GYNECOLOGY

Sensitivity and specificity of a urinary screening test used in an emergency setting to detect abnormal first trimester pregnancies

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OBJECTIVE: To evaluate the performance of a commercial urinary test to screen for abnormal first trimester pregnancies in women presenting to an emergency room.

STUDY DESIGN: In this prospective observational cohort, women with a confirmed first trimester pregnancy (gestational age <12 weeks) provided a urine sample for diagnosing the viability of their gestation. Pregnancy viability and location testing were confirmed by ultrasound and/or laparoscopy.

RESULTS: From 815 eligible patients for the study, 12 were excluded for not having a confirmed pregnancy (n = 6) or were lost to follow-up (n = 6). A total of 803 patients underwent testing and completed follow-up. The pretest probability of an abnormal pregnancy was 44% (9% for ectopic pregnancy and 35% for miscarriage). The test had the following parameters to identify an abnormal first-trimester pregnancy (sensitivity, 13%; 95% confidence interval [CI], 10–17; specificity, 82%; 95% CI, 78–86; positive predictive value, 36; 95% CI, 28–46; negative predictive value, 54; 95% CI, 50–58; accuracy, 47%; positive likelihood ratio, 0.74; 95% CI, 0.53–1.03; negative likelihood ratio, 1.06; 95% CI, 1–1.12). The reproducibility of the test in our study was high (kappa index between readers, 0.89; 95% CI, 0.77–1).

CONCLUSION: In our emergency setting, we were not able to confirm that the commercial test is adequate to detect or exclude an abnormal first-trimester pregnancy.

Key words: abnormal pregnancy, first trimester, Inexscreen, pregnancy, screening test, sensitivity, specificity

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Vaginal bleeding and pelvic pain are common complaints in emergency facilities.[1] Once the diagnosis of pregnancy is established, the next step is to identify the location and viability of this pregnancy.[2] The main differential diagnoses are miscarriage and ectopic pregnancy. The use of vaginal ultrasound (VUS) and quantitative β-hCG are the main diagnostic tools.[3] These diagnostic modalities can be both time consuming and expensive. The search of a biomarker for miscarriage and ectopic pregnancy has been pursued by many researches.[4-6]

The peculiarities of β-hCG and its different plasmatic levels in normal and abnormal pregnancy led the medical industry to launch in the market Inexscreen (Humasis Co, Ltd, Gyeonggi-do, Korea), a point-of-care urinary test. Inexscreen is a urinary lateral flow test with 2 windows, A and B. Window "A" detects the intact hCG (i-hCG) and window "B" detects the β-core fragments, the nicked β-hCG and the β-hCG isoforms. These isoforms are called human chorionic gonadotropin-related protein (hCGRP). The hCGRP:i-hCG ratio is significantly decreased in ectopic pregnancies and miscarriages. As a rapid urinary test, without the need of special equipment or specialized staff, Inexcreen was developed as a screening tool to rule out abnormal pregnancy. In 2011, Mazouz et al[7] published the initial results of Inexscreen in the clinical context. According to their data, Inexscreen had a negative predictive value of 99.3% and 96.6% for ectopic pregnancy and miscarriage, respectively.[7] A negative predictive value >99% for ectopic pregnancy is ideal in the clinical context, given the fact that time and costs can be reduced. However, the authors recognized this first report should be confirmed with a larger prospective study.[7] The objective of this study was to estimate the diagnostic accuracy of Inexscreen test compared with ultrasonography and surgery in diagnosing abnormal pregnancy in the first trimester.

Materials and Methods

Participants

The study population consisted in a consecutive series of subjects who attended the gynecologic emergency department (GED) of Hospital de

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Participant recruitment

Consecutive pregnant women between 14 and 49 years old who attended the GED for any reason were invited to participate in the study.

Inclusion and exclusion criteria

Subjects were included if they had a pregnancy <12 weeks according to the last menstrual period or previous gestational ultrasonography. Subjects were excluded if their pregnancy was not confirmed by urinary β-hCG, if they had a pregnancy of $\geq$12 weeks, or if they did not give written consent to participate in the study. Pregnancy and gestational age were confirmed by urinary and/or serum β-hCG and ultrasound, respectively.

