Tubal subfertility and ectopic pregnancy. Evaluating the effectiveness of diagnostic tests
Mol, B.W.J.
10. Serum human chorionic gonadotrophin measurement in the diagnosis of ectopic pregnancy when transvaginal sonography is inconclusive

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Abstract

Objective: To assess the performance of serum human chorionic gonadotrophin (hCG) measurement in the diagnosis of ectopic pregnancy in patients in whom transvaginal sonography is inconclusive, and to evaluate if patient characteristics influence the diagnostic performance of serum hCG measurement.

Methods: In a prospective study performed in two large teaching hospitals in Amsterdam, The Netherlands, 354 consecutive pregnant patients with suspected ectopic pregnancy in whom transvaginal sonography showed no intra-uterine or ectopic pregnancy were included. Serum hCG measurement was performed in all patients. The performance of serum hCG measurement in the diagnosis of ectopic pregnancy was evaluated with Receiver Operating Characteristic analysis.

Results: Serum hCG measurement performed significantly better in patients with sonographic evidence of an ectopic mass or fluid in the pouch of Douglas than in those without sonographic abnormalities.

Conclusion: The interpretation of serum hCG measurement should depend on additional findings at transvaginal sonography. Serum hCG levels > 1,500 IU/L make the diagnosis of ectopic pregnancy very likely in patients in whom transvaginal sonography showed an ectopic mass or fluid in the pouch of Douglas. Higher cutoff level should be used in patients without these findings at sonography.
Transvaginal sonography and serum human chorionic gonadotrophin (hCG) measurement have proven to be reliable tools in the non-invasive diagnosis of ectopic pregnancy. Transvaginal sonography can discriminate between intra-uterine pregnancy and ectopic pregnancy by visualizing the uterine cavity and the adnexal region, respectively. The presence of an intra-uterine pregnancy virtually rules out the possibility of an ectopic pregnancy. The presence of a gestational sac with a yolk sac and/or cardiac activity in the adnexal region virtually proves the presence of an ectopic pregnancy, justifying start of treatment. The presence of an ectopic mass or fluid in the pouch of Douglas increases the likelihood of an ectopic pregnancy, but does not justify immediate start of treatment. Transvaginal sonography also is inconclusive in case no abnormalities are found in the adnexal region. Serum hCG measurement may be used as an additional diagnostic tool in these patients.

The main concern in the non-invasive management of ectopic pregnancy is to make sure that each patient in whom the diagnosis is made really has an ectopic pregnancy, thereby preventing unnecessary or even harmful treatment. It is less important to identify immediately those patients, who do not have an ectopic pregnancy, because sonography and serum hCG measurement can be repeated until a diagnosis is made with certainty. Consequently, diagnostic tests for ectopic pregnancy should combine an almost perfect specificity with the best possible sensitivity. Optimization of the cutoff value should therefore focus on the serum hCG concentration above which failure to image a gestational sac could be taken as a presumptive index for the presence of an ectopic pregnancy.

In 1981 Kadar et al. proposed the use of a discriminatory zone for serum hCG concentrations in the diagnosis of ectopic pregnancy. The discriminatory hCG zone was defined as the minimal hCG concentration above which the sac of an intra-uterine pregnancy can always be identified by sonography. Consequently, the diagnosis of an ectopic pregnancy can be made when an intra-uterine gestational sac is absent and the serum hCG concentration is above the discriminatory zone.

At its introduction, sonography was performed transabdominally and the discriminatory zone was situated between 6,000 and 6,500 IU/L. Since then, the resolution of sonography has improved greatly, and the discriminatory zone has decreased. Studies reporting on the use of serum hCG measurements combined with sonography in the diagnosis of ectopic pregnancy have recommended cutoff levels varying between 1,000 and 6,500 IU/L. Thus far, no studies have used Receiver Operating Characteristic (ROC) analysis to identify the optimal cutoff value for serum hCG measurement.

