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Viability of intrauterine pregnancy in women with pregnancy of unknown location: prediction using human chorionic gonadotropin ratio vs. progesterone

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KEYWORDS: intrauterine pregnancy; hCG ratio; pregnancy of unknown location; pregnancy viability; progesterone

ABSTRACT

Objectives To find the best predictor of the viability of intrauterine pregnancies of uncertain viability (IPUVs). An IPUV is defined as an empty intrauterine gestational sac measuring less than 20 mm in diameter or an intrauterine gestational sac containing a fetal pole with a crown–rump length (CRL) of less than 6 mm with no fetal cardiac activity.

Methods This was a prospective observational study comparing the following two markers for the prediction of viability in IPUVs: human chorionic gonadotropin (hCG) ratio (hCG 48 h : hCG 0 h); and serum progesterone at first presentation. All women classified with a pregnancy of unknown location (PUL) were followed up until final diagnosis: failing PUL, viable or non-viable intrauterine pregnancy (IUP), or ectopic pregnancy. Those PULs found to have an IPUV at follow-up transvaginal ultrasound scan (TVS) were included in the final analysis. Receiver–operating characteristics (ROC) curves were used to compare the performance of the hCG ratio and progesterone. Using logistic regression, the added value of combining both variables over single-variable prediction was also evaluated. Missing values were imputed using multiple imputation.

Results In total, 12 572 consecutive first-trimester women were scanned. Of these, 1003 (8%) women were classified as having PULs at first scan; 379 (37.8%) PULs were confirmed as IPUVs at follow-up scan, with 82.6% (276/334) found to be viable IUPs and 17.4% (58/334) non-viable IUPs on a second follow-up scan. Forty-five PULs were lost to follow-up. For prediction of viability in IPUVs, the area under the ROC curve (AUC) for the hCG ratio was 0.756 (95% CI, 0.686–0.826) whilst that for progesterone was 0.678 (95% CI, 0.603–0.753). The difference in AUC was 0.078 (95% CI, 0.014–0.169, P = 0.098), suggesting that the hCG ratio was at least as good as initial progesterone. Furthermore, there was no clear threshold for initial progesterone, whilst for hCG a ratio of 2 was suggested (sensitivity 78%, specificity 67%). Logistic regression modeling demonstrated that progesterone did not improve the discriminatory power of the hCG ratio (AUC = 0.758).

Conclusions The hCG ratio may be preferred to single measurements of progesterone to predict the viability of IPUVs in the PUL population. Copyright © 2010 ISUOG. *Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Pregnancies of unknown location (PULs) are defined as early pregnancies with a positive urinary human chorionic gonadotropin (hCG), but no signs of an intrauterine pregnancy (IUP) or an extrauterine pregnancy on transvaginal ultrasonography (TVS)[1,2].

Approximately one-third of PULs are early developing IUPs that are too small to be visualized on initial TVS. A serum hCG increase of more than 66% over 48 h is a good predictor of an IUP[3]. As visualization of fetal cardiac activity is not possible at this early stage, these predicted IUPs should be regarded as intrauterine pregnancies of uncertain viability (IPUVs). Over two-thirds of IPUVs will be viable IUPs when followed up using TVS. To date, a follow-up ultrasound scan is required to determine the viability of these IPUVs. However, the need for one or two weekly follow-up visits may cause prolonged anxiety for the woman, and repeat visits also increase costs and workload of the early pregnancy unit.

In an effort to reduce unnecessary scans in the PUL population, we recently investigated the use of threshold values of serum markers at presentation of women with PUL to predict the viability of pregnancies found to be IPUVs at follow-up scan. We found that the hCG ratio (48 h : 0 h) was significantly higher in those IPUVs which became viable IUPs compared with non-viable IUPs, and this could potentially be used as a predictor of viability[4].

Both the hCG ratio and the baseline serum progesterone levels have been advocated to predict viability in the PUL population[1]. Quantitative measurement of serum progesterone is an indirect way to predict the likely viability of an early pregnancy. It has been suggested that in early pregnancy the production of progesterone by the corpus luteum may reflect the slope of the hCG increase[5]. A high serum progesterone level in early pregnancy reflects a normally functioning corpus luteum and placenta of a viable pregnancy[6]. Conversely, low serum progesterone levels correlate with poor first-trimester outcomes[7].