Data collection

After signing the written consent, a standard questionnaire was obtained. Next, a fresh urine sample was collected from the patient for the index text (Inexscreen). Subjects were followed with serial plasma β-hCG and transvaginal ultrasound. In case of pregnancy of unknown location, subjects were followed every 48 hours or every week until the outcome of pregnancy was identified.

Reference standard and its rationale

A transvaginal ultrasound was used as the reference standard to confirm the presence of a viable, or a nonviable pregnancy. VUS was performed within 4 hours after the Inexscreen test. Pregnancy viability was defined as the presence of intrauterine embryo/fetus with cardiac activity. Miscarriage was defined by the absence of visible heartbeat in an embryo with crown-rump length $\geq$7 mm, or if the mean gestational sac diameter was $\geq$25 mm and no structure was visualized inside. The diagnostic algorithm published by Mol et al[8] was used for diagnosis of ectopic pregnancy. Ectopic pregnancy was confirmed by surgery and pathology report.

Technical specifications

Inexscreen was the index test for screening viable or nonviable first-trimester pregnancy. Urine specimens were collected in a clean, dry, plastic container and Inexscreen was run within 10 minutes of collection. With the disposable pipette provided with the Inexscreen kit, 5 drops of urine were loaded into the sample well of the Inexscreen test device. Interpretation of the test result was performed after 5 minutes, according to the manufacturer's instructions. Briefly, Inexscreen test has 2 windows (A and B), and 2 lines in each window (C and T). Line C is the internal control; if it is absent in one of the windows, the test is discarded and a new test used. The intensity of the T line was defined by visual comparison with standards given by the manufacturer. The intensity of the lines was graduated in a grading system of whole numbers between 0 and 10. The cut-off between normal and abnormal followed the manufacturer instructions. A ratio of the intensity of lines A > B was considered abnormal; a ratio A $\leq$ B was considered normal.

Persons executing and reading Inexscreen

Two experts in Inexscreen reading (J.L.G., R.F.S.) trained and supervised the clinical physicians (n = 8), the residents and medical students that were in rotation every month (total n = 37/30.5 months). The physicians, residents, and medical students evaluated the intensity of line T in windows A and B.

Persons executing ultrasound and pathologic analysis

Board certified radiologists and pathologists were responsible for executing transvaginal ultrasound and pathologic analysis, respectively. Readers of the Inexscreen test, radiologists, and pathologists were blind to the results of each other's test.

Statistical methods and sample size

The performance of the Inexscreen test was calculated using a 95% confidence interval (CI) for sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios. Kappa index was used to assess inter- and intraobserver agreement. Reproducibility of the test, ie, normal or abnormal, was verified between 2 independent observers using 60 cases comprising the whole spectrum of results (ie, from 0 to 10). Digital pictures of lines A and B of these 60 cases were taken and stored in a file. Results from a senior researcher (R.F.S.) were compared with the results of a naïve researcher for interobserver agreement. The senior researcher scored the same 60 cases in 2 different occasions for intraobserver agreement. Analyses were performed using Prism 6.0 (GraphPad, San Diego, CA), online kappa calculator (http://vassarstats.net/kappa.html) and online diagnostic test calculator (http://araw.mede.uic.edu/cgi-bin/testcalc.pl).

Sample size was calculated according to the nomogram described in the literature.[9] The following parameters were used: an estimated incidence of ectopic pregnancy of 10% ($\pm$5%) and an estimated specificity of 95% (with a precision of $\pm$5%). Sample size calculation yielded a total number of 730 subjects. For cases of miscarriage, where the estimated incidence of miscarriage was 40%, the minimal number of subjects was 180. With these parameters we were able to verify with a 95% CI, that Inexscreen has specificity between 90 and 100% to diagnose ectopic pregnancy in a population where the prevalence is between 5 to 15%.

Ethical issues

This study was submitted and approved by Comite de Ética em Pesquisa of Hospital de Clínicas de Porto Alegre, the local institutional review board (no. 11-0113). Inexscreen was evaluated in the GED of the Hospital de Clínicas de Porto Alegre, in Porto Alegre, RS, Brazil.

