Natural Limits of Pregnancy Testing in Relation to the Expected Menstrual Period

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Context
Pregnancy test kits routinely recommend testing “as early as the first day of the missed period.” However, a pregnancy cannot be detected before the blastocyst implants. Due to natural variability in the timing of ovulation, implantation does not necessarily occur before the expected onset of next menses.

Objective
To estimate the maximum screening sensitivity of pregnancy tests when used on the first day of the expected period, taking into account the natural variability of ovulation and implantation.

Design and Setting

Participants
Two hundred twenty-one healthy women 21 to 42 years of age who were planning to conceive.

Main Outcome Measures
Day of implantation, defined by the serial assay of first morning urine samples using an extremely sensitive immunoradiometric assay for human chorionic gonadotropin (hCG), relative to the first day of the missed period, defined as the day on which women expected their next menses to begin, based on self-reported usual cycle length.

Results
Data were available for 136 clinical pregnancies conceived during the study, 14 (10%) of which had not yet implanted by the first day of the missed period. The highest possible screening sensitivity for an hCG-based pregnancy test therefore is estimated to be 90% (95% confidence interval [CI], 84%-94%) on the first day of the missed period. By 1 week after the first day of the missed period, the highest possible screening sensitivity is estimated to be 97% (95% CI, 94%-99%).

Conclusions
In this study, using an extremely sensitive assay for hCG, 10% of clinical pregnancies were undetectable on the first day of missed menses. In practice, an even larger percentage of clinical pregnancies may be undetected by current test kits on this day, given their reported assay properties and other practical limitations.

Methods
We studied 221 healthy North Carolina women who were planning to become pregnant. Women with no known fertility problems were recruited in 1982-1986 from the local community and enrolled at the time they discontinued their method of birth control. Women ranged from 21 to 42 years of age; mean age was 30 years, with 5% older than 35 years. Most were college educated, and 96% were white; further details are provided elsewhere. All participants provided informed consent, and the study was ap...
PREGNANCY TESTING AND EXPECTED MENSTRUAL PERIOD

Table. Estimated Day of Implantation of Clinical Pregnancies Relative to the Expected Onset of the Next Menstrual Period

<table>
<thead>
<tr>
<th>Estimated Day of Implantation Relative to First Day of the Expected Period</th>
<th>Estimated No. of Conceptions Implanted on This Day</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>−7 and earlier</td>
<td>55</td>
<td>40</td>
</tr>
<tr>
<td>−6</td>
<td>15</td>
<td>51</td>
</tr>
<tr>
<td>−5</td>
<td>14</td>
<td>62</td>
</tr>
<tr>
<td>−4</td>
<td>9</td>
<td>68</td>
</tr>
<tr>
<td>−3</td>
<td>7</td>
<td>74</td>
</tr>
<tr>
<td>−2</td>
<td>8</td>
<td>79</td>
</tr>
<tr>
<td>−1</td>
<td>8</td>
<td>86</td>
</tr>
<tr>
<td>Day of expected period</td>
<td>6</td>
<td>90</td>
</tr>
<tr>
<td>+1</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>+2</td>
<td>3</td>
<td>93</td>
</tr>
<tr>
<td>+3</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>+4</td>
<td>2</td>
<td>96</td>
</tr>
<tr>
<td>+5</td>
<td>1</td>
<td>97</td>
</tr>
<tr>
<td>+6</td>
<td>0</td>
<td>97</td>
</tr>
<tr>
<td>+7</td>
<td>0</td>
<td>97</td>
</tr>
<tr>
<td>+8</td>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>+9</td>
<td>0</td>
<td>98</td>
</tr>
<tr>
<td>+10</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>+11 and later</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td></td>
</tr>
</tbody>
</table>

Among the 151 clinical pregnancies in our study, implantation relative to the first day of the missed period. Implantation occurred by the first day of the missed period in 90% of pregnancies (95% confidence interval [CI], 84%-94%). By 7 days after the first day of the missed period, 97% of all clinical pregnancies had implanted (95% CI, 94%-99%). After excluding the 21 women who reported that their cycles were “irregular,” results were unchanged (90% and 97%).

COMMENT

Implantation can occur surprisingly late in relation to a woman’s expected menses. For 10% of clinical pregnancies in our study, implantation occurred after the first day of the next expected period. This represents an insurmountable limitation of hCG-based pregnancy testing on the first day of the missed period. A perfectly sensitive assay for hCG could not have detected 100% of these clinical pregnancies even 10 days after the period was expected (Table).