The aim of this study was to compare the performance of the 48 h : 0 h hCG ratio with that of serum progesterone at the time of presentation to predict viability of IPUVs in the PUL population.

METHODS

This was a non-interventional prospective observational study. All pregnant women who presented to the Early Pregnancy Unit at St George's Hospital, London (between 25 June 2001 and 9 October 2004), underwent a TVS using a 5-MHz probe (Aloka SSD 900, 2000 or 4000; Keymed Ltd, Southend, UK and Aloka Co. Ltd., Tokyo, Japan). This group of women was also analyzed to determine different hCG ratio cut-offs to predict viability in PULs, the results of which have been published elsewhere[4]. Women classified with a PUL (i.e. no signs of either intrauterine or extrauterine pregnancy) or retained products of conception on TVS, were eligible for inclusion. Any woman who was clinically unstable or had an acute abdomen or blood in the pouch of Douglas according to the initial TVS, was excluded from the study.

Indications for sonography included lower abdominal pain with or without vaginal bleeding, dating or a previous miscarriage/ectopic pregnancy. The woman's age, gestational age at presentation, endometrial thickness in millimeters, as well as the appearance of the midline echo (intact or disrupted) and the presence of free fluid in the pouch of Douglas on scanning, were all recorded.

All women classified with a PUL had peripheral blood taken at 0 h (i.e. time at presentation) and at 48 h. Serum hCG levels were measured at 0 h and at 48 h (World Health Organization, Third International Reference 75/537; World Health Organization, Geneva, Switzerland) and serum progesterone levels were analyzed at 0 h (Roche Elecsys 2010 Progesterone II test; Roche Diagnostics, Lewes, UK) using automated electrochemiluminescence immunoassays.

A serum hCG of ≥ 5 U/L represented a positive pregnancy test. The hCG ratio for each PUL was calculated. This was defined as the hCG at 48 h divided by the hCG at 0 h. Women were followed up until a final diagnosis was established: viable/non-viable IUP, failing PUL, ectopic pregnancy or persisting PUL. These diagnoses were established using the criteria described in studies previously published by this unit[8–12]. Women found to have an IPUV at first follow-up TVS were included in the final analysis. G.C. followed up all women and was responsible for collecting data as well as the final clinical outcomes for each PUL.

All PULs with increasing serum hCG levels at 48 h (hCG ratio > 1) were rescanned 1 week later to confirm pregnancy location. PULs with decreasing or plateauing serum hCG levels (hCG ratio < 1) were followed with serial hCG measurements. An IPUV was diagnosed on the second TVS when a gestational sac measuring less than 20 mm in diameter or an intrauterine gestational sac containing a fetal pole with a crown–rump length (CRL) of less than 6 mm with no fetal cardiac activity was visualized. A follow-up scan was performed in this group of women after a further 7 days to determine the viability of these IPUVs. All women with IPUVs therefore underwent assessment of serum hCG levels twice (at 0 h and at 48 h) and serum progesterone level once and three ultrasound scans: one at which PUL was diagnosed, one to confirm an IPUV and the third to determine the viability of these IPUVs. The definition of a viable IUP was based upon the presence of an embryo with visible cardiac activity on TVS. The definition of a non-viable IUP was based upon the following:

- the presence of an embryo with a CRL of ≥ 6 mm without demonstrable cardiac activity on the first scan; or
- the presence of an embryo with a CRL of ≤ 5 mm with no demonstrable cardiac activity at the first scan and then at the second scan, 7 days later, still no fetal cardiac activity was demonstrated; or
- the absence of an embryo in a gestational sac with a diameter of > 20 mm or a gestational sac of < 20 mm with no growth at ultrasound follow-up.

Statistical analysis

Statistical analyses were conducted using the Statistical Analysis System (SAS) (Version 9.1; SAS Institute, Inc., Cary, NC, USA).