RESULTS

Beginning and end dates of recruitment

Data collection was performed between April 14, 2011, and Oct. 28, 2013.

Clinical and demographic characteristics of the study population

Most of the participants were white and the mean gestational age was 8.1, 6.5, and 7.9 weeks for intrauterine pregnancy, ectopic pregnancy and miscarriage, respectively (Table 1).

TABLE 1
Characteristics of the sample

Characteristics	Viable pregnancy n = 450	Ectopic pregnancy n = 73	Miscarriage n = 280
Age, mean (SD)	26.7 (6.5)	28 (6.7)	28.3 (7.8)
Ethnicity, n (%)			
White	315 (70)	44 (60)	182 (65)
Black	131 (29)	29 (40)	88 (31)
Native-Brazilian	3 (0.7)		4 (1.7)
Asian	1 (0.3)		4 (1.7)
Not available			2 (0.6)
Gestational age, mean (SD)	8.1 (2.1)	6.5 (2)	7.9 (2.1)
Risk factors (no/yes)			
Hx ectopic pregnancy	425/25	59/14	269/11
Hx tubal surgery	429/21	64/9	257/13
Smoking	334/116	47/26	214/66
Hx PID	414/36	58/15	258/22
Hx ≥3 miscarriages	425/25	70/3	271/9
Hx infertility[a]	405/45	52/21	228/52
>5 sexual partners	338/112	48/25	197/83
Hx intrauterine dispositive	408/42	66/7	261/19
Signs and symptoms, n (%)			
Asymptomatic	71 (16)	5 (7)	49 (18)
Pelvic pain	163 (36)	8 (11)	31 (11)
Vaginal bleeding	75 (17)	14 (19)	54 (19)
Pelvic pain + bleeding	141 (31)	46 (63)	146 (52)

Hx infertility, history of infertility; *Hx PID*, history of pelvic inflammatory disease.

[a] Defined as failure to achieve a clinical pregnancy after ≥12 mo of regular unprotected sexual intercourse.

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Number of participants

A total of 815 Inexscreen tests were used in the study and none of them were discarded for being faulty (ie, negative control line). Six subjects did not satisfy the inclusion criteria and were excluded; six were lost to follow-up. A total of 803 subjects satisfied inclusion criteria and had a VUS or were submitted to surgery (Figure). The median follow-up was 3 days (range, 1–60 days).

Time interval between tests results

The time interval between the Inexscreen test and the vaginal ultrasound was 4 hours or less. No treatment was administered in between.

Distribution of the severity of the disease

A total of 125 subjects were asymptomatic with no or ≥1 risk factor. Asymptomatic subjects without risk factor comprised 6.6% (53 of 803) of the study population. From these 53 subjects, 39.6% had an abnormal pregnancy (3 cases were ectopic pregnancy; 18 cases of miscarriage). The majority of subjects (93.4%) had pelvic pain and/or vaginal bleeding.

Cross tabulation of the Inexscreen and transvaginal ultrasound

All 803 subjects included in the study underwent transvaginal ultrasound and had a urine sample analyzed by Inexscreen. Positive Inexscreen (abnormal) in abnormal pregnancy was seen in 47 cases; abnormal Inexscreen in normal pregnancy was seen in 81 cases. Normal Inexscreen in abnormal pregnancy was seen in 306 cases, and normal Inexscreen in normal pregnancy was seen in 369 cases. There were no missing or indeterminate results.

Adverse events from Inexscreen and vaginal ultrasound

No adverse event occurred by performing the index test (Inexscreen) or reference standard (transvaginal ultrasound).

Estimates of the test

The test performance to identify an abnormal first trimester pregnancy was as follows: sensitivity: 13% (95% CI, 10–17%); specificity: 82% (95% CI, 78–85%); positive predictive value: 37% (95% CI, 28–46%); negative predictive value: 55% (95% CI, 50–58%); accuracy: 52%; positive likelihood ratio: 0.74 (95% CI, 0.53–1.03); negative likelihood ratio: 1.06 (95% CI, 1–1.12). The pretest probability of an abnormal pregnancy was 44%; the posttest probability after a positive and negative test was 37 and 45%, respectively (Table 2). Similar results were observed in a subgroup of symptomatic subjects with gestational age between 5 and 8 weeks of pregnancy (Table 3).