These data describe the physiologic limits of early pregnancy testing. However, we did not directly test the performance of home test kits. The performance of a single, qualitative assay in the hands of a layperson depends on many factors. One factor is the assay detection limit. While detection limits are not routinely provided with test kits, a Web site lists assay detection limits for 40 commercial kits as determined by telephoning the manufacturers.2 These reported assay detection limits of hCG range from 15 to 100 mIU/mL, with most kits between 25 and 50 mIU/mL. Detection limits for some kits may be slightly more sensitive than reported by manufacturers.8 Even so, an assay detection limit of 15 mIU/mL is about 100 times less sensitive than the assay we used to define implantation (0.13 mIU/mL). This implies that most current test kits would not reliably detect hCG on the day of implantation. Chard9 estimates that an assay with a detection limit of 25 mIU/mL will begin to detect pregnancy around 3 or 4 days after implantation.

Other factors also affect the performance of test kits. Urinary dilution may reduce detection limits.10 Not all test kits measure the same components of hCG; some measure only intact hCG, while others measure intact plus the free beta.11,12 The ratio of these hCG components may vary from pregnancy to pregnancy,13 which could affect the detection limits of specific tests. In addition, user errors have been reported to contribute to false-negative findings.13

The instruction to test on the “first day of the missed period” may not mean the same thing to all women. Women do not know their period is late until the second day of their expected period. If women think the first day of their missed period is after they know their period is late, the percent of false-negative test results would decrease slightly.

How all these factors affect actual test kit performance is hard to predict. If kits do reliably detect pregnancy by the third...
day after implantation, then about one fourth of clinical pregnancies would produce a false-negative test result on the first day of the missed period. Previous studies do not provide information on this point because they lacked proper attention to the timing of the test in relation to expected menses. It should not necessarily be the goal of home test kits to achieve the lowest possible limits of hCG detection. About one fourth of all pregnancies detectable at implantation fail very early. The detection of these events by highly sensitive home test kits would be of uncertain benefit to women.

The interpretation of a negative pregnancy test result on the first day of the missed period deserves comment. Some package inserts state that if a test is negative, “you are probably not pregnant.” One says “you are NOT PREGNANT” (emphasis in the original). This unfounded assurance could have important consequences. For example, women with a negative test result may fail to protect themselves from exposures to toxicants in the workplace or to medications that could damage a developing embryo.

In summary, the timing of implantation varies widely in its relation to the expected period. Many women will test positive a week or more before their period is expected, while a few women will test positive only a week or more afterward. Adolescents and young women are frequent users of test kits but may be especially prone to false-positive test results because they are at high risk for delayed ovulation. Better, information on the limits of early testing can help balance the costs and benefits of early detection against the risks of a false-negative test result.

Author Contributions: Study concept and design: Wilcox. Acquisition of data: Wilcox, Baird, McChesney, Weinberg. Analysis and interpretation of data: Wilcox, Baird, Dunson, McChesney, Weinberg. Drafting of the manuscript: Wilcox. Critical revision of the manuscript for important intellectual content: Wilcox, Baird, Dunson, McChesney, Weinberg. Statistical expertise: Dunson. Administrative, technical, or material support: Wilcox, McChesney. Study supervision: Wilcox.

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REFERENCES
CORRECTIONS

Incorrect Wording: The Brief Report entitled “Natural Limits of Pregnancy Testing in Relation to the Expected Menstrual Period” published in the October 10, 2001, issue of THE JOURNAL (2001;286:1759-1761) contained incorrect wording. On page 1761, last paragraph, the third sentence should be: “Adolescents and young women are frequent users of test kits but may be especially prone to false-negative test results because they are at high risk for delayed ovulation.”

Incorrect Wording and Omissions: In the Caring for the Critically Ill Patient article titled “High-Dose Antithrombin III in Severe Sepsis: A Randomized Controlled Trial” published in the October 17, 2001, issue of THE JOURNAL (2001;286:1869-1878), several errors were printed. On page 1874 in the first column, second paragraph, the sentence should read “Antithrombin III resulted in a 15% relative improvement in 90-day mortality,” not “absolute improvement.” On page 1875, the title of Table 3 should read “Incidence of New Organ Dysfunction According to Logistic Organ Dysfunction Score….” Also on page 1875, the legend for Figure 5 should indicate that the patients represented in the graph are the percentage discharged alive from ICU in relation to all patients (surviving and dead). On page 1876, the footnote to Table 4 was inadvertently omitted. It should have read “Number of patients actually treated with antithrombin III or placebo.” This footnote clarifies the different patient numbers for the primary efficacy analysis (n=1157 per group) vs the safety analysis (placebo: n=1155; antithrombin III: n=1161). On page 1876, the top paragraph in the third column should end with this sentence: “Up to and beyond the second interim analysis, an overall nominal survival benefit for antithrombin III prevailed, despite the recognized increased bleeding risk in that group.”

Incorrect Date: In the Original Contribution entitled “Survival by Time of Day of Hemodialysis in an Elderly Cohort” published in the December 5, 2001, issue of THE JOURNAL (2001;286:2690-2694), there was an incorrect date in the abstract under “Design.” The year after the word “baseline” should be 1988, not 1998.