2 patients with invalid tests and 22 patients with a false negative pregnancy test, that is, a rate of 8.8% (22/249). Of the remaining 227 patients, 13 were lost to follow-up. Overall, the analysis was performed on 214 patients (Figure). Of these 214 patients, 102 (47.7%) had a final diagnosis of progressive IUP, 96 (44.8%) had a final diagnosis of NPP, and 16 (7.5%) had a final diagnosis of EP (Table 1).

The rate of abnormal pregnancy (NPP and EP) in patients with PUL was 52.3% (112/214) (Table 1). The sensitivity and specificity of Inexscreen for the diagnosis of an abnormal pregnancy (NPP and EP) in patients with PUL were 56.3% (95% confidence interval [CI], 47.0%–65.1%) and 62.8% (95%

FIGURE
Flow chart of the trial



EP, ectopic pregnancy; *IUP*, intrauterine pregnancy; *NPP*, no progressive pregnancy; *PUL*, pregnancy of unknown location.

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TABLE 1
Distribution of Inexscreen test results in pregnancy of unknown location

Variables	EP	NPP	Abnormal pregnancies (NPP + EP)	IUP	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
Evolutive IUP	3 (1.4)	46 (21.5)	49 (22.9)	64 (29.9)	113 (52.8)
Miscarriage or EP	13 (6.1)	50 (23.4)	63 (29.4)	38 (17.8)	101 (47.2)
Total	16 (7.5)	96 (44.9)	112 (52.3)	102 (47.7)	214 (100.0)

EP, ectopic pregnancy; *IUP*, intrauterine pregnancy; *NPP*, no progressive pregnancy.

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CI, 53.1%−71.5%), respectively. The positive predictive value (PPV) and negative predictive value (NPV) of Inexscreen for an abnormal pregnancy were 62.4% (95% CI, 52.6%−71.2%) and 56.6% (95% CI, 47.4%−65.4%), respectively. The Youden index was 0.19. The positive and negative likelihood ratios were 1.51 (95% CI, 1.40−1.63) and 0.70 (95% CI, 0.66−0.74), respectively (Table 2).

The rate of EP in patients with PUL was 7.5% (16/214). The sensitivity and specificity of Inexscreen for the diagnosis of EP in patients with PUL were 81.3% (95% CI, 57.0%−93.4%) and 55.6% (95% CI, 48.6%−62.3%), respectively. The PPV and NPV of Inexscreen for EP were 12.9% (95% CI, 7.7%−20.8%) and 97.4% (95% CI, 92.5%−99.1%), respectively. Thus, after the test, the rate of patients who had EP with a negative test was 2.6% (3/113). The Youden index was 0.37, and the positive and negative likelihood ratios were 1.83 (95% CI, 1.73−1.94) and 0.34 (95% CI, 0.17−0.66), respectively (Table 2).

Discussion

Here, the Inexscreen test was under evaluation, and therefore, its result was not considered in the management of the patient. The performance of the Inexscreen test in diagnosing pregnancy is limited by a false negative test rate of 8.8%. We found that the performance of the Inexscreen test was limited in diagnosing an abnormal pregnancy (NPP or EP) in patients with PUL. The test does not indicate whether the pregnancy is an abnormal pregnancy (NPP or EP) or an IUP. In contrast, the test's performance in diagnosing EPs in case of PULs was more interesting with an NPV of 97.4%. Thus, in an initial diagnosis of PUL with the Inexscreen test indicating a normal pregnancy (P1), the risk of not recognizing an EP is 2.6% for an initial population that included a 7.5% EP rate.

Moreover, we defined IUP as the outcome of an evolving intrauterine PUL. Once the diagnosis was confirmed, we did not perform a longer follow-up to evaluate the outcome of this pregnancy. We defined NPP as a nonevolving PUL with a decrease in hCG and without a final diagnosis of localization. It is likely that this group of NPPs included pregnancies of nonevolving IUP and EP.[12] To limit a bias in counting EPs, no treatment was performed in the absence of visualization of EP signs as described in the definition of EP.

The number of patients was limited in this pilot study, and results must be confirmed in larger series.