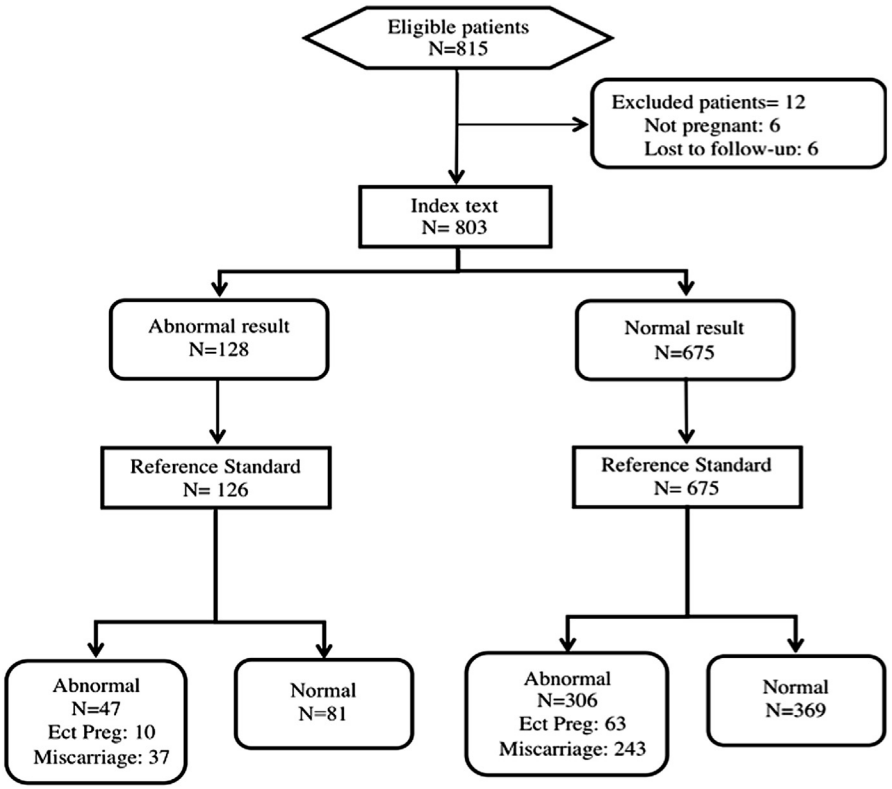
No indeterminate results or outliers were observed. The 6 cases that were lost to follow-up were excluded from the analysis.

Kappa index for inter- and intraobserver agreement was 0.89 (95% CI, 0.77–1) and 0.93 (95% CI, 0.84–1), respectively.

Comment

Our results revealed that Inexscreen correctly identified 6 cases of women that did not have a positive β-hCG (Figure). Similar to the results published by Mazous et al,[7] the specificity of the test was around 82%; in our setting, however, the test was neither sensitive, nor specific to rule in or to rule out abnormal pregnancy with a precision higher than 95% (Table 2). The lower

FIGURE
Flowchart of the study



Ect Preg, ectopic pregnancy.

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TABLE 2
Performance of Inexscreen test compared with VUS, or surgery/pathology in 803 women

Parameter	Total	Ectopic pregnancy	Miscarriage
Pretest probability	44	9	35
Sensitivity	13 (10–17)	14 (6–23)	13 (9–18)
Specificity	82 (78–85)	82 (78–85)	82 (78–85)
PPV	37 (28–46)	11 (5–19)	31 (23–40)
NPV	55 (50–58)	85 (81–88)	60 (56–64)
Accuracy	52	72	56
LR+	0.74 (0.53–1.03)	0.76 (0.41–1.4)	0.73 (0.51–1.05)
LR−	1.06 (1–1.12)	1.05 (0.95–1.16)	1.06 (0.99–1.13)
PPT +	37	11	31
PPT−	45	15	40

Numbers are % (95% confidence interval), except for likelihood ratios. Inexscreen; Humasis Co, Ltd, Gyeonggi-do, Korea.