The aim of the present prospective study among consecutive patients with suspected ectopic pregnancy with inconclusive sonographic findings was therefore to assess the discriminative capacity of serum hCG measurement by means of ROC analysis. The impact of different patient characteristics on the discriminative capacity of serum hCG measurement was also assessed.
10.2 Materials and Methods

Consecutively seen pregnant women with clinically suspected ectopic pregnancy in the Academic Medical Center and the Onze Lieve Vrouwe Gasthuis between September 1993 and April 1996 were entered in a prospective study. Both centers are large teaching hospitals that serve a population of mixed ethnicity in Amsterdam, The Netherlands. The study was approved by the institutional review board of both hospitals. Pregnancy had to be confirmed by a urine pregnancy test (Icon-test; Hybritech® Inc., San Diego, CA). Ectopic pregnancy was suspected in patients who had one or more of the following inclusion criteria: (1) clinical symptoms (abdominal pain and/or vaginal bleeding); (2) presence of one or more risk indicators for ectopic pregnancy; (3) routine sonography, performed after a gestational age of 6 weeks, that failed to show an intra-uterine gestational sac; and/or (4) the microscopic absence of chorionic villi after dilatation and curettage. The following risk indicators were considered: a previous ectopic pregnancy, known tubal pathology detected on hysterosalpingography and/or laparoscopy, previous tubal surgery, pelvic inflammatory disease, diethylstilbestrol-exposure in utero, and sterilization or a contraceptive device in situ at the moment of conception. Hemodynamically unstable patients were excluded from the study.

After patients gave informed consent, transvaginal sonography was performed (Hitachi® EUB 415/515, Hitachi® Medical Corporation, Tokyo, Japan). Sonography was performed by one of the study investigators or, during shifts, by the resident on call. The intra-uterine cavity was scanned and an intra-uterine pregnancy was diagnosed in case an intra-uterine gestational sac was visualized. When an intrauterine gestational sac could not be visualized, both adnexal regions were scanned for the presence of an ectopic gestational sac, an ectopic mass, or fluid in the pouch of Douglas. An ectopic gestational sac was defined as the presence of a yolk sac or a fetal pole. In case an ectopic gestational sac or ectopic cardiac activity was visualized an ectopic pregnancy was diagnosed.

After sonography had been performed, serum hCG concentration was determined using Microparticle Enzyme Immunoassay (Imx analyzer, Abbott® diagnostics division, Chicago IL, USA) and expressed in IU/L (conversion factor to SI unit, 1.00 according to the World Health Organization Third International Standard 75/537). An ectopic pregnancy was diagnosed in case the serum hCG concentration was ≥ 1,500 IU/L in patients in whom sonography failed to show an intra-uterine or ectopic gestational sac. An exception was made for patients with a clinical picture suggestive of a complete miscarriage. Those patients were managed expectantly and excluded from the study.

In cases where transvaginal sonography showed no gestational sac but serum hCG concentration was < 1,500 IU/L, the patients were instructed and reevaluated two days later on an outpatient basis, as described elsewhere (see chapter 11).

The diagnosis was verified in several ways. The diagnosis ectopic pregnancy was verified by laparoscopy. The diagnosis intra-uterine pregnancy was confirmed by repeated sonography at a gestational age of 12 weeks or by histopathology in case of miscarriage; when serum hCG concentrations declined, they were measured repeatedly until it declined below the detection threshold. Thus, three final diagnostic categories were
distinguished; ectopic pregnancy, viable intra-uterine pregnancy and a rest category of 'non-viable pregnancy' that included nonviable intra-uterine pregnancies and chemical pregnancies that resolved without treatment.

**Analysis**

The analysis was limited to those patients in whom transvaginal sonography was inconclusive, i.e., to those patients in whom a gestational sac could not be visualized either within or outside the uterus. Thus, patients in whom transvaginal sonography had demonstrated a gestational sac inside or outside the uterine cavity were excluded from the analysis, because sonography had resolved the diagnostic problem. Further, patients in whom pregnancy was a result of in-vitro fertilization and embryo-transfer (IVF-ET) were excluded, because transfer of multiple embryos could influence the cutoff level for test positivity. Patients with missing data were also excluded. Likelihood ratios (LR) and their 95% confidence intervals (CI) were calculated for each category of sonographic findings.

A ROC-curve was constructed, illustrating the capacity of serum hCG measurement to diagnose ectopic pregnancy, and the area under the ROC-curve was calculated. The area under the ROC-curve expresses the performance of a diagnostic test taking values in the range between 0.5 and 1. An area under the ROC-curve of 0.5 implies that the diagnostic test under study has a discriminative capacity that does not exceed chance, whereas an area under the ROC-curve of 1 implies that the discriminative capacity of the test under study is perfect.