This study evaluated the performance of the Inexscreen test in patients with PULs. Mazouz et al[19] evaluated the performance of the Inexscreen test in diagnosing an abnormal pregnancy. The current study was performed in a gynecologic emergency department, with the inclusion of 272 nonpregnant patients and 254 pregnant patients with known diagnoses of IUP or EP. The sensitivity and specificity for the diagnosis of pregnancy were 100% and 100%, respectively. The sensitivity and specificity for the diagnosis of EP were 97% and 83%, respectively. Thus, the authors conclude that, in an emergency department, the Inexscreen test could be the first step in determining the patient's condition to adapt surveillance.

Cole et al[13,14,16] and Borrelli[20] reported that the concentration of hCG isoforms was lower in EP or miscarriage than in pregnancies with a normal course. The value of hCGRP in diagnosing EP was evaluated by quantifying the level of hCGRP in the urine.[17] A receiver operating characteristic curve analysis was performed to assess the cutoff value for distinguishing EP from IUP. A cutoff point of hCGRP−to

TABLE 2
Performance of Inexscreen test results in pregnancies of unknown location

Type of pregnancy	Se (%) (95% CI)	Sp (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	LRP (95% CI)	LRN (95% CI)	Youden index
Abnormal pregnancies (NPP + EP)	56.3 (47.0−65.1)	62.8 (53.1−71.5)	62.4 (52.6−71.2)	56.6 (47.4−65.4)	1.51 (1.4−1.6)	0.70 (0.66−0.74)	0.19
EP	81.3 (57.0−93.4)	55.6 (48.6−62.3)	12.9 (7.7−20.8)	97.4 (92.5−99.1)	1.83 (1.7−1.9)	0.34 (0.20−0.66)	0.37

CI, confidence interval; *EP*, ectopic pregnancy; *IUP*, intrauterine pregnancy; *LRN*, likelihood ratio of negative test; *LRP*, likelihood ratio of positive test; *NPP*, no progressive pregnancy; *NPV*, negative predictive value; *PPV*, positive predictive value; *Se*, sensibility; *Sp*, specificity.

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−intact urine hCG ratio of <16.2% distinguished EP and IUP. The hCGRP concentration and hCGRP−to−intact hCG ratio were significantly lower in EPs than in normal pregnancies, with a sensitivity of 92%, a specificity of 90%, and a positive predictive value of 99.5%.

Teixeira et al[21] evaluated the performance of the Inexscreen test in detecting abnormal pregnancies (miscarriages and EPs) in the first trimester of pregnancy in women presenting to the gynecologic emergency department. Women with a confirmed first-trimester pregnancy performed the urine test. The viability and location of the pregnancy were confirmed using ultrasound. The sensitivity and specificity of identifying an abnormal pregnancy were 13% and 82%, respectively.

In Condous et al's[22] study, patients with a diagnosis of PUL were classified into 3 groups—low-risk NPP, low-risk IUP, and high-risk PUL—based on serum hCG and/or progesterone levels. This single-visit strategy for low-risk pregnancies had correctly eliminated 84% of NPP or IUP from the system. However, because 67% of patients with EP were discharged without adequate follow-up, the authors concluded that a single-visit strategy should not be used as an alternative to the current multiple-visit strategy.

Bobdiwala et al[12] suggested a triage of PUL into 2 groups with the M6 model —low risk and high risk—based on progesterone and hCG levels. In patients classified as low risk, follow-up requires only 2 consultations at 15-day intervals, with ultrasound tests performed by an experienced sonographer.

The studies by Condous et al[22] and Bobdiwala et al[12] were conducted with experienced sonographers. Thus, these results can only be retained for emergency departments with experienced sonographers available, which is not the case in most services managing patients with PUL.

The prevalence of PUL is dependent on the ultrasound quality and the physician's experience.[9] The available ultrasound skills are variable, depending on the technical platform. The Inexscreen test could be interesting, depending on the technical platform and the available skills. In the case of a diagnosis of PUL, the follow-up can be simplified if the Inexscreen test finds a pregnancy with a normal evolution, because the risk of EP is very low (NPV=97.3%), provided that the ultrasound is performed by an experienced sonographer and that all the clinical and paraclinical elements are available, as EP remains a diagnosis that can be life-threatening. In contrast, in the case of a test showing an abnormal pregnancy, the Inexscreen test does not help the diagnosis.

Conclusion

Inexscreen is a test that can specify the risk of EP in case of PUL and allow an adapted follow-up according to the technical platform available in a gynecologic emergency service. This is because it is a fast, inexpensive, and noninvasive test. ■

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