Missing value imputation

Missing values were imputed using multiple imputation (MI). In MI, missing values are imputed several times (in

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this study 10 times), resulting in several complete versions of the dataset[13,14]. Then, all analyses are performed on each complete dataset and the results are combined to obtain the final results[13,14]. In our study, each of the 10 imputations of the missing values was based on an iterative procedure inspired by Siddique and Belin[15]. Variables used to impute missing values in the imputation procedure were: viability*, age, gestational age*, endometrial thickness*, midline echo*, free fluid in the pouch of Douglas*, hCG ratio, average hCG, and initial progesterone*. The variables with an asterisk (*) had missing values that had to be imputed. Only 1.6% of the patients had a missing value for initial progesterone, and therefore the missing values did not affect the comparison of the hCG ratio and initial progesterone, which was the main aim of this study.

Univariate data analysis

Descriptive statistics were computed for the viable and non-viable IUPs separately. These two groups were compared using the Mann–Whitney *U*-test for continuous variables, the likelihood ratio chi-square test for binary variables, or the Cochran-Armitage trend test for vaginal bleeding (ordinal variable with three levels). MI *P*-values were obtained by combining the results for each complete dataset[16].

The performance of the hCG ratio and initial progesterone was further evaluated using receiver–operating characteristics (ROC) curves and balanced accuracy rate curves. In the latter curve, the average of the sensitivity and specificity – this is the balanced accuracy rate – is plotted vs. different threshold levels to determine viability. For both variables, these curves were constructed for each complete dataset. The 10 resulting ROC and balanced accuracy rate curves were then averaged. The final area under the ROC curve (AUC) was the average AUC over the 10 complete datasets. An MI 95% CI and *P*-value were obtained by combining the results for each complete dataset based on the methodology of DeLong *et al.* for comparing paired AUCs[17].

Multivariate modeling

To model the joint effect of several variables on viability, multivariate logistic regression was used. An efficient alternative to the use of a training and test set to estimate the AUC of a logistic regression model was bootstrapping. The model was fitted on all data, and the accompanying AUC was computed. This AUC, called the apparent performance, will be too optimistic because it is computed on the same data that were used for model fitting. Using 200 bootstrap samples, the level of optimism was estimated as described elsewhere[18,19]. By subtracting the optimism from the apparent performance, a corrected AUC was obtained. This was done for each complete dataset, and the results were averaged to obtain the final value of the corrected AUC. Note that no bootstrapping was used to correct single-variable AUCs because no modeling is involved for the AUC based on one variable.

RESULTS

A total of 12 572 consecutive first-trimester women were scanned during the study period, of whom 1003 (8%) were classified as having PULs. Three-hundred and seventy nine (37.8%) were demonstrated to be IPUVs at follow-up scan and were included in the study. Of these 379 IPUVs, 45 were lost to follow-up. Of the 334 women with IPUVs with recorded viability information, 82.6% (276/334) had a viable IUP and the remaining 17.4% (58/334) had a non-viable IUP confirmed at their second follow-up ultrasound scan. Of the 334 PULs that were demonstrated to be IPUVs at the first follow-up scan, 89.8% (300/334) had an hCG ratio of > 1.66, 48 h after initial presentation. This means that of the 334 IPUVs confirmed at follow-up scan, 89.8% had already been classified as IPUVs on the basis of the hCG ratio[8]. All missing values were imputed according to the procedure described earlier.

Descriptive statistics for both viable and non-viable IUPs are presented in Table 1 (continuous variables) and Table 2 (categorical variables).

Compared with non-viable IUPs, women with a viable IUP had a significantly lower age (median, 29 vs. 31 years; MI $P = 0.003$), lower gestational age (median, 31.0 vs. 35.9 days; MI $P < 0.001$), higher hCG ratio (median, 2.265 vs. 1.830; MI $P < 0.001$) and higher initial progesterone levels (median, 71.5 vs. 56.0 nmol/L; MI $P < 0.001$). The prevalence of vaginal bleeding, lower abdominal pain, a disrupted midline echo or free fluid in the pouch of Douglas, as well as endometrial thickness, were all highly similar in the viable IUPs compared with the non-viable IUPs.