Subsequently, the diagnostic performance of serum hCG measurement in association with patient characteristics was evaluated. We compared the distribution of serum hCG concentrations in subgroups of patients with the use of the Kolmogorov-Smirnov test. Subgroups were defined based on presence or absence of abdominal pain, vaginal bleeding, and an ectopic mass and/or fluid in the pouch of Douglas at transvaginal sonography. In all comparisons, a P-value < 0.05 was considered to indicate a statistically significant difference between the distributions. Whenever distributions of serum hCG concentrations were found to be significantly different between patients with and without any of the studied characteristics, these distributions were plotted to visualize the effect of the characteristics on the diagnostic performance of serum hCG measurement. Such differences could have impact on the ROC-curves and therefore could be of clinical significance. Therefore, a scatter plot was constructed that showed the distribution of serum hCG concentrations in patients with and without the characteristics. When the scatter plot indicated a clinical significance impact, ROC analysis was performed in subgroups of patients with and without the specified characteristic, and LRs were calculated for various levels of serum hCG concentrations.

**10.3 Results**

During the study period, 824 consecutively seen patients presented with suspected ectopic pregnancy. Twenty-three hemodynamically unstable patients were excluded.
Transvaginal sonography showed an intra-uterine gestational sac in 333 patients and the diagnosis intra-uterine pregnancy was made. The diagnosis intra-uterine pregnancy was confirmed in 323 patients, whereas 10 patients gad an ectopic pregnancy. Transvaginal sonography revealed an ectopic gestational sac in 74 patients. An ectopic pregnancy was confirmed at laparoscopy in 72 patients. Two patients did not undergo laparoscopy because their serum hCG concentrations were < 200 IU/L.

Ten patients presented with a clinical picture of complete miscarriage, and also were excluded. Among the remaining 384 women, pregnancy resulted from IVF-ET in 26. The serum hCG concentrations were not available in four women, all of whom were determined to have an ectopic pregnancy.

Among the 354 included patients, 223 had abdominal pain, 228 had vaginal bleeding, and 134 had at least one risk-indicator for ectopic pregnancy. In 34 patients an intra-uterine gestational sac could not be detected on routine sonography performed after a gestational age of six weeks, and in 14 patients histopathology showed no chorionic villi in specimens obtained at curettage. An ectopic mass was detected at initial sonography in 58 patients. Fourteen patients showed fluid in the pouch of Douglas on sonography. Twenty patients had an ectopic mass and fluid in the pouch of Douglas.

An ectopic pregnancy was verified in 129 (36%) of the 354 patients, whereas a viable intra-uterine pregnancy was verified in 67 (19%) patients. The other 158 patients had a non viable pregnancy; a chemical pregnancy with declining serum hCG concentrations was verified in 135 (38%) patients, whereas 23 (6%) patients appeared to have had a miscarriage.

The LRs for the presence of ectopic pregnancy were 3.6 (95% CI 2.2 to 5.9) for an ec-
**Table 1:** Distribution of initial serum hCG concentration in patients without a viable pregnancy at transvaginal sonography, stratified for patients with and without an ectopic mass or fluid in the pouch of Douglas at sonography.

<table>
<thead>
<tr>
<th>Initial serum hCG concentration (IU/L)</th>
<th>EP* viable pregnancy</th>
<th>non-viable pregnancy</th>
<th>LR for EP</th>
<th>LR for IUP</th>
<th>95% CI</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 66</td>
<td>n = 66</td>
<td>n = 25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1,000</td>
<td>18</td>
<td>1</td>
<td>21</td>
<td>0.29</td>
<td>(0.18 - 0.45)</td>
<td>2.4</td>
</tr>
<tr>
<td>1,000 ≤ hCG &lt; 1,500</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>0.79</td>
<td>(0.29 - 2.9)</td>
<td>0</td>
</tr>
<tr>
<td>1,500 ≤ hCG &lt; 2,000</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>∞</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 2,000</td>
<td>36</td>
<td>0</td>
<td>1</td>
<td>14</td>
<td>(2.0 - 98)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Patients without an ectopic mass or fluid in the pouch of Douglas at sonography**