LR+, positive likelihood ratio; *LR−*, negative likelihood ratio; *NPV*, negative predictive value; *PPT+*, posttest probability if test is positive; *PPT−*, posttest probability if test is negative; *PPV*, positive predictive value; *VUS*, vaginal ultrasound.

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performance of the test in our scenario can be seen by the posttest probability of a positive and negative test: 37 and 45%, values that did not differ much from the pretest probability, ie, 44% (Table 2). The negative predictive value for ectopic pregnancy was 85% (95% CI, 81–88), compared with the higher 96.6% (95% CI, 91–98%) reported by Mazous et al.[7] However, the sensitivity of the test found in our study differed substantially from that of Mazous et al.[7] Whereas we found a low sensitivity, 13% (95% CI, 10–17), Mazous et al[7] found a sensitivity of 92% (95% CI, 84–97). Possible explanations for these discrepancies could be related to the fact that we used the test in asymptomatic pregnant women that attended the gynecologic emergency department for nonobstetric reasons, which alters the pretest probability. This subgroup represented 6% of the population, but 39.6% of the cases had an abnormal pregnancy, and they would benefit the most from a screening test. However, if only women with pelvic pain and/or vaginal bleeding were included, as it was in the original work of Mazous et al,[7] the negative predictive value for ectopic pregnancy would remain low, ie, 80% (95% CI, 74–85%), as shown in Table 3. Predictive values of a test are influenced by the prevalence of a condition.[10] Therefore, it is important to conduct studies of screening tests in real-life scenarios, in populations that reproduce the true prevalence of ectopic pregnancy and miscarriage. If these steps were not taken, a selection bias would ensue. In our study, the prevalence of ectopic pregnancy was 9% (95% CI, 7–11%), which is in accordance to the literature.[7,11] The prevalence of miscarriage, 35% (95% CI, 31–38%), was higher than in the population presented by Mazouz et al,[7] but similar to a larger study published by Tunde-Byass and Cheung.[12]

Other possibilities for the discrepancies could be related to the inclusion criteria. In our study we included pregnant women with <12 weeks of gestational age. Mazous et al[7] included pregnancies between 5 and 8 weeks according to the last menstrual period. An analysis of this subgroup, symptomatic

TABLE 3
Performance of Inexscreen in 403 symptomatic women (5 and 8 wks)

Parameter	Total	Ectopic pregnancy	Miscarriage
Pretest probability	45	13	33
Sensitivity	16 (11–22)	13 (6–25)	17 (9–18)
Specificity	81 (75–86)	81 (75–86)	81 (75–86)
PPV	42 (30–54)	15 (6–27)	36 (23–40)
NPV	53 (47–59)	80 (74–85)	62 (56–64)
Accuracy	51	68	57
LR+	0.86 (0.6–1.3)	0.72 (0.3–1.5)	0.92 (0.6–1.4)
LR−	1.03 (0.9–1.1)	1.07 (0.94–1.2)	1.02 (0.9–1.1)
PPT+	42	10	31.2
PPT−	47	14	33.4

Numbers are % (95% confidence interval), except for likelihood ratios. Inexscreen; Humasis Co, Ltd, Gyeonggi-do, Korea.

LR+, positive likelihood ratio; *LR−*, negative likelihood ratio; *NPV*, negative predictive value; *PPT+*, posttest probability if test is positive; *PPT−*, posttest probability if test is negative; *PPV*, positive predictive value; *VUS*, vaginal ultrasound.

