Letter to the Editor

Diagnostic accuracy of non-invasive prenatal sex determination: a large-scale study

To the Editor:

Non-invasive prenatal determination of fetal sex offers a promising alternative to invasive diagnosis of X-linked diseases and fetal disorders of ambiguous genitalia (1–4).

Using free-fetal DNA (ffDNA) from maternal plasma (5) and different detection systems for Y chromosome (6–9), researchers achieved high diagnostic accuracy in fetal sex prediction during the first trimester of pregnancy without excluding variable percentage of false positives (FP) and false negatives (FN) (10, 11).

We previously showed higher performance of DYS14 than SRY detection system without reaching an optimal diagnostic accuracy because of the low percentage of FN and FP (12).

The aim of this study was to enhance the performance of our DYS14 detection system by introducing new key elements, increasing the volume of maternal plasma for DNA extraction and employing innovative interpretation criteria of results.

Finally, we audited the overall diagnostic accuracy on a large-scale study to verify whether the new protocol ensured the correct fetal sex determination.

We analyzed, in two different phases, plasma samples from 513 women at 10–15 weeks of gestation.

During the first non-blinded phase, we established the best threshold value (TV) to discriminate male and female fetuses and, in the second blinded phase, we applied it to assess its diagnostic performance. Fetal sex was verified with the analysis of karyotype or confirmed with phenotype at birth.

Genomic DNA was extracted from maternal plasma (500 μl in the first and 1000 μl in the second phase) using QIAamp DSP Virus kit (Qiagen, Hilden, Germany) and analyzed by real-time polymerase chain reaction (PCR) 7300 detection system (Applied Biosystems, Foster City, CA) using DYS14 detection system to measure the quantity of ffDNA (three replicates for each maternal sample) and telomerase reverse transcriptase detection system as a quality control. Real-time PCR [quantitative PCR (qPCR)] reaction was set up as previously described (12).

Results were expressed as median values with range for a descriptive statistics and analyzed by using receiver operating characteristic (ROC) curves, calculated by spss software 17.0 (SPSS Inc., Chicago, IL), to set the TV, in terms of ffDNA concentration and number of DYS14-positive replicates.

Karyotype or phenotype at birth revealed that among the 115 pregnant women analyzed in the first phase of study, 55 delivered one daughter and 60 one son.

By evaluation of qPCR results (Table 1), we set our first TV by analysis of ROC curve calculated using the number of DYS14-positive replicates (data not shown). However, the TV was not satisfactory, except for samples with 0, 1 or 2 DYS14-positive replicates that were clearly identified as female fetus. Only one sample from a male pregnancy had 0 DYS14-positive replicates.

The critical point was the interpretation of those results with three DYS14-positive replicates, as they occurred in either female or male pregnancies. Two samples from female pregnancy gave three positive replicates but with a very low ffDNA concentration, whereas all the samples from male pregnancy gave three DYS14-positive replicates with elevated ffDNA concentration (Table 1). Therefore, we built a new ROC curve combining ffDNA concentration and the number of DYS14-positive replicates and selecting only those samples with three DYS14-positive replicates (2 from female and 59 from male pregnancies). We individuated the best ffDNA concentration value as 1.42 GE/ml as it allowed us to reach a 100.00% diagnostic sensitivity [95% (confidence interval) CI: 93.9–100.0] and 100.00% diagnostic specificity (95% CI: 19.3–100.0) (Fig. 1).

In the second phase of study, karyotype or phenotype at birth revealed that 208 delivered a son and 190 a daughter.
Table 1. Range of ffDNA concentration values (expressed as genome equivalent of ffDNA per milliliter of maternal plasma, GE/ml) and number of positive replicates obtained by DYS14 qPCR observed in two groups of pregnant women during the first and second phase of the study

<table>
<thead>
<tr>
<th>Phase</th>
<th>Female pregnancies</th>
<th>Male pregnancies</th>
<th>Female pregnancies</th>
<th>Male pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of samples</td>
<td>Range of ffDNA</td>
<td>Number of samples</td>
<td>Range of ffDNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>concentration (GE/ml)</td>
<td></td>
<td>concentration (GE/ml)</td>
</tr>
<tr>
<td>First phase</td>
<td>55</td>
<td>0–1.44</td>
<td>60</td>
<td>0–120.46</td>
</tr>
<tr>
<td></td>
<td>Range of samples</td>
<td>(N = 55)</td>
<td></td>
<td>(N = 60)</td>
</tr>
<tr>
<td></td>
<td>Number of positive</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>replicates</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.06–1.44</td>
<td>0.04–7.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.06–1.37</td>
<td>0.04–12.06</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.06–1.37</td>
<td>0.04–12.06</td>
<td></td>
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<td></td>
<td></td>
<td>0.00–1.37</td>
<td>0.04–12.06</td>
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<td></td>
<td></td>
<td>(median 0.00)</td>
<td>(median 0.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>55</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; ffDNA, free-fetal DNA; qPCR, quantitative polymerase chain reaction.

Results are shown in Table 1. Among samples with three DYS14-positive replicates, those from female pregnancy had low ffDNA concentration with only one exception with a concentration of 120.46 GE/ml overlapping the ffDNA concentration range of male pregnancies.

Finally, applying on 398 samples our interpretative criterion of results together with increased plasma volume, we reached the best diagnostic accuracy as shown in Table 2.

As reported in literature, the most commonly used interpretative criterion of results is based on the number of Y chromosome-positive replicates (6, 9, 13), but it has not achieved an optimal performance because of the incidence of FN and FP (12, 14).

Table 2. Diagnostic parameters of DYS14 protocol for fetal sex determination in 398 pregnant women enrolled in the second phase of the study

<table>
<thead>
<tr>
<th>Diagnostic indexes of test</th>
<th>Percentage (%)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100.0</td>
<td>98.0–100.0</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.5</td>
<td>97.2–99.9</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>99.5</td>
<td>97.2–99.9</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>100.00</td>
<td>98.0–100.0</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99.7</td>
<td>99.4–100.0</td>
</tr>
</tbody>
</table>
In this study, combining ffDNA concentration and number of DYS14-positive replicates, we reduced the incidence of FP and by increasing plasma volume we completely eliminated FN.

The importance of using simultaneous values of ffDNA concentration and number of positive replicates was also evidenced by Hill et al. (10) on 511 pregnancies between 4 and 36 weeks of gestation, who achieved a 99.5% diagnostic accuracy. However, by testing each sample in eight replicates for DYS14 amplification and six replicates for SRY, they obtained a 12.9% of assay failure rate and in clinical practice they did not issue any report in 4.3% of cases.

Therefore, comparing our test to that of Hill et al. (10) we can highlight that our assay improvements allowed us to test each sample in only three instead of six/eight replicates and that we obtained conclusive results at the first analysis without any assay repetitions and issuing report to all cases. Moreover, we used only one Y marker instead of two as Scheffer et al. (15). These are important goals because the time-consuming, complexity and cost of the test are reduced without losing its diagnostic accuracy.

In conclusion, the excellent performance and the absence of any risk for mother and fetus shows the diagnostic validity of this test that could be safely translated into clinical practice, seen the importance of fetal sex determination in pregnancies at risk of ambiguous genitalia, X-linked conditions and single gene disorders such as congenital adrenal hyperplasia.

Acknowledgements

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References


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