<table>
<thead>
<tr>
<th></th>
<th>n = 65</th>
<th>n = 66</th>
<th>n = 133</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1,000</td>
<td>36</td>
<td>53</td>
<td>118</td>
</tr>
<tr>
<td>1,000 ≤ hCG &lt; 1,500</td>
<td>2</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
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<td>5</td>
</tr>
<tr>
<td>≥ 2,000</td>
<td>24</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: Number of patients

EP = ectopic pregnancy; IUP = intra-uterine pregnancy; LR = likelihood ratio; CI = confidence interval

The Kolmogorov-Smirnov test statistic indicated that the distribution of initial serum hCG concentrations in patients with an ectopic pregnancy differed significantly between patients in whom transvaginal sonography showed either an ectopic mass or fluid in the pouch of Douglas, and patients with an ectopic pregnancy without sonographic abnormalities (P-value < 0.01). The median serum hCG concentration was 1,320 IU/L (range 85 to 42,000 IU/L) in patients with additional sonographic findings and 668 IU/L (range 68 to 84,000 IU/L) in patients without additional sonographic findings. No statistically significant differences were found between the serum hCG distributions of patients with and without abdominal pain (P-value = 0.17) or between distributions of patients with and patients without vaginal bleeding (P-value = 0.85).

The distribution of initial serum hCG measurements in patients without an ectopic

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**Figure 1** shows the ROC-curve for initial serum hCG measurement in the diagnosis of ectopic pregnancy. The area under the ROC-curve was 0.81 (95% CI 0.77 to 0.85). In seven of 76 patients with an initial serum hCG concentration > 1,500 IU/L, ectopic pregnancy was not confirmed at laparoscopy, whereas in two patients with a serum hCG concentration of exactly 1,500 IU/L, laparoscopy was delayed. Of these nine false-positive findings, one patient had an ectopic mass at sonography, and the other eight had no findings whatsoever at initial sonography (Table 1). The final diagnosis in these patients was molar pregnancy, triplet pregnancy, miscarriage (n=2), chemical pregnancy (n=3) and non-viable intra-uterine pregnancy (n=2), respectively.
Figure 2AB: Left the distribution of the serum hCG concentrations in patients with and without an ectopic pregnancy, stratified for the presence or absence of an ectopic mass or fluid in the pouch of Douglas on transvaginal sonography. Right the corresponding ROC-curves after stratification for additional findings at transvaginal sonography.

pregnancy did not differ significantly between patients in whom transvaginal sonography showed an ectopic mass or fluid in the pouch of Douglas and patients who had no abnormalities in the adnexal region (P-value = 0.06), between patients with and without abdominal pain (P-value = 0.37), and between patients with and without vaginal bleeding (P-value = 0.06).

Figure 2A shows the distribution of serum hCG concentration in patients with and patients without an ectopic pregnancy, stratified for the presence of additional findings at transvaginal sonography. Among 262 patients who did not have an ectopic mass or fluid in the pouch of Douglas on transvaginal sonography, 63 (24%) had an ectopic pregnancy. Among 92 patients who had either an ectopic mass or fluid in the pouch of Douglas at sonography, 66 (72%) had an ectopic pregnancy. The corresponding ROC-curves for serum hCG measurement are plotted in figure 2B. The area under the ROC-curve was 0.74 (95% CI 0.68 to 0.80) for patients without an ectopic mass or fluid in the pouch of Douglas and 0.85 (95% CI 0.83 to 0.87) for patients with an ectopic mass or fluid in the pouch of Douglas.

Table 1 shows the distribution of serum hCG concentrations in patients with and without an ectopic pregnancy, taking into account additional sonographic findings. For patients in whom sonography showed an ectopic mass or fluid in the pouch of Douglas, the LR increased strongly in case serum hCG concentrations were > 1,500 IU/L. For patients without additional findings, the LR increased strongly in case serum hCG concentrations were > 2,000 IU/L. There were no viable intra-uterine pregnancies in case serum hCG levels were ≥ 1,500 IU/L.
10.4 Discussion

Since the first publication on the discriminatory hCG zone concept, the resolution of transvaginal sonography gradually has improved. Several studies have proposed lower cutoff values for serum hCG concentrations than the initial threshold of 6,000 IU/L as proposed by Kadar et al. in 1981. Some have used repeat measurements and others have not focussed on almost perfect specificity. In an earlier smaller study, ROC analysis was not performed.