Averaged ROC curves for the prediction of viability based on the hCG ratio and serum initial progesterone are shown in Figure 1. The hCG ratio had an AUC of 0.756 (95% CI, 0.686–0.826), whereas initial progesterone had an AUC of 0.678 (95% CI, 0.603–0.753) (difference = 0.078, 95% CI = −0.014 to 0.169; MI $P = 0.098$).

Table 1 Descriptive statistics for viable and non-viable intrauterine pregnancies (IUPs) (continuous variables)

Variable	*Viable IUPs*	*Non-viable IUPs*	*MI P-value**
hCG 0 h (IU/L)	474	433	0.568
hCG 48 h (IU/L)	1124	771	0.016
hCG ratio	2.265	1.830	< 0.001
Progesterone 0 h (nmol/L)	71.5	56.0	< 0.001
Age (years)	29.0	31.0	0.003
Gestational age (days)	31.0	35.9	< 0.001
Endometrial thickness (mm)	13.4	12.8	0.749

Data provided are the average median over the 10 completed datasets using multiple imputation (MI). *Combination of Mann–Whitney *U*-test results for each completed dataset according to Li *et al.*[16]. hCG, human chorionic gonadotropin.

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Table 2 Descriptive statistics for viable and non-viable intrauterine pregnancies (IUPs) (categorical variables)

Variable	*Viable IUPs*	*Non-viable IUPs*	*MI P-value**
Disrupted midline echo	6.4	3.4	0.392
Free fluid in pouch of Douglas	28.6	26.7	0.775
Symptomatic (pain or bleeding)	61.1	58.8	0.708
Lower abdominal pain	47.9	47.1	0.849
Vaginal bleeding			0.346
No	76.9	73.3	
Yes: without clots	22.5	22.2	
Yes: with clots	0.6	4.4	

Data are provided as average percentage over the 10 completed datasets using multiple imputation (MI). *Combination of likelihood ratio chi-square test (or the Cochran–Armitage trend test for vaginal bleeding) results per completed dataset according to Li *et al.*[16].

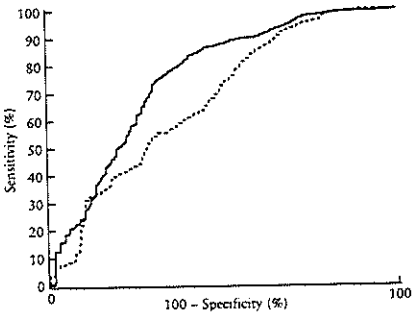

Figure 1 Receiver–operating characteristics (ROC) curves for predicting the viability of intrauterine pregnancies of uncertain viability in the pregnancy of unknown location population using the 48 h : 0 h human chorionic gonadotropin ratio (——; area under the curve (AUC) = 0.756) and initial progesterone level (- - - - -; AUC = 0.678).

The CI suggests that the performance of the hCG ratio is similar to or better than that of initial progesterone, as a difference of 0.014 (in favor of progesterone) is not clinically relevant whilst a difference of 0.169 (in favor of the hCG ratio) is. This is further supported by the observation that a large part of the ROC curve for initial progesterone is parallel to the diagonal line, suggesting random performance within the range of values underlying that part of the curve. This is not seen in the ROC curve for the hCG ratio. This is clear from the balanced accuracy rate curves for the hCG and initial progesterone, which are presented in Figures 2 and 3. Here, values equal to or greater than the threshold lead to the prediction of viability. Figure 2 demonstrates that a threshold of 2.00 for the hCG ratio appeared optimal with a balanced accuracy rate of around 72% (sensitivity 77.9%, specificity 66.6%), whilst for initial progesterone

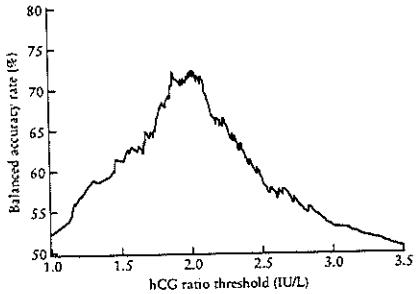

Figure 2 Balanced accuracy rate curve for predicting the viability of intrauterine pregnancies of uncertain viability in the pregnancy of unknown location population using the 48 h : 0 h human chorionic gonadotropin (hCG) ratio.