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women with gestational age between 5 and 8 weeks, revealed that the performance of the test did not change much (Table 3). Reading the test could be an issue, because it compares the intensity of the lines in the cassette to a standard card provided in each kit. The design of this point of care test is simple, and it is easy to read. The end result lies in 2 categories: normal, when intensity of line A $\leq$ B, or abnormal, when intensity of line A $>$ B. These results are straightforward when the intensity of the line is $\geq$3. The high kappa index (κ = 0.89) found in our study reflects this agreement among readers. This result is in accordance with Mazous et al.[7]

Of note, clear diagnosis of ectopic pregnancy, ie, gestational sac or fetal heartbeat outside the uterus, was made by ultrasound in 10 subjects and only 4 tests were abnormal. Lastly, another possible explanation could be related to rationale of the test. Inxescreen is based in the hCGRP:i-hCG ratio; this ratio is significantly decreased in ectopic pregnancies and miscarriages. Borrelli et al[13] analyzed the serum and urine levels of the intact hCG and the hCG fragments in first-trimester pregnancy of women who presented at an outpatient emergency department. They found that urine hCG variants had less significant performance compared with those of serum in distinguishing ectopic pregnancy from viable pregnancy. Indeed, we note that Inexscreen could alternatively be used to estimate hCG levels at urine, as the test provides different levels of hCG in urine: <200 mUI/mL, 200 to 1000, 1000 to 5000, 5000 to 10000 and >10000 mUI/mL. Physicians could benefit from this information to plan the timing of ultrasound, or to evaluate the rising or fall of hCG compared with previous plasma values.

The strengths of the study were the sample size, the low rate of dropout, and the real-world scenario with a prospective follow-up. One of the limitations of this study was that we did not correlate the hCG levels of all cases of ectopic pregnancy to the levels found by Inexscreen. Indeed, this could be further tested as a clinical applicability of the test: to identify the discriminatory zone and the use of vaginal ultrasound.[3]

In conclusion, in this setting, Inexscreen has a specificity of 82%, a value in range of what is published in the literature, but it has 13% of sensitivity. The test is not adequate to rule in or to rule out an abnormal pregnancy in the first trimester in our setting. Further prospective studies are necessary to confirm our results. ■

REFERENCES

1. Matteson KA, Weitzen SH, Lafontaine D, Phipps MG. Accessing care: use of a specialized women's emergency care facility for non-emergent problems. J Womens Health (Larchmt) 2008;17:269-77.
2. Lee AS, Cohen SL, Anderson JR, Chanmugam A, Bienstock JL. The effect of gynecologic algorithm pathways on emergency department visit times. J Emerg Med 2013;44:217-24.
3. Barnhart KT, Simhan H, Kamelle SA. Diagnostic accuracy of ultrasound above and below the beta-hCG discriminatory zone. Obstet Gynecol 1999;94:583-7.
4. Daponte A, Deligeoroglou E, Garas A, Pournaras S, Hadjichristodoulou C, Messinis IE. Activin a and follistatin as biomarkers for ectopic pregnancy and missed abortion. Dis Markers 2013;35:497-503.
5. Senapati S, Barnhart KT. Biomarkers for ectopic pregnancy and pregnancy of unknown location. Fertil Steril 2013;99:1107-16.
6. Rausch ME, Barnhart KT. Serum biomarkers for detecting ectopic pregnancy. Clin Obstet Gynecol 2012;55:418-23.
7. Mazouz S, Lee JK, Fernandez H. Evaluation of a urinary test as a diagnostic tool of a nonprogressive pregnancy. Fertil Steril 2011;95:783-6.
8. Mol BW, van Der Veen F, Bossuyt PM. Implementation of probabilistic decision rules improves the predictive values of algorithms in the diagnostic management of ectopic pregnancy. Hum Reprod 1999;14:2855-62.
9. Malhotra RK, Indrayan A. A simple nomogram for sample size for estimating sensitivity and specificity of medical tests. Indian J Ophthalmol 2010;58:519-22.
10. Grimes DA, Schulz KF. Uses and abuses of screening tests. Lancet 2002;359:881-4.
11. Alkatout I, Honemeyer U, Strauss A, et al. Clinical diagnosis and treatment of ectopic pregnancy. Obstet Gynecol Surv 2013;68:571-81.
12. Tunde-Byass M, Cheung VY. The value of the early pregnancy assessment clinic in the management of early pregnancy complications. J Obstet Gynaecol Can 2009;31:841-4.
13. Borrelli PT, Butler SA, Docherty SM, Staite EM, Borrelli AL, Iles RK. Human chorionic gonadotropin isoforms in the diagnosis of ectopic pregnancy. Clin Chem 2003;49:2045-9.