In the present study, ROC analysis showed that a serum hCG concentration > 2,000 IU/L almost certainly rules in ectopic pregnancy. However, the diagnostic performance of serum hCG measurement depended on additional findings at transvaginal sonography. In patients in whom sonography showed an ectopic mass or fluid in the pouch of Douglas, a serum hCG concentration > 1,500 IU/L indicated ectopic pregnancy with virtual certainty. In the cohort under study, a cutoff value of 2,000 IU/L for patients without any sonographic abnormalities would have prevented five unnecessary laparoscopies, whereas the diagnosis ectopic pregnancy would have been delayed in one patient.

Verification bias, which occurs when verification of the diagnosis depends on the test under study, is a problem in the interpretation of our findings. Confirmative laparoscopy was only performed when the serum hCG concentration initially was > 1,500 IU/L or was > 1,000 IU/L at repeat measurement, or when it reached a plateau after three consecutive measurements. As a consequence, in patients with such serum hCG concentrations ectopic pregnancy was always detected and treated, whereas in patients with lower serum hCG concentrations chemical pregnancies with declining serum hCG levels managed expectantly. Therefore, our study design may have overestimated the specificity of serum hCG measurements, because positive results of serum hCG measurement were followed by confirmative laparoscopy and subsequent treatment, when some of these patients possibly could have been managed expectantly.

However, expectant management in patients with an ectopic pregnancy with such serum hCG concentrations would in our opinion have been unethical. Recently, Korhonen et al. reported that expectant management was successful in 77% of the patients with suspected ectopic pregnancy who had a serum hCG concentration < 5,000 IU/L. Unfortunately, these investigators did not evaluate the relation between serum hCG concentration and failure of expectant management. Future studies are needed to determine the serum hCG concentration at which expectant management is safe.

In the literature the performance of a diagnostic test is commonly reported in terms of sensitivity, specificity and LRs. When such parameters are used to evaluate the performance of a diagnostic test, the crucial underlying assumption is that these indices remain constant for patients with different clinical characteristics. This assumption has been found to be erroneous in several clinical situations in which it has been tested, the best documented example being exercise tests in the diagnosis of ischaemic heart disease.

The present study shows that the patient characteristics abdominal pain and vaginal bleeding had no statistically significant influence on the diagnostic performance of serum
hCG measurement. In contrast, sonographic detection of an ectopic mass and/or fluid in the pouch of Douglas had a strong impact on the diagnostic performance of serum hCG measurement. In fact, the presence of sonographic abnormalities makes the presence of an ectopically nidated pregnancy very likely.

The serum hCG concentration is in these patients used as a prognostic indicator for the need of treatment, with 1,500 IU/L as an initial cutoff level. Because, in our opinion, a diagnosis is only useful when it results in a decision regarding therapy, we decided to classify patients with low serum hCG levels who might have an ectopically nidated pregnancy in the rest category of ‘non-viable pregnancy’.

Furthermore, the prevalence of ectopic pregnancy differed strongly between patients with additional sonographic findings and patients without such findings. In patients with additional sonographic findings the prevalence of ectopic pregnancy, i.e., the pre-test probability, was 72% (66/92). In contrast, in absence of additional sonographic findings this prevalence was only 24% (63/162). This is another reason that serum hCG concentrations should be interpreted with more caution in absence of additional sonographic findings. In fact, the discriminative capacity of serum hCG measurement in absence of these findings should be higher to compensate for the lower pre-test probability. Unfortunately, in this case the discriminative capacity of serum hCG measurement is lower.

In conclusion, the interpretation of serum hCG measurement should depend on additional findings at transvaginal sonography. Serum hCG levels > 1,500 IU/L in patients with an ectopic mass or fluid in the pouch of Douglas make the diagnosis of ectopic pregnancy very likely. Higher cutoff level should be used in patients without these findings at sonography. In chapter 12 it will be demonstrated that sonographic findings and serum hCG concentrations should not be interpreted independent from the pre-test probability for an ectopic pregnancy.

10.5 References