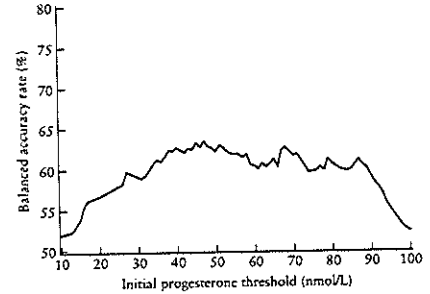

Figure 3 Balanced accuracy rate curve for predicting the viability of intrauterine pregnancies of uncertain viability in the pregnancy of unknown location population using initial serum progesterone level.

the balanced accuracy rate never exceeded 65%, and no clear optimal threshold could be derived (Figure 3).

The corrected AUC for the multivariate logistic regression model to predict viability, based on the hCG ratio and initial progesterone, was 0.758. This shows that initial progesterone did not appear to add predictive value to the hCG ratio because the hCG ratio alone had an AUC of 0.756. Adding the interaction between both markers did not further improve performance.

A priori, possibly useful covariates are maternal age, gestational age and whether the woman is symptomatic (i.e. vaginal bleeding and/or pain). Because the last of these covariates was unimportant in the univariate analysis (Table 2), we focused on maternal age and gestational age. The corrected AUC for a model using hCG ratio and the two covariate variables was 0.775. Adding initial progesterone as a predictor gave a corrected AUC of 0.785.

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DISCUSSION

Based on the results of this study, the performance of the hCG ratio is clinically similar to or better than initial serum progesterone measurements for predicting the viability of IPUVs in the PUL population. Individual clinicians will continue to use either the hCG ratio or serum progesterone for predicting viability in IPUVs based upon their own interpretation of observational evidence.

TVS is regarded as the gold standard for determining pregnancy viability. It is known that the fetal heart pulsation may be visible on TVS from 37 days of gestation in normal pregnancies[20]. The gestational age at which an IUP may be classified as non-viable, in asymptomatic women with sure dates, is 46 days[21]. Performing an ultrasound too early may lead to inconclusive findings (a PUL or an IPUV) with the need for serial serum hCG measurements and further ultrasound assessment to confirm viability[22].

The use of serum markers, such as progesterone or hCG, may be helpful in the prediction of viability when TVS is inconclusive. Historically, high serum progesterone levels (> 55 nmol/L) have been associated with a viable pregnancy[7,23,24]. High serum progesterone levels reflect a persistent corpus luteum and a well-supported placenta[6]. Conversely, low levels of initial serum progesterone (< 30 nmol/L) have been demonstrated to correlate well with pregnancy failure[5,25].

Interestingly in our study, when evaluating the initial serum progesterone, no particular threshold was found to be more predictive of the viability of IPUVs (Figure 3). This was in contrast to the hCG ratio where a cut-off of > 2 correlated well with the viability of IPUVs. We have previously demonstrated that the hCG ratio can predict pregnancy failure in the PUL population. When the hCG ratio is > 2.00, after confirmation of location with a scan at 7 days, we can potentially avoid the viability scan after 14 days and directly book the woman for the nuchal scan at 11 to 13 + 6 weeks. Using the hCG ratio we would be able to diagnose correctly and book for the nuchal scan, without unnecessary interventions and without an increased risk of missing ectopic pregnancies, in 87% of IPUVs. However, ~8% of the IPUVs booked for the nuchal scan will be non-viable at 11 to 13 + 6 weeks[4].

This study demonstrates that combining the hCG ratio and initial serum progesterone is not necessary and does not improve performance. The addition of the covariates gestational age and maternal age to the hCG ratio and/or progesterone did not strongly improve the prediction of viability in IPUVs.

Conclusions

The performance of the hCG ratio for the prediction of viability in the PUL population is similar to or better than that of progesterone measurement at presentation. However, the preferences of the early pregnancy unit, evidence-based protocols, as well as the cost implications of these different biochemical assays, could also influence the choice for either the hCG ratio or progesterone.

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